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Fenugreek

Biology and Applications

Springer

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Preface

Diabetes and cardiac disorders are the two most common diseases prevailing nowadays in human life. The WHO estimates a prevalence of 347 million people suffering from diabetes, which is the cause of 4.6 million deaths every year as per estimates. It is the fastest-growing menace to human life worldwide because it is a major cause of comorbidities and mortality due to its increasing prevalence. World scientists are making unstinted efforts to explore the cure of diabetes by extending research to improve the biological understanding of the disease and development of its more effective treatment. Many allopathic drugs are commercially available for the management of diabetes. However, their side effects and high costs compel the scientists to think of herbal alternative drugs. Fenugreek (*Trigonella foenum-graecum*) is highly important in the management of diabetes and contributes greatly to the alternative systems of medicine. Ongoing global dynamic efforts may find a role of fenugreek in exploring the remedy for such a deadly disease.

Fenugreek plant bears a number of nutritionally and pharmaceutically important bioactive compounds, including trigonelline and diosgenin, which make the plant extraordinarily valuable. The plant reduces the blood and urinary glucose levels by slowing down the digestion and absorption of carbohydrates; it also lowers the blood cholesterol concentration significantly. Besides, due to high fibre-content in the seed, the plant bears beneficial properties to control diabetes. With the current subject in mind, we intend to deliver a content-rich manual to the reader, which can prove as a platform for their further studies, research, or field cultivation. Therefore, it is the need of the hour to embark upon these challenging issues, rising day by day in the life of human beings. Owing to the amazing medicinal properties of fenugreek, we aim to bring forth a comprehensive volume of the book "Fenugreek: Biology and Applications", highlighting its various aspects, including agricultural, pharmacological and pharmaceutical sides, in vitro technology, nutrient management strategies, and other aspects that are being involved in current scenario on this medicinally important plant and its future applications.

The book consists of 22 chapters, mostly review articles, written by the experts from around the globe. We are hopeful that this book would furnish the needs of most researchers, who are working or have a great interest in the concerned field. Undoubtedly, this book will be helpful for the general use of postgraduate students, research students, teachers, traditional practitioners, ethno-botanists, pharmacologists,

and herbal growers, who may have an extraordinary interest in this plant of paramount importance. This book will also prove fruitful in the field of agriculture, agronomy, botany, plant biology, biotechnology, medicinal chemistry, medical sciences, pharmacognosy, pharmacology, and pharmaceutical sciences.

Till date, no comprehensive book is available on this plant. This will be the first book of its kind on "Fenugreek: Biology and Applications", which will serve as an excellent reference-book on the subject that will also give way to future directions for research students, who believe that laboratory research should be oriented with field applications.

We are greatly thankful to Springer Nature Singapore Pte. Ltd. for their prompt acceptance and compilation of this scientific task. Sincere thanks are expressed to the team members of the Springer publisher for their dedication, sincerity, and friendly cooperation in producing this volume. With great pleasure, we extend our sincere thanks to all the contributors for their timely response, outstanding and up-to-date research contribution, support and consistent patience.

Lastly, thanks are also due to well-wishers, research students, and family members for their moral support, blessings, and inspiration in compilation of this book.

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About the Editors

M. Naeem is an Assistant Professor in the Department of Botany, Aligarh Muslim University, Aligarh, India. He received his degrees of BSc, MSc, and PhD in Botany from Aligarh Muslim University, Aligarh. He is recipient of Research Associateship and Young Scientist Award from the CSIR, New Delhi, and State Government of Uttar Pradesh, India, respectively. Also, he has received Postdoctoral fellowship of DST, SERB, New Delhi and UPCST, India. He is member of Indian Society for Plant Physiology, Indian Botanical Society, Society for Medicinal Plant and Natural Product, Germany, American Society for Plant Biologists, USA, etc. His research focuses on escalating the production of MAPs and their active principles using a novel and safe technique involving radiation-processed polysaccharides as well as the application of PGRs under normal and stressed conditions.

Tariq Aftab received his Ph.D. from the Department of Botany at Aligarh Muslim University, India, and is currently an Assistant Professor at the university. He is the recipient of a prestigious Leibniz-DAAD fellowship from Germany, Raman Fellowship from the Government of India, and Young Scientist Awards from the State Government of Uttar Pradesh (India) and Government of India. He also worked as Visiting Scientist at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, and in the

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M. Masroor A. Khan is affiliated with the Department of Botany, AMU, Aligarh, India. After completing his PhD, he joined Ohio State University, USA as a Postdoc. Before he joined as Assistant Professor in this university, he worked as Pool Scientist and Research Associate (CSIR, New Delhi). He has published 10 books and more than 200 research papers in reputed journals and books on the subject concerned. He has guided 14 PhD, 2 M Phil, and 40 MSc students to date. He is working to promote the productivity and active ingredients of medicinal and aromatic plants using different strategies, such as application of mineral nutrition, PGRs, and nanoparticles. He has contributed greatly towards the establishment of radiation-processed polysaccharides (RPPs) as plant growth promoters.

Part I

Agricultural Procedures for the Cultivation and Production of Fenugreek

Historical Background, Origin, Distribution, and Economic Importance of Fenugreek

1

Ayah Rebhi Hilles and Syed Mahmood

Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is named as Trigonella, meaning 'little triangle' in the Latin due to its yellowish-white triangular flowers. It is one of the oldest medicinal plants in history with an exceptional medicinal and nutritional profile. Its description and benefits had been mentioned in the Ebers Papyrus (one of the oldest Egyptian medicinal document maintained) earlier in 1500 BC. It has a moderate tolerance to salinity, drought, and heavy metals and can adapt to different climatic regions and marginal lands. Fenugreek seeds contain a substantial amount of oleic acid, nicotinic acid, linolenic acid, linoleic acid, fibres, glycolipids, phospholipids, choline, and vitamin A, B1, B2, C, and niacin. Empirical studies suggest that fenugreek has many potential therapeutic applications such as antidiabetic, antioxidant, antibacterial, anti-anorexia, and anticarcinogenic agents. Fenugreek also showed a potential for development as a resource of sustained income to cultivators, traders, and allied industrial concerns. Fenugreek currently cultivated in many countries from Asian, Europe, and Africa. India has fenugreek seed production of 1675 kg/ha, while Egypt and United Kingdom produce about 1400 kg/ha and 3700 kg/ha, respectively. The valuable resources of fenugreek can be further commercially exploited to the benefit of the farming community and economy of the country. On the similar note, the current chapter was aimed to discuss historical background, origin, distribution, and economic importance of fenugreek.

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Keywords

Active components · Antioxidants · Therapeutic uses · Fenugreek

1.1 Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an aromatic annual leguminous crop of family Fabaceae exhibiting diploid status with no aneuploidy and is considered one of the oldest medicinal plants. It is used as a herb (fresh leaves or dried), and spice (seeds) (Zandi et al. 2015). Fenugreek has long pods and tender leaves which are used in various medicinal preparations, as well as a spice in Indian food (Baliga et al. 2017). Fenugreek leaves are dark green in colour and the leaf petioles are cartilaginous and thickened at the top (Aasim et al. 2018). The pods of fenugreek are curved with short hair with 10–18 cm length and 3.5 × 5 mm width. The pods are reddish or greenish, turning brown on ripening (Petropoulos 2003). Fenugreek seed has yellow embryo surrounded by a corneous and comparatively large layer of white and semi-transparent endosperm (Wani and Kumar 2018). The height of fenugreek plant is about 30–60 cm with the long pink cylindrical stem while the root has a finger-like structure. It has pinnate, stipules triangular, and long-stalked compound leaves. Its flowers appear axillary and are 15 cm long, 2–8 pods, white to yellowish-white colour with 5 petals which are hermaphrodite and insect-pollinated. Its seeds are hard, smooth, small in size (5 mm long), with yellow to golden-yellow colour, and the germination process of fenugreek takes 5–10 days while the first trifoliate leaf appears at 5–8 days after germination. It is a fast-growing plant and needs 4–7 months to reach maturity. The flowering period is midsummer from June to August and seeds ripen during late summer. It can also grow in a tropical climate with cool summer or mild winter (Ahmad et al. 2016). Fenugreek possesses various medicinal activities; it can be used as a human food supplement due to its nutritional benefits to the health as it contains essential amounts of proteins, carbohydrates, fats, and amino acids. The pharmacological effect of fenugreek seeds is attributed to its bioactive compounds which serve as raw materials for the manufacture of different therapeutic and hormonal drugs (Mahmood and Yahya 2017).

1.2 Origin and Distribution

There are different claims about the origin of fenugreek, Vavilov (1926) suggested that fenugreek originated from the Mediterranean region, while there was another claim of it being Asian in origin (De Candolle 1964). Fazli (1978) suggested that fenugreek is originated in the Asia, while Dangi et al. (2004) claimed that origin of fenugreek was from Turkey. It is also reported that fenugreek was cultivated as a crop in parts of Europe, northern Africa, west and south Asia, North and South America, and Australia (Acharya et al. 2006). Another study recorded that fenugreek is native to India and North Africa (Fuller and Stephens 2015). It was reported that

Table 1.1 Different claims about the origin of fenugreek

Different claims about the origin of fenugreek	Reference
Mediterranean	Vavilov (1926)
Asian	De Candolle (1964), Fazli (1978)
Turkic	Dangi et al. (2004)
Indian and North African	Fuller and Stephens (2015)
West African	Rashid et al. (2018)

fenugreek is native to West Africa and now it is cultivated in India, Pakistan, China, Turkey, Egypt, and Mediterranean countries. It has been stated that fenugreek is used in the medicinal, nutraceutical, and pharmaceutical fields. Different countries around the world used fenugreek seeds and leaves for pharmaceutical purposes and in food industry (Rashid et al. 2018). Such disagreements over the origin of fenugreek suggest that its location is still debatable. There were six species of fenugreek in Asian countries, five species in Europe, one species in Africa, and one species in Australia. Despite the differences in the origin of fenugreek, it was reported that there are 260 species of fenugreek (Petropoulos 2002a, b, c). The most common species of fenugreek currently recognized are: *T. caerulea, T. corniculata, T. anguina, T. rigida, T. arcuata, T. arabica, T. cariensis, T. suavissima, T. torulosa, T. hamosa, T. cretica, T. spinosa, T. occulta, T. polycerata, T. radiata, T. platycarpos,* and *T. striata* (Acharya et al. 2008). It is distributed throughout the world, Asia considered on the top of the continents in the production of fenugreek (Mentioned 80% in the 'Economic Importance' Section) (Jongebloed 2004). Other Asian countries such as Iran, China, and Pakistan also contribute a significant portion in the overall production of fenugreek. Africa ranks second after Asia in terms of fenugreek production (Qadir et al. 2017). It was also recorded that fenugreek is cultivated in parts of West Asia, Australia, the United Kingdom, Mediterranean Europe, Russia, and the USA. Fenugreek is used as a herb (dried or fresh leaves), a vegetable (fresh leaves), spice (seeds), and as an artificial flavouring of maple syrup or in the manufacture of steroids and other hormones (Ouzir et al. 2016) (Table 1.1).

1.3 Historical Background

Fenugreek is one of the oldest known medicinal plant that has been documented in ancient religious scriptures, herbal publications, travel records, and anecdotes dating back in human history (Lust 1986). In the first century BC, the Romans used wine flavoured with fenugreek (Curry 2010). In ancient Rome, it was used as an aid to be inducing labour during delivery and it was cultured as a forage crop in the ancient Greek period (Yoshikawa et al. 1997). It is also mentioned in the texts of Ayurveda, Charaka Samhita and Sushruta Samhita that were written as far back as 1000 BC (Jhajhria and Kumar 2016). Utilization of fenugreek seeds in Chinese medicine was

first introduced during the Song Dynasty (1057 BC). It was used in traditional Chinese medicine as a tonic and treatment for weakness and oedema of the legs (tissue swelling due to excess lymph fluid) (Basch et al. 2003). In China (1060 BC), fenugreek was used to dissipate cold and relieve pain. It was also used in Europe to treat skin inflammations and temporary lack of appetite (Yao et al. 2020). The whole grass, seeds, and flowers of fenugreek are used in traditional Chinese medicine for the treatment of hyperlipidaemia, hypertension, and immune diseases (Luan et al. 2018). Fenugreek leaves were used in Egyptian incense Kuphi to produce a holy smoke used in fumigation, and embalming rites (Rosengarten 1969).

Fenugreek seeds were found in the tomb of the Egyptian Pharaoh, Tutankhamun (1333 BC–1324 BC) (Fazli 1978 cited in Petropoulos 2002a, b, c). It was reported that fenugreek was used by people of Harappan civilization in India around 2000–1700 BC (Saraswat 1984). Fenugreek seeds are considered as a major constituent of Indian spices; they possess various medicinal activities and a good source of dietary fibre and protein. In India, soaking and germination of fenugreek seeds in water or curd were practised traditionally since ancient days, and this traditional germination process enhances the antioxidant activity and lowers glycemic index (Chaubey et al. 2018). It was documented that fenugreek was used dating back to the fifteenth century. The details about it were compiled in the well-known Kolozsvar Herbarium in 1578 (Petropoulos 2003). It was used worldwide since ancient times as a smelling agent and spice. In the fifteenth century in Africa, fenugreek seeds were used as coffee-substitute to control insect infestations in grain storages. Additionally, it was used in traditional healing process and in cosmetic industries. Dried seeds and leaves were used for curing various illness such as eczema, rash, or inflammatory conditions using a paste prepared with ground fenugreek seeds. It was also well documented the use of fenugreek in ethnoveterinary purposes (Chaudhary et al. 2018). Fenugreek was later introduced to Central Europe at the beginning of the nineteenth century but the cultivation was recorded in England in the sixteenth century (Petropoulos 2002a, b, c). Some uses of fenugreek were documented in the sixteenth century by Hidvegi et al. (1984). Howard (1987) mentioned that fenugreek seeds powder was used in the seventeenth century to expel the placenta after giving birth. In the nineteenth century, it was used for postmenopausal and dysmenorrhoeal symptoms, reducing blood glucose level and promoting lactation (Wani and Kumar 2018) as mentioned in Fig. 1.1.

Fenugreek seeds were used traditionally for various health benefits such as improving the digestion, reducing blood glucose levels in diabetic patients, and maintaining plasma cholesterol level. These health benefits were attributed to the bioactive compounds found in abundance in the fenugreek (Salarbashi et al. 2019). Another study recorded that fenugreek was used as a traditional herbal medicine in the treatment of many health conditions such as diabetes, hyperlipidaemia, and to increase milk production in lactating women (Fuller and Stephens 2015). Dried fenugreek seeds have been traditionally used in India, Egypt, China, and in some parts of Europe for their benefits to the health such as antibacterial, galactagogue, anti-inflammatory, insulinotropic, and rejuvenating effects. It is also used for food flavouring, curry powders, spice blends, and teas (Khorshidian et al. 2016).

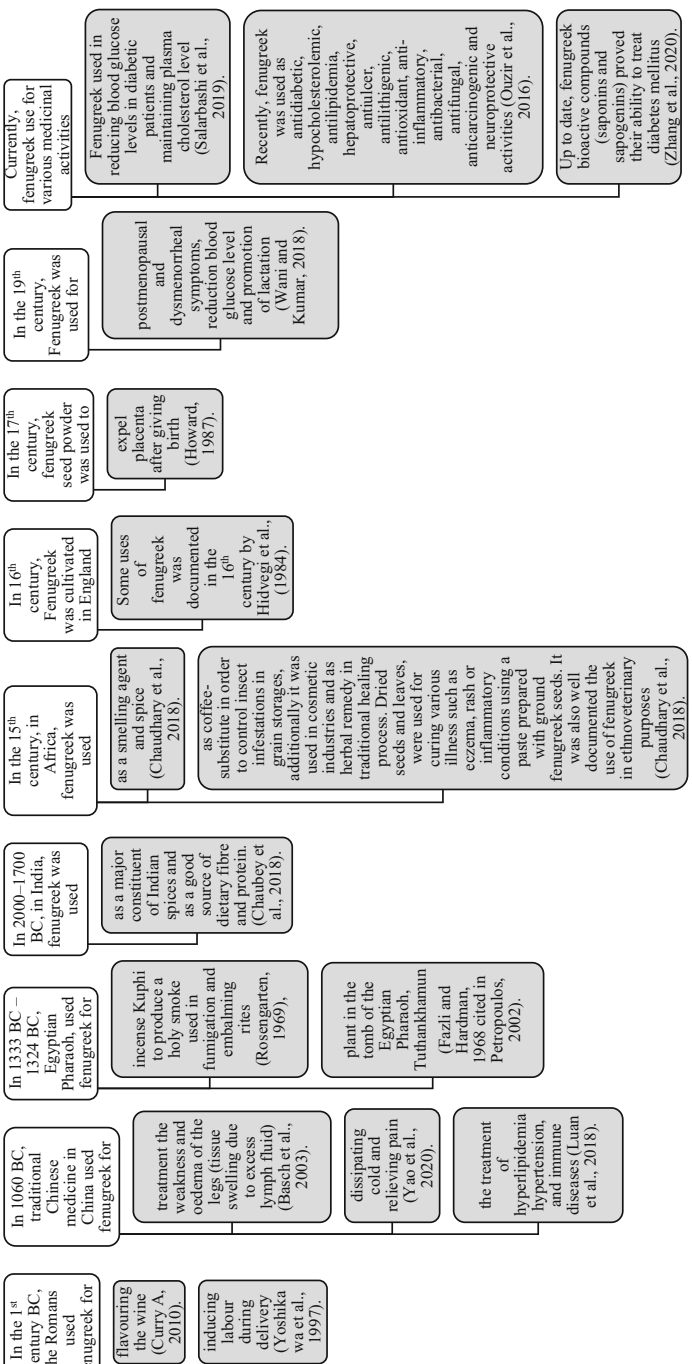

Fig. 1.1 Flowchart shows the history of fenugreek uses from the first century to the twentieth century

Currently, fenugreek is cultivated in different countries around the world including India, China, Egypt, Morocco, Ethiopia, Turkey, Ukraine, Greece, and Canada mainly as a beneficial medicinal plant (Dadrasan et al. 2015). Recently, fenugreek is being used for its antidiabetic, hypocholesterolaemic, antilipidaemic, hepatoprotective, antiulcer, antioxidant, anti-inflammatory, antibacterial, antifungal, anticarcinogenic, and neuroprotective activities (Ouzir et al. 2016). Fenugreek is used as a spice in Iran, in Switzerland, for preparing flavour cheese, in Egypt, as seed powder with flour used for making flatbread, and in India and Pakistan, it is used as a vegetable (Moradi and Moradi 2013). Up to date, fenugreek bioactive compounds (saponins and sapogenins) proved their ability to treat diabetes mellitus (Zhang et al. 2020).

1.4 Economical Importance

Fenugreek is economically important as a culinary ingredient and medicinal herb, which continues to grow extensively throughout its native regions. India is the leading fenugreek producers in the world, accounting for 80% of the world's production (Mentioned '90%' in the 'Origin and Distribution' Section; Kindly check and be consistent) (HerbaZest Editorial Team 2020). Its production has reached up to 45,000–55,000 tonnes per year (Jhajhria and Kumar 2016). In 2010–2011 fenugreek cultivation was 80,378 ha, and it showed 94,200 metric tonnes of its production (Vidyashankar 2014). It was reported that fenugreek seed yields of 500–3320 kg/ha and that yields of 1800 kg were economically feasible (Banyai 1973). It offers an economical advantages besides its benefits to the health, especially in reducing blood glucose level (Goyal et al. 2016). In Morocco, fenugreek plays an important role in the socio-economical framework (Brogi et al. 2019). The seed yields of 3700 kg were reported from Bath, England and 1000 kg from Morocco. Main exporters of fenugreek are India, France, Lebanon, Egypt, and Argentina (Duke 2012). It has high impact on soil renovator in Asia and Africa (Duke 1986). Its seeds are a source of diosgenin, which is a base to produce oral contraceptives. It is rich in protein and fixed oils which could make a two-fold economical contribution to the world increase of population problems by assisting in birth control and at the same time providing additional food. Moreover, fenugreek as a plant can save energy by fixing atmospheric nitrogen, contribution to the world's food supply, reducing hunger and improving health care. Thus, the future of fenugreek seems promising and beneficial.

1.5 Conclusion

Although the origin of fenugreek is still debatable, it is definitely one of the oldest medicinal plants in the written history. It is traditionally consumed for medicinal purposes since prehistoric time. It is extensively used in food and medicine. Fenugreek seeds were applied traditionally for impotence and lowering blood glucose;

besides, it was used in treating many symptoms such as burns, gout, eczema, stomach discomfort, and diarrhoea. Interestingly, fenugreek is cultivated and consumed in different forms in various parts of the world including India, northern Africa, the Mediterranean, and Canada.

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2 Fenugreek Cultivation in the Middle East and Other Parts of the World with Emphasis on Historical Aspects and Its Uses in Traditional Medicine and Modern Pharmaceutical Science

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the botanical family Papilionaceae, and its native geographic range is the area extending from Iran to Northern India, but it is presently cultivated also in other regions of the world. Historically, fenugreek has been used as an important traditional, multipurpose medicinal herb in Iranian, Indian, Chinese, and Tibetan Medicinal Practices for several centuries. The most important compositions of fenugreek seeds are neutral detergent fiber, protein, gum, moisture, lipids, starch, and ash. Fenugreek seeds and leaves are anti-cholesterolemic, anti-inflammatory, anti-tumor, carminative, demulcent, deobstruent, emollient, expectorant, febrifuge, galactogogue, hypoglycemic, laxative, parasiticide, restorative, and uterine tonic and useful in burning sensation. Traditional uses of fenugreek seeds around the world are in bone and muscles, respiratory system, gastrointestinal system, female reproductive system, cardiovascular system, endocrinology, and hepatic. The most important modern health benefits of fenugreek are in appetite suppressant and weight loss, reduce cholesterol, reduce cardiovascular risk, control diabetes, a good relief

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for sore throats, it cures acid reflux or heartburn, relieves constipation, prevents colon cancer, good for kidney trouble, good for skin infection, increases milk production, reduces menstrual discomfort, and it minimizes symptoms of menopause. Integrative use of modern science and traditional medicine with novel technologies and discoveries will secure production of medicinal herbs and promote sustainability in a long-term and a wide-range. Treatment with natural medicine especially barberry as non-synthetic drug is recommended.

Keywords

Trigonella · Fenugreek · Traditional medicine · Modern pharmaceutical science

2.1 Introduction

2.1.1 Fenugreek—History, Occurrence, and Cultivation

Herbal medicines have played a major role in the health care system of many countries throughout the world (Ogbaji et al. 2013, 2018; Shahrajabian et al. 2019a, b, c, d; Sun et al. 2019a, b; Shahrajabian et al. 2020a, b, c, d, e, f, g, h, i, j; Sun et al. 2020a, b). Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the legume family (Alaghemand et al. 2017). This crop is native to an area extending from Iran to northern India, but is now widely cultivated in China, north and east Africa, Ukraine, and Greece (Petropoulos 2002). It was called as *Trigonella* from Latin language that means little triangle due to its yellowish-white triangular flower (Shashikumar et al. 2018). Hippocrates considered it a soothing herb. Fenugreek is a Greek hayseed originating in the Mediterranean, Southern Europe, and Western Asia (Altuntas et al. 2005). The Near East region, extending from Israel through Syria and southern Turkey into Iran and Iraq, and the Mediterranean center including Spain, Morocco, and Turkey are the centers of origin of *Trigonella*, *Trifolium*, and *Medicago* species. It is also part of Traditional Persian Medicine (TPM), and more than 32 species of this plant have been found in central regions of Iran. Fenugreek is one of the oldest cultivated medicinal plants identified in written history. According to historical facts, the classical texts of Ayurveda, Charaka Samhita, and Sushruta Samhita were written around 1000 BC and these include 600 medicinal plants along with therapeutics (Jhajhria and Kumar 2016). Fenugreek was introduced into Chinese medicine in the Sung dynasty, 1057 AD. Dioscorides, a Greek physician of Anazarbus in Cilicia and who considered father of pharmacology (65 AD), write that fenugreek is an active compound of ointments and mentions fenugreek as a spice crop in his texts. Both the foliage and seeds of fenugreek are edible and are especially integral to Indian cuisine, found in curries and chutney. The seeds are also sometimes roasted and used in India as a substitute for coffee. The leaves are used to make an herbal tea, and the seeds are sprouted and used as a vegetable in many African nations. Fenugreek is also used in the Jewish version of halvah, a sweet confection. Fenugreek was also used to embalm the dead

Table 2.1 Botanical classification of *T. foenum graecum*

Domain	Eukarya
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Sub-family	Trifoliae
Genus	Trigonella
Sub-genus	Foenum graecum
Species	*Trigonella foenum-graecum*

of the ancient Egyptians. The herb was used by the Jewish defenders of Jerusalem during the first Jewish-Roman war to repel the invaders from the city wall. Fenugreek was combined with boiling oil and then poured down upon the city walls, making them too slick for the Romans to climb. In Moroccan cuisine, it is the whole fenugreek seeds which are primarily used as a spice. These fragrant, golden seeds most famously show up as a key ingredient in the Moroccan chicken and lentil dish of Rfissa. Due to fenugreek's ability to promote lactation, this particular dish is traditionally prepared for new mothers several days after giving birth. Helba is an ingredient in a number of other cuisines, including Indian, Pakistani, Egyptian, Chinese, Greek, Turkish, and Middle Eastern. Ghormeh sabzi is the quintessential Iranian recipe. It is a stew prepared with fresh herbs that is considered to be the national dish of Iran, and fenugreek leaves are the most important ingredients of it. Fenugreek is resistant to a wide variety of crop diseases; but is susceptible to the fungal pathogen powdery mildew (*Erysiphe polygoni* D. C.) and does not grow well in moist and humid areas that facilitate the fungal infection. This plant is recommended for the semi-arid regions of Asia, Sub-Saharan Africa, and Latin America as a low input, dryland, annual forage legume. The genetics, physiology, and highly specialized arid and semi-arid climate adaptations allow fenugreek to work as an active nitrogen-fixer with low water requirement and allow the crop to grow in arid or semi-arid climatic regimes under low input agriculture system practices in several poor developing and under-developed nations. India is the largest global producer of fenugreek in the world. Since fenugreek is a low input crop marginal lands in Iran could be used for commercial fenugreek production and can contribute towards income generation for farmers with lower capacity for agricultural investments. Also, the rising popularity for fenugreek products in the international markets could be an important opportunity for Iranian farmers for earning extra profit for a low input industrial crop. Basu and Zandi (2017) stated that fenugreek has the potential to become a chemurgic crop suitable for supply of raw materials rich in phytochemicals for the emergent global food and nutraceutical industries. They have concluded that fenugreek foods and fenugreek products show significant promise for sales into global niche markets; if the target consumers are made aware of the important health benefits of fenugreek comprehensively. Botanical classification of fenugreek is shown in Table 2.1. The diversity and current

Table 2.2 The diversity and current distribution of the crop across major continents

Continent	Countries
Asia and the Middle East	Turkey, Israel, Lebanon, Jordan, Syria, Saudi Arabia, Bahrain, Qatar, the UAE, Kuwait, Oman, Yemen, Iraq, Iran, Afghanistan, Turkmenistan, Azerbaijan, China, Taiwan, India, Pakistan, Nepal
Africa	Egypt, Morocco, Tunisia, Algeria, Sudan, Libya, Ethiopia, Eritrea, Kenya, South Africa
Europe	Russia, the UK, France, Spain, Portugal, Greece, Italy, Sweden, Germany, Switzerland, Austria, Hungary, Poland, Ukraine, Romania, Croatia, Slovenia
North America	Canada, the USA
South America	Argentina
Oceania	Australia

Table 2.3 Major *Trigonella* species and their distribution

Trigonella species	Distribution
T. Arabica Delile (Syn. T. pectin Schenk)	N. African, especially in Arabia; Syria to NE Egypt
T. caerulea (L.) Ser. (syn. *Melilotus caeruleus* Desr. *Trifolium caeruleum* Moench., *Trigonella melilotus caoerulear* L. Aschers. Et Graebn)	E. Mediterranean region; SE Europe, origin in Mediterranean region
T. caerulea (L.) Ser. ssp. *Caerulea*	C., W., and S. Europe; N. Africa, widely cultivated in gardens
T. corniculata (L.) L. (syn. *Medicago corniculata* L. Trautv., *Trifolium corniculata* L., *Trigonella esculenta* L.)	Mediterranean region; Near East countries
T. stellata Forsk	N. Africa, Arabia, Egypt, Tunisia, Algeria, Morocco, Canary Islands, W. Asia, Iran, Iraq, Middle East, Israel, Lebanon, Kuwait
T. foenum-graecum L. (syn. *Foenum graecum officinale* Moench, *T. graeca* St. Lag.)	Caucasus, ex Soviet Union, C. Asia, E. Europe

distribution of the crop across major continents is shown in Table 2.2. Major *Trigonella* species and their distribution are shown in Table 2.3.

Ogbaji et al. (2018) reported that soaking and germination of fenugreek seeds resulted in significant changes in bioactive components and antioxidant activity. They have found from their results that germinated fenugreek possess more health potential compared to non-germinated fenugreek seeds. Kaur (2016) showed that fenugreek accumulated Pb and translocated it in the harvestable parts of the plants. Metal accumulation increased consistently with increasing concentration of Pb in the treatments. Dry matter yield of plant increased with decreasing Pb concentration in the treatment, and fenugreek can be used for remediation of Pb contaminated soil. Singh et al. (2018) observed that increased green leaf and seed yields occurred when nitrogen level increased and cutting was delayed, and the maximum harvest index (0.44) was when nitrogen was applied at 60 kg/ha and plants were cut at 60 DAS.

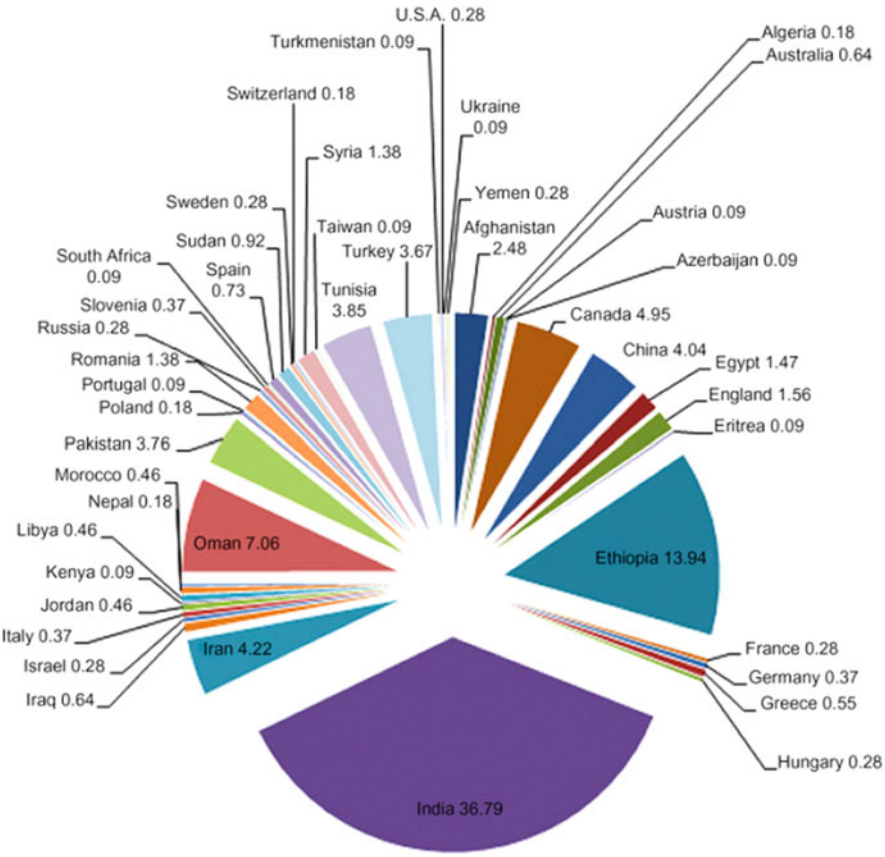

Fig. 2.1 Pie chart indicating relative frequency of fenugreek diversification distributed across different countries

They concluded that delaying cutting increases nitrogen requirement in fenugreek if supplied in proper amount and it results to increased yield. Pie chart indicating relative frequency of fenugreek diversification distributed across different countries is presented in Fig. 2.1.

2.2 Chemical Constituents and Nutritional Composition

Fenugreek is a natural source of iron, silicon, sodium, and thiamine and it contains mucilagins which are known for soothing and relaxing inflamed tissues. Fenugreek seeds contain alkaloids, including trigonelline, gentianine, and carpine compounds; the seeds also contain fiber, 4-hydroxyisoleucine, and fenugreekine, a component that many have hypoglycemic activity (Moradi kor et al. 2013). Seed of fenugreek contains lysine, and L-tryptophan, proteins, mucilaginous fiber and saponins,

Table 2.4 Important phytochemicals reported from fenugreek as a medicinal herb

Trigonelline
Tigogenin
Diosgenin
Apigenin
Kaempferol
Luteolin
Atroside
Yamogenin (Steroidal sapogenins)
Graecunin B, C, D, E, and G (Spirostanol Saponins)
Gitogenin (dihydroxy-sapogenins)
Triterpenoids
Galactomannan (complex carbohydrate)
4-hydroxy isoleucine (essential amino acid)
Fenugreekine (alkaloids)
Saponins
Quercetin (flavonoids)

coumarins, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin, and trigonelline (Bukhari et al. 2008). Important phytochemicals reported from fenugreek as a medicinal herb are shown in Table 2.4.

Fenugreek seeds are a natural source of vitamins such as thiamine, biogenic elements such as Fe, Si, and Na, and a rich source of P and S (El-Nasir and El-Tinay 2007). In the research conducted by Kochhar et al. (2006), fenugreek seeds contained 25.8% crude protein and 6.53% oil. Seed dry matter had the following chemical composition: 3% ash, 6.28% crude fiber, and 58.13% total carbohydrates. El-Nasir and El-Tinay (2007) estimated the protein content of fenugreek seeds at 28.4%, crude fiber at 9.3%, and crude fat at 7.1%. The fatty acid profile was dominated by unsaturated acids: oleic, linoleic, and alpha-linolenic acids which account for 16.3%, 50.0%, and 24.4% of total fatty acids, respectively. The unique mineral and organic properties of fenugreek are exploited in the production of functional and nutritional foods as well as nutraceuticals and cosmetics (Hooda and Jood 2005; Lubbe and Verpoorte 2011). Bienkowski et al. (2017) investigated that fenugreek seeds grown in north-eastern Poland contained 26.0% protein and 4.8% oil. They have observed that total unsaturated fatty acids accounted for 80% of the fatty acid profile, with a predominance of essential fatty acids in oil: linoleic acid (37.9%) and α-linolenic (28.2%) acid. Sowing date and weed control were responsible for up to 3.1%–4.5% of differences in concentrations of essential fatty acids between treatments in their experiment. Composition of fresh fenugreek leaves and mature fenugreek seeds is shown in Table 2.5.

Chandra et al. (2018) revealed that consumption of fenugreek fiber flakes (5 and 10 g) with a standard breakfast increased satiety satisfaction and fullness. Fenugreek fiber flakes reduced hunger and desire to consume food and prospective food consumption in 10 g significantly compared to 5 g. There were no significant

Table 2.5 Composition of fresh fenugreek leaves and mature fenugreek seeds

Component	Leaves	Seeds
Moisture	86.0 g	–
Protein	4.4 g	30 g
Fat	1.0 g	7.5 g
Fiber	1.0 g	50 g
Sapogenins	–	2 g
Trigonelline	–	380 mg
Ca	395 mg	160 mg
Mg	67 mg	160 mg
P	51 mg	370 mg
Fe	16.5 mg	14 mg
Na	76 mg	19 mg
K	31 mg	530 mg
Cu	0.26 mg	33 mg
S	167 mg	16 mg
Cl	165 mg	165 mg
Mn	–	1.5 g
Cr	–	0.1 mg
Choline	1.35 g	50 mg
Vitamin C	52 mg	43 mg
Beta carotene	2.3 mg	96 μg
Thiamine	40 μg	340 μg
Riboflavin	310 μg	290 μg
Nicotinic acid	800 μg	1.1 μg
Folic acid	–	84

Values expressed per 100 g

changes observed in glucose homeostasis with fenugreek fiber flakes. Fenugreek fiber flakes 5 g and 10 g showed an acceptable safety profile. In their study fenugreek fiber flakes have a role in the control of food intake in normal individuals who want to use diet as a method to control energy intake through their effects on appetite suppression and food intake.

2.3 Traditional and Modern Pharmaceutical Sciences

Fenugreek seeds are used in remedies for diabetes and hyper-cholesterolemia in Indian, Arabic, and Chinese medicine. Traditional Persian medicine (TPM) is one of the ancient traditional medicines, recommended prescriptions that are still in use in Iran and different countries in west and center of Asia for many diseases like asthma (Emtiazy et al. 2018). Zakariya al-Razi has used fenugreek to treat diabetes and Sheikh Bu Ali Sin has presented some information about therapeutic properties and benefits of this plant in eliminating mouth odor, undesired odor of body and sweat in his book named Medicine Law, and he has also mentioned some other properties and

therapeutic benefits for this plant (Bahmani et al. 2016). The nature of this plant is dry and warm and has laxative properties; its oil is useful for hair. Its mucilage in seeds, especially if mixed with oil of flower, treats striae created by cold. This plant is used to treat skin diseases like black spots and annoying odor of body, mouth, and sweat. It can treat dandruff if it is used as a shampoo. Boiled form of fenugreek helps treat the red spot of eye and helps soften throat and chest and provides relief from cough (Bahmani et al. 2016). Using this plant in the form of powder, infusion, decoction, and pomade has been very common in traditional medicine of Iran from ancient times (Bu Ali Sina 1988). This plant is locally used as an emollient in treatment of pellagra, loss of appetite, gastrointestinal disorders, and it is also used as a general tonic (Sweetman 2009). The plant has been used for centuries in Indian Ayurvedic Treatment (IAT) as well as traditional Chinese medicine (TCM), and Unani System of Medicine (USM) as important medicinal herb (Thomas et al. 2006; Khan et al. 2017). Traditional Chinese herbalists used this plant for kidney problems and conditions affecting the male reproductive tract. The seeds also function as a preservative and are added to pickles, chutneys, and other similar products (Vortex health, Fenugreek). In modern food practice, the seeds or the extract are used in bakery products, frozen dairy products, meat products, relish, condiments, candy, gravy sauces, gelatin puddings, and in alcoholic and non-alcoholic beverages. The nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia. The seeds freshen bad breath and help restore a dulled sense of taste. The oil in the seeds is used as a skin softener and emollient. In China, the fenugreek seeds are used treat cervical cancer. In the Middle East and the Balkans, the aerial parts of plants are a folk remedy for abdominal cramps associated with both menstrual pain and diarrhea or gastroenteritis. They are also used to ease labor pains (Indian food, Fenugreek) (Moradi kor et al. 2013). In addition to its medicinal properties, fenugreek is also recognized for its culinary value, and the plant is widely used as a spice that not only improves the taste of food, but also contributes to metabolic functions and overall health (Zuk et al. 2017). Wijaya et al. (2013) found that in traditional medicine, fenugreek is used to prepare infusions, water and alcohol extracts, tinctures, meads, tonics with antidepressant and psychotonic properties, and muscle growth supplements. Fenugreek is used in the treatment of seborrhea, acne, and dermatitis, and it is widely used n cosmetology (Wijaya et al. 2013). In traditional Persian medicine is used as an appetite stimulant, lung tonic and chest wall analgesic, also enhances breathing and lung secretion, clears the voice, and induces menstruation (Emtiazy et al. 2018). Yao et al. (2019) reported that fenugreek extract and its bioactive compounds showed excellent anti-diabetic activity and anti-obesity activity in studies; they have concluded that, although the fenugreek seed has been used as TCM raw materials for a long time of period in China, China is lacking the research of fenugreek in both depth and width. The rich medicinal properties of fenugreek is attributed to the presence of a wide diversity of important phytochemicals in the seeds and leaves of the plant like trigonelline, fenugreekine (alkaloids), atroside, quercetin (flavonoids), diosgenin, tigogenin, yamogenin (steroidal sapogenins), gitogenin (dihydroxy-sapogenins) triterpenoids, galactomannan (complex carbohydrate), and 4-hydroxy isoleucine (essential amino acid). Both

leaves and seed have important medicinal properties and are known to reduce blood glucose (anti-diabetic), and blood cholesterol (anti-hypercholesterolemic) levels in both human subjects and in experimental animals. Acharya et al. (2006) reported that leaves and seeds of fenugreek have been used extensively for medicinal purposes. Its seeds are known to exhibit anti-diabetic and anti-nociceptive properties and effects such as hypocholesterolemic, anti-cancer, and thyroxine-induced hyperglycemia. Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses (Basch et al. 2003). Its utility has been proved experimentally in diabetic humans (Sharma and Raghuram 1990). Fenugreek is reported to have anti-diabetic, anti-fertility, anti-cancer, antimicrobial, anti-parasitic, and hypocholesterolemic effect (Al-Habori and Raman 2002). In India, fenugreek is used as a lactation stimulant (Tiran 2003). Fenugreek seed in powder or germinated form exhibits anti-diabetic properties (Broca et al. 2004; Devi et al. 2003), hypocholesterolemic (Suboh et al. 2004; Devaraj and Devraj 2003), anti-cancer (Devasena and Menon 2003), effect on thyroxine-induced hyperglycemia, and protective effect on ethanol toxicity (Thirunavukkarasu et al. 2003). Kaviarasan et al. (2006) suggested that the polyphenolic compounds of fenugreek seeds can be considered cytoprotective during ethanol (EtOH)-induced liver damage. Badale et al. (2019) concluded that diosgenin alone did not cause any particular body weight or fat gain, but is likely to interact in a complex manner with the other ingredients of the fenugreek seeds. Fenugreek seeds are healthy but very bitter legumes, making these seeds difficult to be consumed as it is or to formulate fenugreek-enriched food products. When incorporated into food products, including breads, cookies, pastas, and tortillas, fenugreek seeds can improve insulin sensitivity.

Walli et al. (2015) suggested that extracts of fenugreek may have antibacterial activity against some human pathogens. They have shown that only the boiling water extract contains the antimicrobial active ingredients of fenugreek seeds, while both cold water extract and methanol extract are not suitable for such purposes. Hassani et al. (2019) noticed that fenugreek is effective for fasting blood sugar (FBS) and HgA1C control, lowering body mass index (BMI0, waist circumference, blood pressure and improving quality of life in type 2 diabetes mellitus (T2DM) patients. They have found that it can be ingested simply without adverse effects for blood glucose control in such patients. Avni et al. (2019) considered fenugreek as a useful medicinal plant for treatment of various dysfunctions and diseases in recorded history and in Ayurveda. They have mentioned that fresh leaves are also used as vegetables in the diets and the leaves is known for its medicinal qualities such as anti-diabetic, anticarcinogenic, hypocholesterolemic, and antioxidant. Lamfon (2012) discovered that fenugreek treatment leads to a significant decrease in the level of malondialdehyde (MDA) and increase in the activity of superoxide dismutase (SOD) and catalase (CAT). It is concluded that fenugreek extract can improve the testicular toxicity of carbendazim and this effect may be attributed to its antioxidant properties. Helal et al. (2019) observed that the high levels of fenugreek and soymilk intake can cause hormonal disturbance and decrease sperm count. They have suggested that the fenugreek oil and soy milk have high potential of negatively altering the lipid profile and increasing the health risks associated with a poor lipid

profile. Furthermore, both the fenugreek oil and soymilk possess potentials that can impair hormonal functions and fertility as demonstrated in their experiment. Badr (2017) concluded that 2.5% fenugreek is safe to be used as a hypocholesterolemic agent without any side effect for better kidney structure and function. El-Hak and Elrayess (2018) noticed that fenugreek seeds are rich with different benefits and medical compounds that have antioxidant and anti-inflammatory activity. Fenugreek seeds not recommended to be used to increase the male fertility, and if will be used should be used in low doses for a short time as using low doses of phytoestrogen neither affects the semen quality nor the reproductive function.

Kaya et al. (2019) observed that fenugreek prevented the proliferation of the parasite at certain times, and they thought that the dose can be increased when a rapid effect fenugreek extract on the parasite is desired (LD90 = 36.92 mg/mL), and the dose can be decreased if a long-term effect is expected (LD90 = 16.42 mg/mL). Sundaram et al. (2018) showed that fenugreek powder can be used adjunctive to scaling and root planning (SRP) to control the glycemic status and serum lipid levels in uncontrolled noninsulin-dependent diabetes mellitus (NIDDM) patients. Sharma et al. (2017) discovered that from bacteriological point of view fenugreek leaves and stem appear to play a great role in clinical as well as antibacterial agents. Poole et al. (2010) concluded that 500 mg of proprietary fenugreek extraction had a significant impact on both upper- and lower-body strength and body composition in comparison to placebo in a double blind controlled trial. Chourasiya et al. (2019) presented that not only substantiates the folklore use of the seed of fenugreek, but also suggests its inclusion in the treatment of anemia as it exhibited significantly anti-anemic activity. Kiss et al. (2019) did do a research and their study shed light to that chronic consumption of fenugreek seed is able to influence the complex interplay of anabolic hormones, their results also indicate that apart from its proven insulin sensitizing effect fenugreek might have a therapeutic potential in the adjuvant treatment of thyroid diseases. Singaravelu et al. (2018) announced that fenugreek seed extract is rich in polyphenol which protects the erythrocyte from oxidative damage and maintains the hemoglobin and PCV values. They have concluded that fenugreek seed extract has both gastro-protective and antioxidant property. Devi et al. (2013) in their study proved that a regular intake of fenugreek could reduce the oxidative stress by reducing the lipid per oxidation.

Bae et al. (2015) emphasized that fenugreek tea might be helpful on appetite control by reducing further food intake in overweight women. Abeysekera et al. (2018) concluded that seed extract of fenugreek had both anti-glycation and glycation reversing activities in BSA-glucose model. They have concluded that glycation reversing activity of fenugreek seed is a novel finding for anti-diabetic properties of fenugreek and indicates potential use in managing advanced glycation end products associated pathologies in diabetic patients. The most important health benefits of fenugreek are listed in Table 2.6.

Ajaya and Paramahand (2009) revealed that fenugreek in the diet showed a marked decrease in diabetes induced polydipsia, polyuria, urine sugar, hyperglycemia, renal hypertrophy, and glomerular filtration rate. The results of their experiment showed the beneficial effects of fenugreek in reducing kidney damage during

Table 2.6 The most important health benefits of fenugreek

It is used as appetite suppressant, and it facilitates weight loss
It reduces cholesterol
It reduces cardiovascular risk
It helps to control diabetes
It is a good relief for sore throats
It cures acid reflux or heartburn
It relieves constipation
It prevents colon cancer
It is good for kidney trouble
It is good for skin infection
It increases the milk production
It reduces menstrual discomfort
It minimizes symptoms of menopause

diabetes. Walli et al. (2015) indicated that only the boiling water extract contains the antimicrobial active ingredients of fenugreek seed, while both cold water extract and methanol extract are not suitable for these purposes. Sadak (2019) demonstrated the effect of silver nanoparticles on fenugreek plant. In his experiment, different concentrations increased plant growth, photosynthetic pigments, IAA contents, and yield quantity and quality. Among various concentrations used in the study, 40 mg/l AgNPs was the most effective treatment for the improvement in growth, biochemical parameters studied, and yield of fenugreek.

Zhou et al. (2019) successfully overcame the limitation of hydrophilicity of fenugreek gum by conjugating stearic acid as a hydrophobic chain through a simple esterification reaction. The obtained FG-C_{18} could self-assemble into spherical nanomicelles with narrow size distribution. And FG-C_{18} showed low hemolysis with the hemolytic ratio less than 5%. In vitro cytotoxicity studies showed high cell viability either on $HepG_2$ cells or on MCF-7 cells. Cellular uptake showed that C6-FG-C_{18} NMs with galactose residues could specifically recognize ASGP-R receptor on HepG2 cell surface compared to C6 solution. Finally, they reported that FG-C18 NMs showed enormous potential applicability as nanocarriers for intravenous administration of poorly soluble drugs due to biocompatibility, low toxicity, and liver-targeting potential. Figer et al. (2019) reported that in the human gastric carcinoma epithelial cells, fenugreek protected against the damage induced by ethanol at 5 μg/mL; whereas a protection of 67% at the dose of 1000 mg/kg was observed in the animal studies. The flavonoid derivatives, namely vitexin-7-O-glucoside, vicenin-2, orientin, and luteolin showed good interactions on H^+/K^+ ATPase while the saponins lacked good interaction in *in silico* analysis. Fenugreek seed extract showed gastroprotection in both: *in vitro* and *in vivo* studied and the possible mechanism of action for the extract was elucidated by *in silico* studies. Singh et al. (2013) stated that fenugreek leaves and seeds have been used extensively for medicinal purposes. Fenugreek seed is known to exhibit anti-diabetic properties and effects such as hypcholesterolemic, anti-cancerous, and thyroxine-induced hyperglycemia. They differ in morphology, growth habit, biomass and seed

production capability, and chemical constituents of the seed, for example, polyphenol, phytic acid, saponin, carbohydrate, protein, and proximate analysis contents also differed markedly.

Khan et al. (2018) confirmed that the study of pharmacology and phytochemicals may help to understand the effect of sprouted fenugreek seeds in traditional as well as future use of medicinal plants. Fenugreek can be recommended for the diet and must use in daily habit for its medicinal health benefits and its safe use. The side effects of fenugreek are nausea, gastrointestinal discomfort which includes diarrhea and gas. Wani and Kumar (2018) recommended fenugreek as daily diet as its liberal use is safe and various health benefits can be drawn from this valuable natural herb, on the basis of several health usefulness and various scientific findings reported in the past. Fenugreek can enhance breast milk production. However, with regard to studies performed, breast-feeding women are recommended to consider the following when consuming fenugreek seeds (Shahrajabian et al. 2020a): (1) Fenugreek should be consumed carefully by women who have signs of asthma or digestive disorders, (2) Minimum amount of consumption that provides effect should be considered, (3) It should be avoided in women with blood pressure and patients with cardiovascular diseases, (4) Women who have sensitive skin should check sensitivity to fenugreek, (5) Women who use warfarin plus aspirin should use fenugreek with caution, (6) Women who use fenugreek for their milk supply increase should avoid long-term use of it. It is recommended to check coagulation time and blood glucose test during the consumption period (Turkyilmaz et al. 2011).

2.4 Conclusion

Fenugreek (*Trigonella foenum-graecum* L.) is an aromatic, medicinal plant rich in several important phytochemicals. Historically, fenugreek has been used as an important traditional, multipurpose medicinal herb in Iranian, Indian, Chinese, and Tibetan Medicinal Practices for several centuries. It is named as Methi (Hindi, Urdu, Punjabi, and Marathi), Hulba (Arabic), Moshoseitaro (Greek), Uluva (Malayalam), Shoot (Hebrew), Dari (Persian), and heyseed in English. Fenugreek plant history dates back at least to around 4000 BC. Its name is derived from its Latin name, *Trigonella foenum-graecum*, meaning Greek hay, in reference to the Greek tradition of amending inferior hay for livestock with fenugreek. Historical uses for fenugreek were predominantly medicinal and were thought to cure a gamut of disorders from fever, colic, flatulence, dysentery, coughs, tuberculosis, oedema, rickets, ulcers, gout, diabetes, and even baldness. The seeds also have been used to promote lactation and as an aphrodisiac. The plant is traditionally grown in major parts of South Asia, Middle East, North Africa, and Mediterranean Europe as a spice crop, and as an ingredient of the famous East Indian curries or as a part of the traditional curry mix powder of the Indian subcontinent. It is a very well-known traditional spice which is famous to South Asians and South Asian diaspora spread across the globe. The highest number of fenugreek cultivars is reported from India, Pakistan, and China; followed by North African countries (Egypt, Tunisia, Morocco, Algeria,

Libya, Sudan), Horn of Africa (Ethiopia, Eritrea), sub-Saharan African nations (Kenya, South Africa), the Middle East nations (Turkey, Israel, Oman, Jordan, Syria, Yemen, Iran, Iraq), and European countries (United Kingdom, France, Italy, Spain, Portugal, Germany, Greece, Romania, Slovenia, Poland, Austria, Switzerland, Hungary, Azerbaijan). Chemical constituents of fenugreek are proteins (Globulin, Albumin, and Lecithin), lipids fatty acids (Linoleic acid, A-Linolenic, Oleic, Stearic acids, Palmitic and Sterols, B-Sitosterol, Campesterol, Triunsaturated, Cycloartenol, and Diunsaturated Triacyl Glycerides), carbohydrates (Mucilage or Gum: galactomannan), saponins (Graecunins, Fenugrin B, Fenugreekine, Trigofoenosides A-G), Steroidal saponins (Diosgenin, Yamogenin, Gitogenin, Tigogenin, Neogitogenin, Smilagenin, Sarsasapogenin, Yuccagenin), flavonoids (Apigenin, Luteolin, Vitexin, Isovitexin, Quercetin, Kaempferol-Dirhamnoside, Kaempferol Rhamnoside, Orientin, Biochanin A, Formononetin, Irilone, Tricine, Daidzein, Calycosin), alkaloids (Trigonelline, Gentianine, Carpaine, Choline), fibers (Gum, Neutral Detergent Fiber Lipids Triacylglycerols, Diacylglycerols, Monoacylglycerols, Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol, Free Fatty Acid), and amino acids (Isoleucine, 4-Hydroxyisoleucine, Histidine, Leucine, Lysine, L-Tryptophan, Arginine). The general uses of fenugreek are for bread, biscuits, extruded product, culinary use (color, flavor, aroma), spice and seasoning, organoleptic character improver, maple syrup and artificial flavoring, dietary fiber, galactomannan, curries, condiments, pickles, chutneys as a flavoring, food stabilizer, adhesive, and emulsifying agent. Traditional herb and medicine are making inroads into diets with their promises to improve health and nutrition. Consumers should choose nutritional and healthy food to maintain general health and reduce the risk of health problems. Traditional medicines and super-food and fruits play important role in sustainable agriculture and food system, it also offers a holistic approach to prevent diseases while making appropriate use of organic and herbal products especially growth by consumers.

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3 Employing Modern Technologies in the Cultivation and Production of Fenugreek (*Trigonella foenum-graecum* L.)

Mahmut Camlica and Gulsum Yaldiz

Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant of Fabaceae family. It has various medicinal properties. The plant is used as spice or forage crop. It is native to the Mediterranean countries and it is also used as a spice plant in many parts of the world. The seeds and leaves of the fenugreek have been used commonly in traditional medicine because of including some important phytochemicals such as diosgenin, trigonellin, and fenugreekine. The used area of the fenugreek should be increased in different industry as food containing health-giving additives and having medicinal benefit as its phytochemicals. Fenugreek cultivation can increase the diversity of farming systems, improve its profitability, and make an important contribution to human health. This plant has been grown in arid and semi-arid region of the world as easily. However, yield, production and sowing area of fenugreek have lower values compared to other legume plants. Thus, best scientific strategies including breeding programs or cultural application should be implemented to improve the high yield of fenugreek species. Applications of different agricultural system can increase the fenugreek yield and phytochemical properties, thus it can contribute both crop production and soil management. This chapter emphasizes on the various scientific prospective including agricultural, agronomical, nutraceuticals, and industrial uses of fenugreek from past to present. In addition to this, it will moot for new research areas and different used areas of fenugreek.

Keywords

Agronomical properties · Cultivation · Fenugreek · Industrial uses

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3.1 Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is called methi, Greek hayseed, and bird's foot is an annual dicotyledonous and self-pollinated plant belongs to Fabaceae family including 260 species in worldwide. It is one of the famous spices, and green leaves and seeds of fenugreek are used for medicinal purposes throughout history. Also, it has been used to increase on color, flavor and texture of food materials as well as the medicinal uses (Acharya et al. 2006a, b; Arivalagan et al. 2013). Studies on karyotype and genome of fenugreek are limited as few data. Somatic chromosome numbers of *Trigonella* taxa were determined as 2n = 14, 16, 30, and 46 with B chromosomes. *Trigonella foenum-graecum* L. has 2n = 16 chromosomes among the *Trigonella* taxa (Martin et al. 2011; Vaidya et al. 2013; Khan et al. 2014).

In the last years, a number of studies on fenugreek emphasized that biological activities and therapeutical properties of this species especially associated to bioactive secondary metabolites such as alkaloids, flavonoids, steroids, and saponins. Extracts and powders of this plant can be used in the treatment of hypocholesterolemic, lactation aid, antibacterial, gastric stimulant, for anorexia, antidiabetic agent, galactagogue, hepatoprotective effect, and anticancer. These useful properties of fenugreek have promising nutraceutical values (Basch et al. 2003; Srinivasan 2006; Meghwal and Goswami 2012; Chaudhary et al. 2018). The fiber, gum, protein and other properties and volatile compounds are well known in this plant. Recently, it is used for food stabilizer, adhesive, and emulsifying agent because of including fiber, protein, and gum content (Meghwal and Goswami 2012). Locally, fenugreek seed has been used as a natural dye material, cosmetic production as well as medicinal properties. It was also used for soil improvement and has commonly been used as green manure in agricultural systems (Abdelgani et al. 1999).

According to Oncina et al. (2002), this plant has 6, 5, 1, 1 species in Asia, Europe, Africa, and Australia continentals, respectively. It is also noted that fenugreek is cultivated in part of Europe, northern Africa, west and south Asia, North and South America, and Australia (Jongebloed 2004; Acharya et al. 2006a, b) (Fig. 3.1). *Trigonella* species were extended from Israel to Syria, southern Turkey, Iran, Iraq, and Mediterranean center including Spain, Morocco. Turkey is also center of these species. The large seeded cultivar of *T. foenum-graecum* is abundant around the Mediterranean region, but the small seeds cultivars are predominant eastward (Malhotra 2011). Jain et al. (2013) reported that cultivation of this plant is widely grown in India, Iran, Nepal, Bangladesh, Pakistan, Ukraine, Russia, Greece, Argentina, Egypt, France, Spain, Turkey, Morocco, and China (Fig. 3.1).

This plant has different morphological properties, growth habit, biomass, and yield performance based on growing at different places. Chemical components of the fenugreek seeds as saponins, fiber, protein, amino acids, and fatty acid contents also show differences saliently (Taylor et al. 2000).

Fenugreek can grow in different areas such as dry grasslands, cultivated or uncultivated lands, hillsides, and plains, field edges, and needs enough sunlight. It can germinate among the 5–10 days; after germination the first trifoliate leaf comes

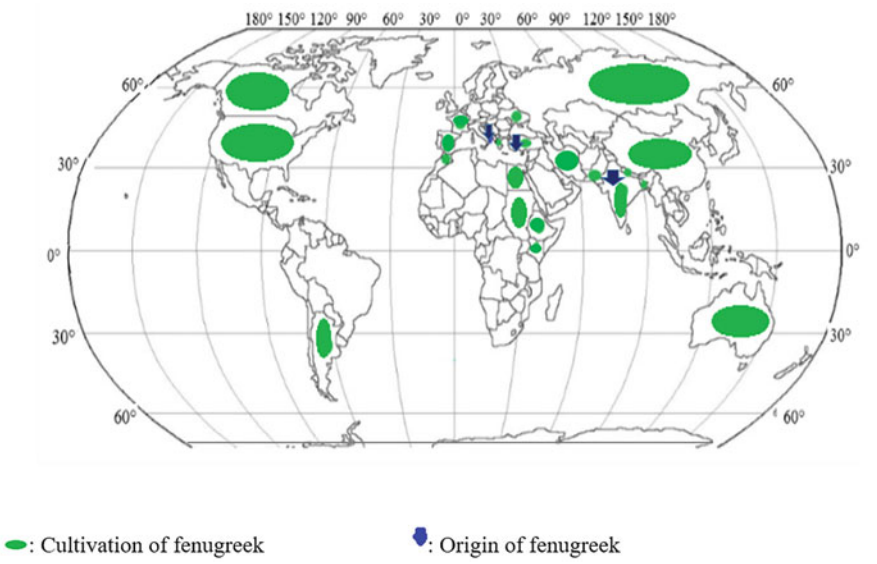

Fig. 3.1 Map of the cultivation and origin of fenugreek

up in 5–8 days. Maturity time is between 4–7 months (Petropoulos 2002; Montgomery 2009; Mehrafarin et al. 2011). Other vegetation periods can change in the different ecological conditions. Fenugreek is resistant to drought stress and it is also well adapted on mild winter and summer conditions (Chayut et al. 2014). pH ratio changed between 5.3–8.2 and it needs watering in dry conditions (Min 2011). This study primarily includes knowledge on the agricultural, agronomical, nutraceuticals, and industrial uses of fenugreek, production methods, origin of plant germplasm, and available genetic resource and biochemical properties for crop development and medicinal properties.

3.2 Agricultural Perspective of Fenugreek

The legumes are preferred by farmers or producer compared to the other crops. These plants can be used both as grain and fodder. They also have the higher yield under low water stress because of fixing atmospheric nitrogen. Intercropping system is the most important agricultural system. It provides improvement in the yield and returns and reduces the risk of sole cropping system depending on crop failure (Tandon 1986). Fenugreek is between these legumes. Different agricultural systems were carried out on fenugreek by many of researchers in the worldwide. These studies aimed to increase crop productivity, quality properties, and biocompounds of fenugreek.

Various researches were conducted to increase yield and quality parameters of fenugreek in different agricultural systems of the world as applying some useful

materials, against stress conditions. Fenugreek production was cultivated under native or similar agro-climatic growing conditions and it was great pledged for the dry and semi-arid regions of the world such as Africa, Latin America. It has also been grown in the Mediterranean regions of the continents of Europe and Africa, West Asia, and the Indian subcontinent in South Asia. This plant is suitable for growing under rain fed conditions as predominantly dryland crop. It is used as a forage or spice or medicinal and aromatic plant in the low input agricultural systems of some Africa countries or American countries in the same agro-climatic conditions. Further, it could be an important crop evaluated in rural areas of these regions because of having immense medicinal values, multi-purpose uses, and incoming generating venture for the rural poor (Zandi et al. 2015).

Crop production technology is important subject to obtain the standard plant quality, produced the medicinal value, and resistant against diseases for the fenugreek. Agricultural applications affect from sowing to harvesting time such as field preparation, time of sowing and harvesting, application methodology as fertilizer, micro-macro nutrients doses, quantity of irrigation water, and application time (Rajan et al. 2002; Mathur et al. 2006; Ghasolia and Shivpuri 2007). In agriculture, resource use efficiency can affect the plant growth and yield in intercropping. It was reported that fenugreek could produce similar dry matter depending on sowing ratio in intercrops. Fenugreek and buckwheat were grown in intercrops systems and dry matter yields of these plants had more advantage compared to pure stands of the crops (Salehi et al. 2018). In addition to this, chemical fertilizers and broiler litter were applied to the growth and yield properties of fenugreek and buckwheat and broiler litter was found more effective than chemical fertilizer. Application of broiler fertilizer in intercrops growing increased the biomass production of fenugreek in semi-arid environments (Salehi et al. 2018).

Fenugreek is a low-cost production in Africa and Latin America. As a cheaper source and raw materials or products, these conditions make the crop attractive for growth in pharmaceutical and nutraceutical industries (Zandi et al. 2015). This plant was cultivated in Asia, Africa, and Latin America in the dry and semi-arid places for agricultural production (Acharya et al. 2008; Basu and Agoramoorthy 2014; Solorio-Sánchez et al. 2014; Zandi et al. 2015).

3.3 Agronomical Applications in Fenugreek

There are numerous studies on agronomical properties of fenugreek reported by researchers (Ahmad et al. 2016). These studies carried out different ecological conditions (different countries, regions, provinces), growth conditions as applying fertilizer, stress conditions (drought, salt, cold stresses, etc.), intercropping application, and genetic differences in fenugreek genotypes to determine the adaptation, morphological, yield, and quality properties of fenugreek species (Acharya et al. 2006a, b). Same genotypes of fenugreek showed different properties in the same location and they did not follow each other in yearly trend more than one year according to the literature studies (Acharya et al. 2006a, b).

3.4 Agronomical Properties in Different Fenugreek Genotypes

Different agronomical reports have been noted in different agro-climatic regions of India and some parts of Egypt that increased the productivity of fenugreek (Ahmad et al. 2016). It was suggested that fenugreek seed is spaced out 20–30 cm separately to obtained optimal productivity. Also, it is planted in early October and November to optimize crop productivity (Baswana and Pandita 1989; Bhatt 1988; Gill et al. 2001; Korla and Saini 2003). Acharya et al. (2008) reported that late April to mid-May is selected for the sowing of fenugreek to obtain well performance like other parts of the world and cultivation of fenugreek in temperature climate region as western Canada has enhancement of fenugreek yield depending on its dryland conditions. It was also reported that agronomic applications such as forage cutting, and minimum irrigation time maximized the fenugreek yield (Ram and Verma 2000; Sheoran et al. 2000; Lal et al. 2003; Moyer et al. 2003). Moreover, the ideal soil properties for fenugreek cultivation can be preferred well-drained loam soils and 8–8.5 pH by the crop. Potash has been used to adjust soil pH to increase nutrient uptake of fenugreek (Yadav and Kumawat 2003).

According to Shah et al. (2018) a study was conducted on 110 fenugreek populations to determine the spatial genetic structure in inter-regional populations of *Trigonella foenum-graceum* L. species through phenotypic variation and seed protein profiling including 14 different countries populations. The results were reported between 23.3–114.5 cm, 71–127 days, 4.1–13.5 mm, 2.4–4.3 mm, 1.0–93.6 g/plant for plant height, days to first flowering time, pod length, pod width, seed yield, respectively. These characteristics and others divided into 10 cluster groups and four major and six minor classes by Euclidean distance-based cluster analysis. Also, they analyzed the total seed protein polymorphism profiling in 106 populations using SDS-PAGE technique.

In the other similar study, Jain et al. (2013) reported a significant genetic variability in 50 fenugreek germplasms based on morphology, yield, and yield properties in Ajmer, India. Plant height was noticed in between 43.83 and 68.47 cm, branch number was found 3.71–9.47, pod length was in between 8.89 and 12.58 cm, pod number per plant was recorded in between 22.43 and 81.03, test weight ranged from 4.12 to 18.41 g, and seed yield was found 1.39–13.99 g.

3.5 Organic, Inorganic, and Other Applications in Fenugreek

The application of organic and inorganic fertilizers such as N, P, farmyard manure, kitchen waste compost, and agricultural field waste has been reported to significantly increase the fenugreek yield (Detoroja et al. 1995; Petropoulos 2002; Khiriya and Singh 2003; Yadav and Kumawat 2003). Acharya et al. (2008) suggested that a beef or dairy operation can reduce the cost of production by using a fertilizer source as farm waste maintaining a good acreage of field under rotation of fenugreek. Further, it was reported that combined application of nutrients as inorganic fertilizer and

organic manure increased the seed yield, nutrient, and protein content of fenugreek (Shamsun Naher et al. 2016).

3.6 Organic Fertilizer in Fenugreek

Organic fertilizers can affect positively yield, yield component, and yield efficiency of fenugreek. In addition to this, arid and semi-arid regions have good possibilities to obtain fenugreek organic production. There is not enough information on effectiveness of rhizobium cultures on fenugreek. However, rhizobium cultures have provided to improving the soil nitrogen property and they increased the fenugreek yield as high (Farook et al. 2012).

Three different organic fertilizer (7 t/ha of cow manure, vermicompost, and vermiwash) control were used to evaluated plant height, pod length, pod fresh and dry mass, 1000-seed mass, plant fresh and dry mass, internode length and percents of leaves protein and nitrogen in fenugreek. Organic fertilizer effected the numbers of pods/plant, number of seed/pod, number of nodes, number of lateral branches, antioxidant capacity, total phenols, and dry yield lower than control (no fertilization), but these fertilizers affected as highly compared to control (Alaghemand et al. 2017).

In another similar study, fertilizers effects on growth and yield components of fenugreek vegetable (*Trigonella foenum-graecum* L.) were investigated in field conditions by using five different applications: Vermicompost (0.6 kg/plot), NADEP (Narayan Deotao Pandharipande) compost (1.25 kg/plot), pit compost (1.25 kg/plot), chemical fertilizer (80:40:40 kg of NPK/ha), and control (soil without fertilizer). It has been reported that plant height (28.75 cm), fresh (4.88 g/plant) and dry weight (1.06 g/plant) were found in application of vermicompost treatment. The highest pod number was found in control treatment with 2.66 number. Total pod weight, mean weight per pods and yield were recorded 0.78 g/plant, 0.30 g/pod/plant, and 1.653 kg/plot, respectively, in chemical fertilizer. They also noted that chemical fertilizer application is the best and sustainable for the fenugreek yield compared to other fertilizer treatments (Vedpathak and Chavan 2016).

Dutta et al. (2011) also determined the effect of biofertilizer (Rhizobium) and nitrogen levels (70, 100, 100% N, P, K + FYM (farmyard manure) + Rhizobium, 75, 100, 100% N, P, K + FYM + Rhizobium, 80, 100, 100% N, P, K + FYM + Rhizobium, 85, 100, 100% N, P, K + FYM + Rhizobium, recommended NPK + FYM, recommended NPK) (control) on growth, yield, and quality of fenugreek (Rmt-1) with farmyard manure. The treatments were reported between 62.2 and 74.5 cm for plant height, 4.43 and 5.73 branches number, 30.7 and 41.4 number pod/plant, 9.96 and 12.1 g/1000 seed weight, 7.38 and 12.8 q/ha seed yield, 1.66–2.57% essential oil. The highest seed weight, seed yield, and essential oil of fenugreek were found in 85, 100, 100% N, P, K + FYM + Rhizobium treatment.

Likewise, effect of different organic sources on fenugreek was reported by Malav et al. (2018) in two years (2007–2008 and 2008–2009). Ten treatments such as T_1: 100% Recommended Dose of N (RDN) through FYM, T_2: 100% RDN through

castor cake (CC), T_3: Rhizobium treatment, T_4: Phosphate solubilizing bacteria (PSB) treatment, T_5: Rhizobium+PSB treatment, T_6: 50% RDN through FYM + Rhizobium, T_7: 50% RDN through CC + Rhizobium, T_8: 50% RDN through FYM + Rhizobium+PSB, T_9: 50% RDN through CC + Rhizobium+PSB, and T_{10}: RDN were used as different organic and inorganic sources. They found that the highest seed yield was observed from 50% RDN through CC + Rhizobium+PSB application with 1212 and 1106 kg/ha in two vegetation years.

A study was carried out on fenugreek using different nitrogen fertilization doses (N_0 = control, $N_{0.5}$, and $N_{1.0}$ g/pot) and rhizobium inoculation (non-inoculated (control), inoculation with *Rhizobium meliloti*) on the yield and quality of fenugreek by Wierzbowska and Zuk-Gołaszewska (2014). As a result of the study, the fenugreek traits were determined as 25.90–48.95 cm plant height, 11.50–16.36 seed number, 7.55–14.50 g thousand seed weight, and 0.101–0.211 g seed weight. Plant height and seed number values were noted the highest and lowest in non-inoculated and the highest thousand seed weight value was seen in inoculated. The highest seed weight was found in non-inoculated and the lowest value was seen in inoculated.

In addition, wheat straw biochar was used in the growth and yield of fenugreek in cadmium spiked soil in plastic pot by Tanveer et al. (2019). Different biochar doses (Control, 2.5% and 5% w/w) and NPK fertilizer (60, 80, 40 kg/ha) were used in their study. After 90 days, it was reported that biochar affected the germination rate and growth of fenugreek. Cadmium accumulation decreased with increasing biochar concentrations in different parts of fenugreek. Germination rates and dry leaf weight were changed as 53.33%, 76.67%, 92.22%, 1.6, 4.25, 5.85 g based on control, 2.5% and 5% w/w biochar doses, respectively.

Bieńkowski et al. (2016) determined the effect of the agro technical factors as application of *Rhizobium meliloti* bacteria in fenugreek seeds, seed sowing date (the earliest possible date, delayed by 10 days, delayed by 20 days), row spacing (15, 30, 45 cm), weed control (control of weed infestations depends on mechanical, chemical), and protect against the pathogen (normal seeds, chemical matter, seed dressing and not using chemical matter, seed dressing with chemical matter) on productiveness of fenugreek as yield components and seed yield. They noted six yield components of fenugreek such as plant height (cm), branch number, pod number per plant, seed number in pod, 1000 seed weight (g), seed yield (g/plant). Plant height changed between 29.1–32.0 cm, branch number ranged from 4.07 to 5.45, pod number per plant changed between 10.86–13.74, seed number in per pod was seen between 6.38–7.37, 1000 seed weight was found between 14.2–15.1 g, and seed yield was reported as 1.04–1.31 g/plant depending on five key agro technical factors. At the end of their study, they noted that weed control was the most important factor among the applications to be responsible for the highest seed yield variations.

Kurubetta et al. (2018) studied on the effect of four spacing (S1: 22.5 × 10 cm, S2: 30 × 10 cm, S3: 37.5 × 10 cm, S4: Broadcasting (40 kg/ha seeds) and four fertilizer doses (F1–50:50:0 kg, F2–75:50:0 kg, F3–100:50:0 kg $NP_2O_5K_2O$/ha, F4-only organic fertilizer as farmyard manure (FYM) and vermicompost in three vegetation years. Mean plant height, number of branch, number of pod in per plant,

seed yield per plant, seed yield were determined as 41.1–43.9 cm, 4.4–5.0 number, 18.0–24.1 number, 1.5–3.7 g, 11.6–13.9 q/ha, respectively, in different row spacing and fertilizer doses. In the result of the study, they recommended the 30 × 10 cm row spacing with 50:50:0 kg $NP_2O_5K_2O$/ha for the obtained higher seed yield with 15.5 q/ha.

3.7 Inorganic Fertilizer in Fenugreek

Inorganic fertilizers such as nitrogen are used for the growth and yield of plants as an essential nutrient. In this way, a study was conducted by Baricevic and Zupancic (2002) to determine the effect of drought stress and nitrogen fertilizer on the yield and secondary metabolite substance of fenugreek cultivars (Margaret, Paul). It was reported that seed weight of fenugreek cultivars changed as 11.46 and 13.55 g/plant in stress condition and it changed as 19.19 and 20.79 g/plant in non-stress condition. In addition, diosgenin content changed as 0.12 and 0.19% in stress condition and 0.18 and 0.20% in non-stress condition depending on fenugreek cultivars. Salehi et al. (2019) studied on effect of intercropping and nitrogen (N) source on yield and yield components of fenugreek (*Trigonella foenum-graecum*)-buckwheat (*Fagopyrum esculentum*) intercrops through 2 years. Treatments included sole cropping of fenugreek (F), sole cropping of buckwheat (B), and three intercropping (F:B; 2:1, 1:1, 1:2) ratios. Second factor was N fertilizer type: mineral chemical fertilizer (CF) or broiler litter (BL). Intercropping increased the total above ground dry matter (F:B, 2:1, with 9 and 29%), total seed yield (F:B, 1:1 with 3751 kg/ha), N and P concentrations (14 g/kg), and uptake of these concentrations at the flowering and harvest time.

Beyzi et al. (2019) reported that foliar boron treatment affected the yield and yield components of fenugreek. Different foliar doses (control, 100, 200, 400, and 800 mg/l) were applied to the fenugreek cultivar (Güraslan). Results were evaluated by PCA (Principal Component Analysis) and the highest biological and seed yields were determined in 800 mg/L foliar boron dose in two growing seasons. Morphological and yield properties were determined as from 53.65 to 71.25 cm for plant heights and branch number per plant was found from 2.35 to 4.60, pod number in per plant changed between 6.33–11.13, first pod heights changed between 27.46–34.04 cm, seed number per pod ranged from 10.04–12.74, 1000 seed weight changed between 13.26–18.57 g, and seed yield varied between 126.48–259.07 kg/da. Generally, the obtained highest values in examined properties were obtained from second year.

Another study was conducted by Basu et al. (2008) to determine the effect of phosphate fertilizer and harvest management for improving fenugreek seed and forage yield in a dark brown soil zone of Canada. Different doses of phosphate fertilizer (0, 30, 40, 50, and 60 kg/ha) were used on forage and seed yield in two different conditions (under fed and irrigated conditions). The seed yield changed between 415–988 kg/ha under rain fed conditions and forage yield ranged from 2581–3279 kg/ha under irrigated conditions. The effective phosphate doses were

found as 40–50 kg/ha for seed yield and 50–60 kg/ha doses were found available for forage yield. In addition to these, two harvest methods as swathing and direct combining of seed were used for these properties. The swathing of seed method was effective than direct combine method. Average 975 kg/ha swathing yield was obtained from two years and average 898 kg/ha was found from direct combine methods.

In a similar study, Tunçtürk (2011) reported that different row spacing (20, 30, and 40 cm) and phosphorus doses (0, 30, 60, and 90 kg/ha) affected the fenugreek yield and quality properties in 2006 and 2007 vegetation years in Van Turkey ecological conditions. Plant height, branch number, first pod height, pod number, seed number in per pod, pod length, 1000 seed weight, seed yield were reported as 34.7–41.3 cm, 2.4–3.2 branch/plant, 14.4–16.3 cm, 6.7–9.3 pods/plant, 11.1–12.6 seeds/pod, 10.8–12.2 cm, 17.1–18.1 g, 612.0–809.0 kg/ha, respectively. It was reported that phosphorus fertilizer applications affected all fenugreek characters, except pod length.

According to Aminifard et al. (2019), foliar application effects of different levels of salicylic acid phrasing (0, 75, 150, and 300μm) and glycine betaine (0, 50, 100, and 150 mg/L) were used on pigments, phenolic compounds and total antioxidants content and some vegetative and reproductive growth indices of fenugreek under greenhouse condition. Plant height, leaf dry weight, root dry weight, and stem dry weight were observed as 21, 15, 31, and 24% higher than noted in the control treatment. Also, lateral branches number (4.6 and 6.3), pod length (9.5 and 12.2 cm), pod number in per plant (13.3 and 25), seed number in per pod (14.6 and 17.6), 1000 seed weight (15.8 and 18.4 g), nitrogen content of leaf (1.8 and 3.0%), chlorophyll a (1.1 and 1.4 mg/g fresh weight), total phenol content (45 and 60 mg 100 g/fresh weight), and total antioxidant content (45.6 and 56.6%) were found depending on foliar applications and different salicylic acid and glycine betaine. The highest level of glycine betaine was reported the best application compared to other applications.

3.8 Other Application in Fenugreek

Different sowing date affected the yield and quality characters of fenugreek. Anitha et al. (2016) reported that five sowing dates (at 15-day interval) from 15th October to 15th December were evaluated for five fenugreek varieties (Hissar sonali, Rmt-1, Co-1, Rajendrakanthi, and Co-2). The highest seed yield per plant, 1000 seed weight, and pod weight per plant were found as 9.99 g, 15.60 g, and 14.36 g on 15th October 2016, respectively.

A study was conducted to determine the interaction of five seed rates (16, 18, 20, 22, 24 kg/ha) and three row spacing (20, 30 40 cm) on the growth and yield of fenugreek. Plant height changed between 83.24–97.93 cm, branch number ranged from 9.60–12.93, days to 50% flowering time changed between 81.47–90.87, number of pods per plant changed between 78.47–98.57, pod length changed between 7.53–9.70 cm. Other observations were found for number of seeds per

Fig. 3.2 Different growth stages of fenugreek; (**a**) seeds, (**b**) pod setting stage, (**c**) before flowering stage, (**d**) flowering stage, (**e**) after flowering stage, (**f**) pods (Image credit: Yaldiz, G. and Camlica, M.)

pod, seed yield/plot, seed yield/ha, biological yield, and harvest index as 11.61–15.70, 1.70–2.63, 17.59–27.29, 3.89–5.37, and 43.65–2.17, respectively. Result of their study, 20 kg/ha seed rate with 40 cm row spacing was reported as the best application to obtain higher seed yield. However, 24 kg/ha seed rate and 40 cm row spacing were noted as the best application to obtain better growth and quality properties under semi-arid conditions (Kumar et al. 2018) (Fig. 3.2).

In line with these findings, when evaluated previous studies, different researchers around the world were conducted studies for agronomical properties in different fenugreek genotypes and germplasms or different applications as organic, inorganic, and other applications grown for their morphology, yield, and yield components both in vitro and in vivo methods (Table 3.1). Fenugreek has a large variation in the world and all different applications affect the morphological, yield, and yield components of fenugreek genotypes.

3.9 Agronomical Breeding of Fenugreek

Genetic variability in the plant population is very important for the crop improvement program. The desirable genetic property can be selected for improvement and options to improve selected property. Different fenugreek genotypes are found in the worldwide in terms of phenotypic and genotypic properties. Growth habit,

Table 3.1 Different agronomical studies on fenugreek in some countries

Country	Character	Value	Experimental area	Application	Reference
Canada	Seed yield (kg/ha)	19–4119	Field	Irrigated-dry land	Acharya et al. (2006a, b)
	Plant height (cm)	7.5–81.2	Greenhouse	Mutation-EMS-Mutant 1	
	Node number	4–29			
	Pod number	0–90			
	Pod length (cm)	0.2–24.3			
	Seed number	0–1352			
	Seed weight (g/plant)	0–10.1			
	Plant height (cm)	5.6–83.4		Mutation- EMS-selectedMutant 2	
	Node number	3–77			
	Pod number	4–82			
	Pod length (cm)	1.5–13.7			
	Seed number	0–759			
	Seed weight (g/plant)	1.1–9.8			
India	Plant height (cm)	67.07–121.23	Field	Different genotypes (150 number)	Mamatha et al. (2017)
	Primary branch (no/plant)	1.06–6.067			
	Secondary branch (no/plant)	6.23–12.53			
	Pod length (cm)	7.34–14.42			
	Pod number (no/plant)	40.7–164.4			
	Seed number (no/plant)	7.06–12.60			
	Seed weight (g/plant)	8.49–26.20			
	Test weight	4.86–14.53			

(continued)

Table 3.1 (continued)

Country	Character	Value	Experimental area	Application	Reference
Iran	Days to flowering (day)	46–66; 47–97	Field	Rainfed-Irrigation (20 Iranian landraces)	Sadeghzadeh-Ahari et al. (2010)
	Days to maturity (day)	94–108; 87–118			
	Plant height (cm)	11–25.2; 13.8–28.7			
	Pod number	2.3–12.5; 3–14			
	Seed number	16–144.5; 24.9–144.3			
	Seed weight (g)	1.7–16.5; 2.6–20.1			
	Biological yield (kg/ha)	0.7–2.8; 1.35–4.41			
	Grain yield (kg/ha)	0.25–1.40; 0.38–1.46			
	Harvest index (ratio)	0.27–0.53; 0.22–0.46			
	1000 seed weight (g)	6.7–17; 9–22			
Tunisia	Germination percentage (%)	35–100	Plates	Arid bioclimate of Mediterranean type with a mild winter (38 genotypes)	Marzougui et al. (2007)
	Leaves number	10.56–73.12			
	Central leaflet length	1.26–1.90			
	Central leaflet width	0.68–1.03			
	Branch number by stem	0.38–6.89			
	Pods number by stem	1–8.7			

	Pods length (cm)	10.0–11.45			
	Seeds number by pod	9–14			
	200 collected seeds weight	2.04–4.24			
India	Plant height (cm)	46.20–74.83	Research farm	Different genotypes (30 number)	Gurjar et al. (2016)
	Pod number (plant)	51.10–80.14			
	Seed number (no/pod)	13.86–17.47			
	Seed weight (g)	9.77–17.03			
	Biological yield (g/plant)	26.20–46.83			
	Harvest index (ratio)	27.89–50.24			
	1000 seed weight (g)	11.10–19.10			
	Number of branches	11.23–16.20			
	50% flowering time (day)	43.67–53.33			
	Pod length (cm)	9.82–12.19			
India	50% flowering time (day)	34–57	Field	Different genotypes (40 number)	Patahk et al. (2014)
	Days to maturity (day)	81–90			
	Plant height (cm)	32.7–56.8			
	No of primary branches	3.66–8.16			
	Pod number (plant)	8.49–31.29			
	Seed number per pod	9–25			
	Pod length (cm)	5.61–12.21			

(continued)

Table 3.1 (continued)

Country	Character	Value	Experimental area	Application	Reference
	100 seed weight (g)	0.51–1.69			
	Seed yield (g/plant)	2.97–10.02			
Iran	Pod length (cm)	8.21–8.546	Field	Different in-row spacing-nitrogen levels	Zandi et al. (2011)
	Pod number (plant)	35.17–45.39			
	Seed number (no/pod)	14.04–14.75			
	1000 seed weight (g)	11.50–12.12			
	Seed yield (kg/ha)	1301–1468			
	Biological yield (kg/ha)	5402–6336			
	Harvest index (%)	22.84–25.37			
	Productivity index (%)	38.09–40.90			
Turkey	50% seedling day	7.09–29.09	Field	Different genotypes (88 genotypes)	Çamlıca and Yaldız (2019)
	50% flowering day	40.41–57.91			
	First pod setting (day)	49.32–70.82			
	First pod height (cm)	12.28–37.75			
	Plant height (cm)	24.95–85.15			
	First pod height (cm)	17.00–35.78			
	Pod weight (g/plant)	0.63–63.05			
	Seed number per pod	3.56–14.30			
	Pod length (cm)	7.01–36.10			
	Pod weight (g/plant)	1.11–63.05			
	1000 seed weight (g)	0.49–56.31			
	Seed yield (g/plant)	0.21–27.44			

Iran	Plant height (cm)	38.21–139.50	Field	Nitrogen fertilizer and plant density	Keivanrad and Zandi (2012)
	Seed yield (kg/ha)	1861–13,490			
	Biological yield (kg/ha)	952–12,840			
	1000 seed weight (g)	2.47–115.6			
	Harvest index (%)	10.07–30.44			

morphological, yield, and quality criteria of fenugreek show differences (Table 3.1). In terms of desired properties, different fenugreek genotypes can be collected and used to obtain new variety by using traditional or molecular breeding techniques for breeding.

Fenugreek is known as a self-pollinated plant due to its flower structure and synthetically crossing is not easy. Emasculation and manual pollination have been applied in different fenugreek lines effectively. Mutation breeding has been suggested to develop new fenugreek varieties genetically and hybridization also has been used for the improvement of desired properties of this plant effectively (Snehlata and Payal 2012).

The germplasm pool of fenugreek should be known very well for the improvement of new species. This situation will bring out the gene reserve for the whole properties, especially yield, quality, and stress factor (biotic and abiotic) in breeding of this plant. It was reported that genetic information on fenugreek qualitative and quantitative are insufficient and limited with variability, heritability, and genetic advance (Malhotra 2011). Fenugreek germplasm were collected to study in the different countries of the world by researchers. In this context, some collections were created by researchers in Turkey (Oltraco and Sabanci 1996), Australia (McCormick et al. 2000), Canada (Basu et al. 2004; Acharya et al. 2006a, b), India (Malhotra 2010), and Russia (Provorov et al. 1996). These genetic diversities can be used for the improvement of new fenugreek varieties and eliminated the insufficient information about genetic diversity, relationship, intra and interspecific variability of fenugreek (Malhotra 2011). Level of fenugreek yield potential is possible with either plant breeding method or application of cultural modification treatments (Zandi et al. 2010; Basu et al. 2009). In addition, the plant is subjected to biotic and abiotic stress factors such as insects, fungi, bacteria, salinity, drought, flooding, insufficient micronutrients (Basu et al. 2006a, b; Chakraborty et al. 2014). This new fenugreek species should be grown against such stress factors by using traditional breeding methods or molecular genetic methods.

Fehr (1993) reported that mutation breeding can be used for a new fenugreek variety in exiting gene pool. In this context, a study was conducted on mutation breeding by Acharya et al. (2006a, b). It was applied to determine the nutraceutical properties of fenugreek genotypes. Levels of 10, 20, 30, 40, 50, 100, 150, 200, and 300 mM concentrations were used in Ethyl Methane Sulfonate (EMS) and after growing period in pots, mutation 1 (M1) plants were selected. Mutation 2 (M2) plants were obtained from M1plant seeds. Generally, they reported that all treatments of fenugreek genotypes such as plant height, no of nodes, no of pods, pod length, no of seeds, and seed weight were affected positively in selected M2 mutant plants under different EMS conditions. In addition to mutation breeding, different genetic identification technologies such as AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence), RAPD (Random Amplification of Polymorphic DNA) have been applied to find genetic diversity in fenugreek in different countries as India, Pakistan, Iran, and Oman (Dangi et al. 2004; Sundaram and Purwar 2011; Kumar et al. 2012; Ahari et al. 2014; Al-Maamari et al. 2014; Hora et al. 2016). Breeding studies on fenugreek have been studied based on different

agronomic, biotechnological, and molecular techniques. The techniques aimed to determine the highest genotypes among the examined fenugreek genotypes.

3.10 Nutraceutical Properties of Fenugreek

Fenugreek has been known as a medicinal plant in traditional Ayurvedic medicinal practices; as well as in traditional Chinese and Tibetan medical systems (Tiran 2003; Acharya et al. 2010; Basu et al. 2007). Many medicinal uses of this plant were recorded such as ancient literatures, religions scripture, travel records, and anecdotes from different countries and from different periods of human history (Lust 1986). Nutraceutical properties are lactation aid, immunological activity, hypoglycemic effect, hypocholesterolemic effect, antioxidant activity, anticancer effect, antibacterial and antifungal effect, advantages of fenugreek on digestion (Wani and Kumar 2018). Fenugreek has many phytochemicals such as essential oil, steroidal sapogenins, complex carbohydrates, essential amino acids. Steroidal sapogenins, galactomannans, and isoleucine are the most important chemical components among phytochemicals. These three components seem to produce health effects and have placed fenugreek among the most commonly known nutraceutical or health food products. This plant had many medicinal properties such as antioxidant, antidiabetic, antimicrobial, anti-leukemic, and hypocholesterolemic (Basu et al. 2007; Acharya et al. 2008, 2010; Agarwal et al. 2013) (Table 3.2).

Seeds of fenugreek are rich in terms of gum, alkaloids, essential content, fiber, saponins, and flavonoids. It can be used for food stabilizer, adhesive, and emulsifying agent to alteration of food content for some special aims because of having high component of fiber (Khorshidian et al. 2016).

Fenugreek seeds include high iron content and A, B, C vitamin contents of fenugreek seeds increase in the period of the fenugreek germination. 4-hydroxyisoleucine a peculiar free amino acid extracted from seeds potentiates an insulinotropic activity through a direct effect on pancreatic B cells in rats and humans (Billaud and Adrian 2001).

Some advantages of fenugreek were given under the main advantages as health and food advantages in Fig. 3.3.

3.11 Steroidal Sapogenins of Fenugreek

Fenugreek species have important major steroidal sapogenins as diosgenin, yamogenin, tigogenin, neotigogenin, smilagenin, sarsasapogenin, and minor sapogenins (Dihydroxy steroidal sapogenins) as yuccagenin, gitogenin, and neogitogenin which are used in pharmaceutical and nutraceutical industries (Taylor et al. 1997; Arivalagan et al. 2013).

Diosgenin is generally used as a raw pioneer material for steroidal drugs and hormones production such as testosterone, glucocorticoids, and progesterone (Fazli and Hardman 1968; Raghuram et al. 1994). Determination of high diosgenin level in

Table 3.2 Some chemical components of fenugreek

Main group	Sub-group	Main group	Sub-group
Pyridine alkaloid	Trimethylamine	Minerals	Iron
	Neurin		Zinc
	Choline		Phosphorus
	Gentianine		Magnesium
	Capaine		Manganese
	Betaine		Selenium
	Trigonelline		Calcium
Flavonoids	Vitexin	Vitamins	Vitamin A
	Tricin		Vitamin C
	Naringenin		Niacin
	Quercetin		Pyridoxine
	Luteolin		Thiamine
Saponins	Graecunins		Riboflavin
	Fenugrin B		Nicotinic acid
	Fenugreekine		Folate
	Trigofoenosides A-G	Protein and amino acids	Globulin
Steroidal sapogenins	Diosgenin		Albumin
	Yamogenin		Lecithin
	Sarsasapogenin		Histidine
	Smilagenin		lysine
	Gitogenin		4-hydroxyisoleucine
	Yaccagenin	Volatile oils	n-hexanol
	Saponaretin		Heptanoic acid
	Tigogenin		Dihydroactiniolide
	Neotigogenin		Dihydrobenzofuran

fenugreek may be provided economically competitive compared to *Dioscorea tubers* and this diosgenin is used as traditional source for the steroid drugs synthesis (Taylor et al. 2002).

3.12 Use of Diosgenin and Its Extraction from Fenugreek

Diosgenin component has many biological activities. Some of them are anticancer, hypolipidemic, anti-inflammatory, and antidiabetic ones. It plays an important role beneficially in the cardiovascular system and alleviates the loss of bone in osteoporosis (Chen et al. 2015). In addition to this, effects of diosgenin and other steroidal saponins on human sex hormones are uncertain or even incoherent (Król-Kogus and Krauze-Baranowska 2012). Reliable analytic methods are determined in used fenugreek seeds for multidirectional biological activity of diosgenin (Król-Kogus et al. 2018). To obtained high steroid yield, high yielding genotypes can be used in plant

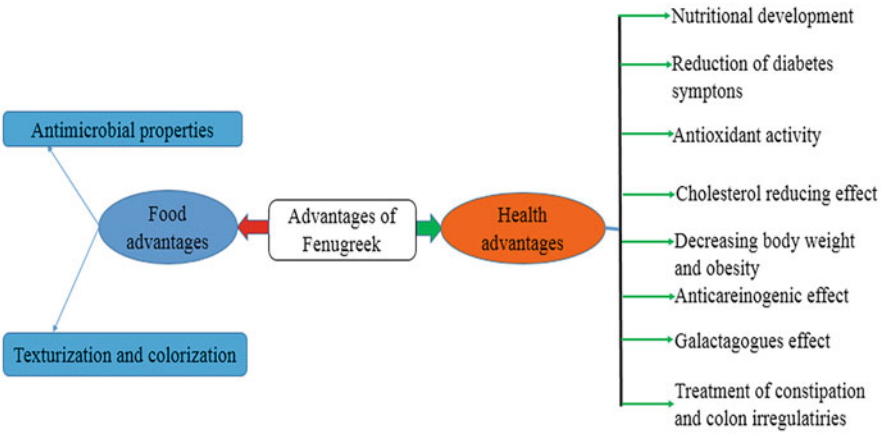

Fig. 3.3 Advantages of fenugreek in food and health

breeding as initially material. Also, fenugreek steroidal saponins have important potential value because of including hypocholesterolemic activity and other interesting properties (Saunders et al. 1986; Petit et al. 1995; Sauvaire et al. 1996; Benichou et al. 1999).

It was reported that fenugreek seeds have been used in alternative medicine including clinical trial to control diabetes and associated complications as effectively. Additionally, many experimental studies should be carried out to the use of diosgenin in diabetes and its related complications (Raju and Rao 2012).

Several chromatographic methods were set up to determine the diosgenin quantity. Some of them as HPLC and GC need special and expensive equipment, TLC can be used in diosgenin analysis as useful tool for alternative and hyphenated with densitometry in plant material (Taylor et al. 2000; Trivedi et al. 2007; Amir et al. 2012; Ghosh et al. 2012; Kharat et al. 2015; Król-Kogus et al. 2018).

An extraction method of diosgenin content in fenugreek was given in Fig. 3.4.

3.13 Breeding Nutraceutical Properties of Fenugreek

The diosgenin content in some different plants such as *Costus speciosus, Smilax menispermoidea, Trigonella foenum-graecum,* species of *Paris*, *Aletris,* and *Trillium*, and many species of *Dioscorea* is found as high level (Patel et al. 2013; Chen et al. 2011). The diosgenin is the main precursor component for the manufacture of some synthetic steroidal drugs in pharmaceutical industry (Chen et al. 2015).

Therefore, some conditions or applications have been tried to increase the diosgenin occurrence such as treatment with elicitors or under biotic and abiotic stress factors. It was reported that ethylene and ethephon plant growth regulators may provide the diosgenin content in plants and quantity of diosgenin in fenugreek elicited as 195% and 126% after application of 25 and 5 ppm ethephon doses

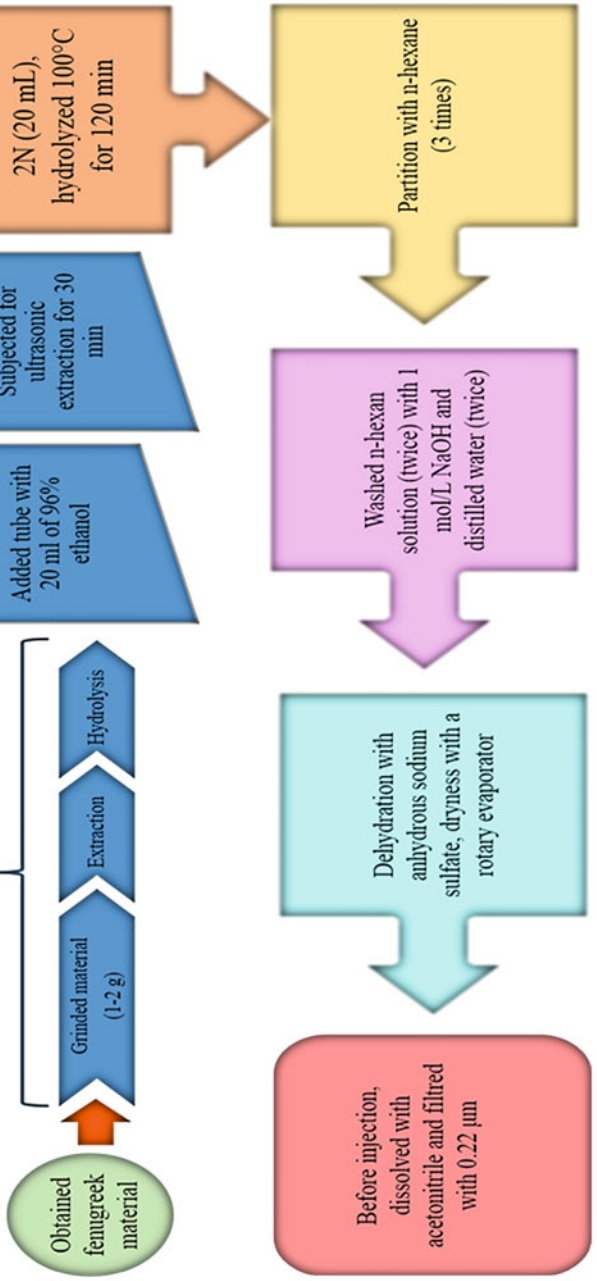

Fig. 3.4 Extraction of diosgenin content in fenugreek

(Oncina et al. 2002; Gomez et al. 2004). Taylor et al. (2002) reported that genotype, genotype × year, and location × year affected the diosgenin levels of fenugreek. It was reported that water stress affects the diosgenin yield of fenugreek. Baricevic and Zupancic (2002) reported that diosgenin level was seen lower than fenugreek grown without water stress. Biotechnological methods can be used to increase chemical compositions of fenugreek besides cultural applications. Mahna et al. (1994) used the mutation breeding to increase the diosgenin level in *Trigonella corniculata*.

Another study was conducted by Bitarafan et al. (2019) to determine the medicinal compounds of fenugreek depending on drought stress and the effect of charcoal on six fenugreek ecotypes. They carried out their studies in field and greenhouse conditions to observe the diosgenin and trigonelline contents. Trigonelline and diosgenin content changed between 0.0307–0.0554 mg/g and 0.795–1.019 mg/g in greenhouse conditions, respectively. Trigonelline content was not affected statistically from charcoal treatment in field conditions and diosgenin content was found between 0.721 and 0.564 mg/g. Tissue and cell cultures have been used for the plant regeneration or production of the secondary metabolites for product economically of fenugreek. The important secondary metabolites are diosgenin as saponin and trigonelline as alkaloid. These products are used in therapeutic properties and they are components of fenugreek seeds (Jain et al. 1977; Oncina et al. 2000).

Shams et al. (2014) reported that different nitrogen (urea) and copper (copper sulfate) doses affected the dry matter yield and diosgenin content in fenugreek. Five nitrogen levels (0, 50, 100, 150, 200 kg/ha) and four copper levels (0, 10, 20, 30 kg/ha) were applied by researchers. The highest diosgenin content was found in 100 kg/ha nitrogen application and 30 kg/ha Cu applications with 56.52 mg/g dry weight diosgenin content in 45-day old leaves and 15.42 g/plant dry matter yield in flowering stage.

Anitha et al. (2016) reported that different sowing time affected the diosgenin and protein content of fenugreek. The diosgenin contents changed between 0.30–0.62% and protein content ranged from 8.95–12.90% in different five fenugreeks (Hissar sonali, Rmt-1, Co-1, Rajendrakanthi, and Co-2) varieties. The highest diosgenin content was found as in seeds (0.62%) in Co-1 variety by the plants sown on 15th October and the minimum diosgenin content (0.30%) was recorded in the 15th December sown plots in Rmt-1 variety. The highest protein content was found by the plants sown on 15th October in Co-1 variety and the lowest protein content was determined from Rmt-1 fenugreek variety seeds.

Different phosphorus fertilizer doses and row spacing affected the protein content (%) and protein yield (kg/ha) of fenugreek. Protein content varied from 21.5–23.1% and protein yield changed between 141.0–187.0 kg/ha in Van Turkey ecological conditions in 2006–2007 growing seasons. The highest protein content was found in 20 cm row spacing and 30 kg/ha P fertilizer application and the highest protein yield was obtained from 30 cm row spacing (Tunçtürk 2011). Different fenugreek varieties (39) showed the content between 0.07–0.75% on the dry weight basis using spectrophotometric assay (Kamal et al. 1987). 31 fenugreek collected from gene banks of different countries were evaluated for the diosgenin levels (plus yamogenin the 25S stereoisomer) in Russia as detailed. Diosgenin levels were

reported between 1.14–1.64% by using by thin-layer chromatography (Provorov et al. 1996).

Diosgenin content was found between 0.12–0.18% by using HPTLC-densitometric method (Król-Kogus et al. 2018), 0.07–0.16% in field-grown foliage sampled during a season at 9, 15, and 19 weeks postseeding by Capillary Gas Chromatography and Combined Gas Chromatography/Mass Spectrometry (Taylor et al. 1997). Diosgenin contents were reported from 10 accessions of fenugreek seeds grown for two consecutive years at three sites in western Canada. The diosgenin analysis was carried out by GC with a Hewlett-Packard 6890 instrument and HP-5 column. Diosgenin levels were found between 0.28–0.92% in mature seeds of fenugreek accessions (Taylor et al. 2002).

3.14 Industrial and Other Uses of Fenugreek

The fenugreek can form initial raw herbal material as safety and effectively in industry. Therefore, more needed studies and preparations must be done for increasing the popularity and find out multiproperties of this plant. Chemical properties of this plant should be desirable status to use in industry. Fenugreek is cultivated for the different many uses. One of them is the high diosgenin content. Diosgenin is used for steroidal industry. Second of them is high mucilage (galactomannan) content with 4:1 mannose/galactose ratio for the uses in industry. Others of them can be listed as cured oils, aromatic and spice uses, and use in pharmaceutical components (Petropoulos 2002; Basu 2006; Acharya et al. 2008). Basu et al. (2017) reported that fenugreek presents rich raw materials depending on its phytochemicals and it can be used in the emergent global industry (food and nutraceutical) as a chemurgic crop.

Seeds, leaves, and gum of fenugreek have been used in various food products especially bakery and extruded product (Wani and Kumar 2018). This plant can be used in many areas of industry. Seeds, leaves, stems, straw, seed oil, galactomannan, fiber, extract, and trigonellin of fenugreek have been used in various industry areas as bread making (Raju et al. 2001; Meghwal and Goswami 2012), food gum, forage (Sowmya and Rajyalakshmi 1999), general food, flavoring agents, perfumes, fumigant, paints (Srinivasan 2006), vegetable (Balch 2003), functional food, cosmetics, insect repellant syrups, stabilizer and adhesive (Meghwal and Goswami 2012), dyes, paper industries, alcoholic beverages, emulsifying agent (Jani et al. 2009).

Fenugreek seeds also have a major polysaccharide species and they include galactomannans polysaccharide nearly 50% of the seed weight (Raghuram et al. 1994). Different studies on galactomannans of fenugreek showed positive and beneficial effects of fenugreek in the control of two diabetes in both animals (Puri et al. 2002; Raju et al. 2001; Vats et al. 2002, 2003) and humans (Puri et al. 2002; Sharma et al. 1996) by reducing hyperglycemia in these individuals. They have a high water-binding capacity and are able to form highly viscous solutions at relatively low concentrations. They also decrease the glucose absorption in the digestive tract (Raghuram et al. 1994). Fenugreek extract can be evaluated for the positive

effect such as antibacterial and antimicrobial properties (Alwhibi and Soliman 2014; ElNour et al. 2015).

According to Alwhibi and Soliman (2014) fenugreek seed extracts were applied to determine the antibacterial activity against a selection of gram-positive and gram-negative pathogenic bacteria. Distilled water, acetone, chloroform, ethanol, and methanol extracts were tested in two fenugreek cultivars. Chloroform and methanol extracts had significant antibacterial activity against *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Salmonella typhi* ATCC14027, and *Klebsiella pneumonia* ATCC700603. Chloroform zone inhibition changed between 27.4–39.8 mm and methanol zone inhibition ranged from 25.2–37.3 mm in two fenugreek cultivars.

ElNour et al. (2015) screened the phytochemical properties and antimicrobial activities of fenugreek seeds and callus extracts. The antimicrobial activities were tested against *Bacillus subtilis* (NCTC 8236 G + Ve), *Staphylococcus aureus* (ATCC 25923 G + v), *Escherichia coli* (ATCC 25922 G-V), *Pseudomonas aeruginosa* (ATTC 27853 G-V), *Aspergillus niger* (ATCC 9763), and *Candida albicans* (ATCC7596). Methanolic and petroleum ether extracts of seeds and callus were used. The petroleum ether extract of seeds had the highest antimicrobial activity and antibacterial activity with 17 ± 0.33 mm and 15 ± 0.57 mm of inhibition zone rather than methanolic extracts in 250 mg/mL concentration. The extract of petroleum ether showed antifungal activity against *Aspergillus niger* and *Candida albicans* with maximum inhibition zone with 20 ± 0.88 mm against *Aspergillus niger* and with 17 ± 0.57 mm against *Candida albicans* in 250 mg/mL concentration.

According to Chalghoumi et al. (2016) fenugreek seed crude extracts were used as an antibacterial such as tannins and flavonoids against a rabbit *Escherichia coli* isolate depending on used solvents as aqueous and methanolic (hexane, chloroform, acetone, ethanol, and methanol) extracts for extraction. Aqueous extract of fenugreek did not show any antibacterial effect. However organic extracts as chloroform, acetone, and methanol showed low and moderately high growth inhibitory effect ($8.33 \text{ mm} \leq \text{IZ} \leq 20 \text{ mm}$) in equal to or upper 5 mg/ml concentration. Sharma et al. (2016) indicated that fenugreek leaves, seeds, and stem extract (Methanol, Acetone, and aqueous extract) had antimicrobial effect against *E.coli* and *Staphylococcus*. The maximum inhibition zone was found in methanol extract with 20 mm and 19 mm. As a result of their study, they recommended that the fenugreek leaves, stem have an important role in clinical besides antimicrobial agents.

A study was conducted on polyphenolic content, antibacterial and antioxidant activities of fenugreek (*Trigonella foenum-graecum* L.) leaves by Premanath et al. (2011). The extracts of fenugreek leaves were obtained from chloroform, hexane, methanol, ethanol, and water extracts. The highest phenolic and flavonoid contents were determined from ethanol extract with 4.9 and 0.47 mg/g, respectively. Various antibacterial activities were screened by ethanol and methanol extracts and ethanol extract was carried out against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ethanol extract of fenugreek leaves had the highest activity among the tested other solvent extracts. Total antioxidant activity was found 0.3 and 0.7 mg/mL

ethanol extract, and this extract had the highest activity compared to other solvent extracts.

In other research, the aqueous and hexane extracts of the leaves of fenugreek are determined for their activity on ten clinical isolates as Methicillin Resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter divergens, Shigella flexneri, Salmonella paratyphi* A, *Salmonella paratyphi* B, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. All extracts had antibacterial activity against all tested isolates at different concentrations (50, 75, 100, 150, 200, 300 mg/mL). The inhibition zone diameters were found to be in the range of 5.0 mm for MRSA to 21.67 mm for *Salmonella paratyphi* A with aqueous extract and 5.0 for *Shigella flexneri* to 20.67 mm for *Salmonella paratyphi* B with hexane extract. It was reported that all extracts could be used for treatment of infections caused by the examined clinical isolates (Dathar et al. 2017).

As a result, many studies reported the antimicrobial, antibacterial, antifungal, etc., activities of fenugreek parts such as seeds, leaves, and stem. These activities were determined by using various solvents and the best solvents were found as ethanol, methanol, chloroform, and hexane. Generally, examined literatures in this study showed that ethanol, methanol, chloroform, and hexane were used as organic solvents to obtain extract from different parts of fenugreek.

3.15 Conclusion

Different cropping management systems should be applied to determine the yield performance and available bioactive composition in the same or different fenugreek species. In addition to this, effects of different agronomic and environmental conditions should be studied to the varying properties of fenugreek seeds or leaves. Although, key properties of this plant were examined in the previous studies, but more effective agriculture production system for fenugreek is highly required. In the future, studies on fenugreek are needed on some modern applications to increasing the popularity of fenugreek. In this context, researchers should be focused on to improve the yield and yield components, quality criteria as primary or secondary metabolites, harvest time, different cultural and molecular approaches, different techniques from sowing to harvesting time of fenugreek. In addition, fenugreek germplasm can be collected and subjected to intensive selection via modern breeding programs. Previous studies also showed that high level variability was obtained among the fenugreek species in terms of morphology, yield, and quality properties. Moreover, by selecting genotypes with superior characteristics, gene transfer can be made by determining suitable genes among these genotypes and new fenugreek genotypes with desired properties can be obtained. The widespread use of the products obtained from fenugreek plants is required. Therefore, cultivation area, production and export, import quantity and values may be increased. All agricultural, agronomical, nutraceutical properties, industrial, and other uses of the fenugreek can be further developed for the economic, industrial, and medicinal uses of the natural source plant. This compilation will certainly help the researchers to obtain

optimal production, optimum biochemical components, and adapt to different environmental and specific farming conditions of fenugreek.

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4 The Role of Agricultural Practises on Quality Characteristics of Fenugreek (*Trigonella foenum-graecum* L.) as Medicinal and Aromatic Plant

Erman Beyzi and Adem Güneş

Abstract

Fenugreek is a medicinal and aromatic herb that is used in many areas. Its importance is increasing day by day. It is known that fenugreek has been used medically since ancient times. It has been emphasized in studies that fenugreek has sedative, carminative, diuretic, tonic, restorative, antimicrobial, antiviral, and aphrodisiac effects. It is reported that fenugreek contains diosgenin and saponin components, anti-diabetic properties, and antioxidant effects. Seeds contain important components such as protein, crude oil, ash, moisture content, mucilage, alkaloid, sterol, and fatty acid components. Fenugreek cultivation can increase the diversity of farming systems, improve its profitability, and make an important contribution to human health. Thus, best scientific strategies including breeding programs or cultural application should be implemented to improve the high yield of fenugreek species. Applications of different agricultural system can increase the fenugreek yield and phytochemical properties.

There are various agricultural practices that may have positive or negative effects on quality properties in fenugreek. They can affect the quality characteristics in every stage of the plant, from sowing to harvesting. For example, sowing times may have positive (in crude oil, protein, and ash contents) or negative (in protein and diosgenin contents) effect on the quality characteristics of the plant and some fertilizer applications (in trigonelline concentration) may also have some effects on the plant. In addition, some increased stress conditions in plants may have positive or negative effects on quality properties of the plant.

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Keywords

Quality characteristics · Anti-diabetic · Antioxidants · Agriculture practices · Fertilizers

4.1 Introduction

Fenugreek is a plant that has been widely known and used since ancient times for its leaf and seeds (Chauhan et al. 2017). It is widely produced in places such as India, Asia, Europe, Africa, America, and Australia (Acharya et al. 2008). Fenugreek is an important medicinal and aromatic plant belonging to the Fabaceae family. It is an herbaceous plant with 30–60 cm tall, triple leaves, and hollow stems. The leaflets are in reverse egg form. There are an average of 10–20 seeds with a color ranging from dirty yellow to dark brown in the pods (Gençkan 1983; Baytop 1984). Fenugreek plant has found raw material and usage value in food (Akgül 1993), spices (Srinivasan 2006), and pharmacy fields (Beyzi 2016). This plant is also used for cosmetic and medicinal purposes (Abdelgani et al. 1999). Medically, the plant has been used in folk and modern medicine since ancient times (Beyzi 2016).

Medicinal and aromatic plants are generally used in modern and traditional medicine as a drug for the prevention, improvement, and maintenance of diseases (Temel et al. 2018). The studies have been reported that fenugreek seeds protect against certain diseases and this protection is thanks to secondary metabolites, also known as phytochemicals (Al-Oqail et al. 2013; Snehlata and Payal 2012).

In addition to the agronomic properties (plant height, pod length, number of pod, thousand seed weight, etc.) in fenugreek plants (Beyzi 2016), there are also some quality properties (protein, crude oil, ash, moisture, mucilage, alkaloid, vitamin, sterol, fatty acid compositions, etc.) that have important effects on the yield of the plant (Akgül 1993). Medicinal and aromatic plants are generally obtained by nature collection and cultural cultivation. It is not possible to obtain high quality products from these plants by collecting them from nature. A quality and standard product, is obtained through regular cultivation, selection, and breeding from these plants (Bayram et al. 2010). For this reason, as with all medicinal and aromatic plants, the determination of standards in quality characteristics in fenugreek plants is very important.

There are some agricultural practices that may have positive or negative effects on quality properties in fenugreek. These agricultural practices can affect the quality characteristics in every stage of the plant, from sowing to harvesting. For example, sowing times may have positive (in crude oil, protein, and ash contents) (Gökçe 2015) or negative (in protein and diosgenin contents) (Anitha et al. 2016) effect on the quality characteristics of the plant and some fertilizer applications (in trigonelline concentration) (Dadrasan et al. 2015) may also have some effects on the plant. In addition, some increased stress conditions in plants may have positive (in diosgenin content) (Saxena et al. 2017) or negative effects (germination rate) (Ghorbanpour et al. 2011) on quality properties, and specific applications such as bacterial inoculation (Żuk-Gołaszewska et al. 2015) may also have some effects on the plant. All

these examples are just a few examples that can be encountered in the plant-quality-agricultural application triangle. This study was prepared to reveal the effects of agricultural practices on the important quality characteristics of fenugreek with medicinal and aromatic value.

4.2 An Overview of Fenugreek as a Medicinal and Aromatic Plant

Fenugreek is a medicinal and aromatic herb that is used in many areas. Its importance is increasing day by day. It is known that fenugreek has been used medically since ancient times (Altuntas et al. 2005; Moradi Kor and Zadeh 2013). It has been emphasized in studies that fenugreek has sedative, carminative, diuretic, tonic, restorative, antimicrobial, antiviral, and aphrodisiac effects (Duke 1986; Moradi Kor and Moradi 2013). Apart from these, this plant also has healing effects on lung, dyspnea, and asthma diseases. This plant also has expectorant and breast softening properties (Koç 2002).

Fenugreek seeds are known to have anti-diabetic properties (Nilufar 1993; Devi et al. 2003) and antioxidant effects (Belguith-Hadriche et al. 2010). Fenugreek was also used for wound treatment in bovine animals such as cows and horses (Er and Yıldız 1997). Saponin components in fenugreek seeds have anticarcinogenic effects (Sur et al. 2001). Diosgenin, which is a saponin, in fenugreek has preventive effects on colon cancer (Raju et al. 2004). As a medicinal and aromatic plant, fenugreek is still used in the pharmaceutical industry, modern and folk medicine. More studies are needed to emphasize the medicinal effects of fenugreek which is alternative plant to other plants.

4.3 Important Quality Characteristics of Fenugreek

Fenugreek is a plant that uses seeds and vegetative parts. Seeds contain important components that can change with agricultural practices and affect the quality of the plant. These components can be generally classified as protein, crude oil, ash, moisture content, mucilage, alkaloid, sterol, and fatty acid components. Apart from these, it is known that fenugreek seeds have substances such as saponin, lecithin, essential oil, choline, vitamin, fitin, flavonoid, nicotinamide (Akgül 1993).

Fenugreek has high protein content in seeds. Abdel-Nabey and Damir (1990) reported protein content of fenugreek seed as between 23.25 and 27.00%. In another study, Acharya et al. (2006) stated that protein content in fenugreek lines varies between 26.0 and 31.6%. In another study comparing the protein contents of *Trigonella* species, Niknam et al. (2004) reported protein content of *Trigonella foenum-graecum* L. species as 29.93%. Al-Jasass and Al-Jasser (2012) reported protein content of fenugreek as 12.91% (Table 4.1). Based on these studies, it can be said that the protein content of fenugreek varies between 12.9 and 31.6%. This

Table 4.1 Protein and crude oil contents of fenugreek according to some study results

References	Protein content (%)
Abdel-Nabey and Damir (1990)	23.25–27.00
Acharya et al. (2006)	26.0–31.6
Niknam et al. (2004),	29.93
Al-Jasass and Al-Jasser (2012)	12.91
	Crude oil content (%)
Küçük and Gürbüz (1999)	4.01–5.89
Çiftçi et al. (2011)	5.8–15.2
Kıralan et al. (2017)	7.01–8.82
Sulieman et al. (2008)	8.4
Al-Jasass and Al-Jasser (2012)	4.51

high protein content of fenugreek makes it an important food in human and animal nutrition. In addition, because this plant has a high quality feed content and does not cause bloating in cattle, it can be used as a feed plant in cattle breeding (Acharya et al. 2006). Since it contains high amounts of protein, vitamins, essential amino acids and provides good digestibility to cattle, fenugreek has a forage value (Blumenthal et al. 2000; Çiftci et al. 2011).

Fenugreek seeds have a golden yellow hard oil with an unpleasant smell and bitter taste (Sulieman et al. 2008). Its oil is evaluated in the perfumery and cosmetics industry, it has insect and pest repellent effects (Srinivasan 2006; Fillips and Foy 1990). Küçük and Gürbüz (1999) reported crude oil content of fenugreek as between 4.01 and 5.89%; Çiftci et al. (2011) reported as between 5.8 and 15.2%; Kıralan et al. (2017) reported as between 7.01 and 8.82%; Sulieman et al. (2008) reported as 8.4% and Al-Jasass and Al-Jasser (2012) reported as 4.51% (Table 4.1).

Fenugreek seeds are rich in unsaturated fatty acids such as linoleic, oleic, linolenic and saturated fatty acids such as palmitic, stearic, arachidic (Shahat 1947; Sulieman et al. 2008; Ali et al. 2012; Çiftci et al. 2011). Küçük and Gürbüz (1999) found linoleic acid (44.64%) as the highest fatty acid in fenugreek, and this component was followed by linolenic (23.12%), oleic (19.34%), and myristic (10.24%) acid. Çiftci et al. (2011) reported linoleic acid content as between 45.1 and 47.5%, a-linolenic content as between 18.3 and 22.8%, oleic acid content as between 12.4 and 17.0%, palmitic acid content as between 9.8 and 11.2%, and stearic acid content as between 3.8 and 4.2%. Sulieman et al. (2008) reported myristic acid as 0.2%, palmitic acid as 11.0%, palmitoleic acid as 0.2%, stearic acid as 4.5%, oleic acid as 16.7%, linoleic acid as 43.2%, linolenic acid as 22.0%, arachidic acid as 1.5%, eicosamonoenoic acid as 0.1%, behenic acid as 0.5%, and lignoceric acid as 0.1%. Ali et al. (2012) determined 7 components in fenugreek and reported linoleic acid as 42.5%, oleic acid as 20.0%, and linolenic acid as 18.0%. In another study, Dinu et al. (2013) detected 13 components in fenugreek plants and reported linoleic acid as 43.15%. All these studies show that the rate of unsaturated fatty acids in fenugreek oil is much higher than saturated fatty acids. It is rich in unsaturated fatty acids of fenugreek, especially in terms of linoleic acid. Therefore, fenugreek oil enters into

Table 4.2 According to some study results, major fatty acid compositions of fenugreek plants

References	Linoleic acid (%)	Linolenic acid (%)
Küçük and Gürbüz (1999)	44.64	23.12
Çiftçi et al. (2011)	45.10–47.50	18.30–22.80
Kıralan et al. (2017)	45.10–46.19	17.93–20.69
Sulieman et al. (2008)	43.2	22.0
Al-Jasass and Al-Jasser (2012)	34.85	30.8

Table 4.3 Diosgenin, β-sitosterol, and campesterol contents of fenugreek according to some study results

References	Diosgenin content (%)
Saxena et al. (2013)	1.3–1.5
Taylor et al. (2002)	0.28–0.92
	β-sitosterol content (%)
Kıralan et al. (2017)	59.94–68.24
Çiftci et al. (2011)	31.8–49.6
	Campesterol content (%)
Kıralan et al. (2017)	11.78–16.14
Çiftçi et al. (2011)	8.7–20.5

the drying oil category. Drying oils are oxidized to form an elastic and hard film when exposed to air (Aljuhaimi et al. 2018) (Table 4.2).

Fenugreek is one of the few plant sources that produce diosgenin (Evans 1996), its seeds are an important source of steroidal sapogenins such as diosgenin (Acharya et al. 2008; Mehrafarin et al. 2010). Diosgenin has an important value in the pharmaceutical industry. Diosgenin is generally used in the production of some steroidal drugs and hormones (Mehrafarin et al. 2010). Saxena et al. (2013) reported diosgenin content as between 1.3 and 1.5%, Taylor et al. (2002) reported as between 0.28 and 0.92%, and Dangi et al. (2014) reported as 5087 mg g^{-1} (Table 4.3).

Plant sterols have important roles in human and plant functions. These affect the absorption of cholesterol in the human body and reduce the level of blood serum while controlling the membrane permeability in the plant (Kmiecik et al. 2011). The most common plant sterols are β-sitosterol, campesterol, stigmasterol, and avenasterol (Kozłowska et al. 2016). Fenugreek seeds have sterol contents. Its seeds are rich in β-sitosterol (Beyzi et al. 2019). Kıralan et al. (2017) reported that the β-sitosterol content ranged from 59.94 to 68.24% and camplesterol content varied between 11.78 and 16.14%. In another study, Çiftci et al. (2011) identified β-sitosterol as the major component and reported that β-sitosterol content ranged from 31.8 to 49.6%. In the same study, campesterol was found as the second component and reported that campesterol content ranged between 8.7 and 20.5% (Table 4.3).

Fenugreek contains secondary metabolites that are drug sources such as mucilage and trigonelline (Danesh Talab et al. 2014). Thanks to the mucilage, which is insoluble in liquid and also found in fenugreek, when fenugreek seeds come into

Table 4.4 Other quality characteristics of fenugreek according to some study results

References	Mucilage content (%)
Aydın (2010)	19.7–24.3
Rao and Sharma (1987)	20
Mutlu (2011)	19.11–32.75 24.00–35.67
	Trigonelline content (%)
Mutlu (2011)	0.66–1.40 0.91–1.93
Mathur and Yadav (2011)	27
	Moisture content (%)
Bouhenni et al. (2019)	3.0
Çalık (1996)	7.0–10.0
	Ash content (%)
Bouhenni et al. (2019)	3.0
Aydın (2010)	3.1–7.9
Çalık (1996)	3.11–8.86
Mutlu (2011)	3.94–5.36 3.72–5.70

contact with water, it becomes swell and sticky (Sindhu et al. 2012). Trigonelline alkaloid, which is also found in fenugreek seeds, plays an important role in the medicinal effects of the plant (Dadrasan et al. 2015).

Aydın (2010) reported mucilage content as between 19.7 and 24.3% and Rao and Sharma (1987) reported as 20% in fenugreek plants. In a study carried out at different locations in fenugreek plants, Mutlu (2011) reported mucilage content in Samsun and Çarşamba locations in Turkey as between 19.11 and 32.75% and 24.00 and 35.67% respectively, and trigonelline content as between 0.66 and 1.40% and 0.91 and 1.93%, respectively. In another study, Mathur and Yadav (2011) reported trigonelline content as 27% (Table 4.4).

Other factors known as quality properties in fenugreek are moisture and ash contents of the seed. Many studies have been carried out on moisture and ash content in fenugreek seeds. In these studies, Bouhenni et al. (2019) reported that the moisture and ash contents was 3%. Aydın (2010) reported ash content as between 3.1 and 7.9% and Çalık (1996) reported as between 3.11 and 8.86%. Also Çalık (1996) reported moisture contents as between 7.0 and 10.0%. Mutlu (2011) reported ash content in Samsun and Çarşamba locations in Turkey as 3.94–5.36% and 3.72–5.70%, respectively (Table 4.4).

4.4 Effective Agricultural Practices on Quality Characteristics of Fenugreek

4.4.1 Different Sowing Applications

Sowing time and plant density applications may affect the quality characteristics of the plant in fenugreek cultivation. Although studies on determining the most appropriate sowing time (Sheoran et al. 2000; Bhutia and Sharangi 2016; Gürbüz et al. 2000) and plant density (Zandi et al. 2011) have been carried out in fenugreek, studies on quality characteristics have been limited. Fenugreek is a temperate climate plant that can withstand hot and drought and can be grown for the winter. Although fenugreek production in Turkey is done as a summer planting in cold climates, it is carried out as a winter planting in hot climates (Kızıl and Arslan 2003). Since planting at different times in crop production has different climatic parameters, this can significantly change the growth, yield, and quality of the plant. Especially early sowings causes the plant to be sensitive to frost and extreme cold factors, and late sowings can lead to negativities in the yield and quality of the plant (Sheoran et al. 2000; Seghat Aleslami and Ahmadi Bonakdar 2010; Sharangi and Roychowdhury 2014; Bhutia and Sharangi 2016). Another factor that affects the quality of the plant and varies according to the purpose of cultivation and soil properties is the plant density applied during planting. Both factors (sowing time and plant density) can affect the quality of fenugreek plants.

It has been stated in some studies that fenugreek plants react differently to different sowing time and plant density applications in terms of quality characteristics (fixed oil, protein, protein and mucilage rate) (Tokbay 2007; Gökçe 2015; Uğur 2016). Anitha et al. (2016) investigated the effects of sowing time (October 15th, November 1, November 15th, December 1, December 15th) on the quality characteristics (diosgenin ve protein contents) of fenugreek. In this study, the highest (0.54%) diosgenin content in seeds was obtained from October 15th and the lowest (0.40%) from December 15th and the highest (10.91%) protein content was obtained from October 15th and the lowest (9.55%) from December 15th. Uğur (2016) reported the crude oil content as 7.41% at the first sowing time (February 26th), 6.69% and 4.63% at the second (March 12th) and third sowing times (March 30th), respectively. Tokbay (2007) examined the effects of different sowing time (October 15th, November 15th, December 15th, January 15th, February 15th, March 15th, April 15th) and row spacing on the yield and quality characteristics of the fenugreek. In this study, it was reported that the highest crude oil content was obtained from November 15th and the highest mucilage content was obtained from December 15th (Table 4.5).

In another study, Gökçe (2015) investigated the effects of sowing time (November 13th and March 7th) on the quality characteristics of fenugreek. In this study, mucilage, crude oil, crude protein, moisture, and crude ash contents were reported as 25.4%, 5.4%, 26.3%, 6.4%, and 3.2%, respectively, during the winter planting date (November 13th) and 24.9%, 6.3%, 29.3%, 6.4%, and 4%, respectively, during the summer planting date (March 7th). In the same study, fatty acid

Table 4.5 Some study results on different sowing time and plant density

References	Diosgenin content (%)
Anitha et al. (2016)	0.40–0.54
	Protein content (%)
Gökçe (2015)	26.3 29.3
Anitha et al. (2016)	9.55–10.91
Buçak (2016)	28.91–31.02 29.35–31.03
	Crude oil content (%)
Gökçe (2015)	5.9 6.3
Buçak (2016)	4.21–4.35 4.23–4.96
Uğur (2016)	4.63–7.41
	Mucilage content (%)
Gökçe (2015)	24.4 24.9
	Moisture content (%)
Gökçe (2015)	6.4
	Ash content (%)
Gökçe (2015)	3.2 4

components were examined and linoleic acid content (major component) reported as 38.1% in the winter planting date (November 13th) and as 38.7% in the summer planting date (March 7th) (Table 4.5).

When all studies are examined, it shows that different applications applied during planting have an effect on the quality characteristics of the plant and various results have been obtained.

4.4.2 Fertilization Applications

Plant nutrient elements have significant effects on yield and quality parameters such as tissue and organ development, biomass production, and seed quality in plants. In some studies, it has been stated that depending on the genotype characteristics of the plants and environmental conditions, plant growth, and plant biomass increase or decrease according to the high or low concentrations of plant nutrients (Hassan 2012; Savvas and Gruda 2018).

Macro and micronutrients contribute to plant growth by affecting different metabolic and cellular functions in the plant. Among the macro elements, nitrogen especially in primary metabolites such as amino acid, protein, and chlorophyll amounts (Giorgi et al. 2009); *phosphorus* in many metabolism processes such as ATP, nucleic acids, phospholipids, energy transfer, protein activation (Marschner 1995; Wu et al. 2003); *potassium* in different metabolic events such as plant growth

and stress resistance, secretion of high molecular compounds and quality (Wang et al. 2013); *magnesium* plays an important role in processes such as ATP synthesis, chlorophyll formation, photosynthesis, and photo oxidation (Cakmak and Yazici 2010).

Microelements play a structural role in stabilizing proteins and activate enzymes (Hansch and Mendel 2009). In particular, microelements such as Fe, Cu, Mn, and Zn play an important role in the formation of secondary metabolites necessary in plant development and quality (Isah 2019). Fertilizer management systems have been established in fenugreek and other plants to obtain the desired properties and increase yield. The main purpose of these systems is to increase the quantity and quality of the product taken per unit area. However, as a result of unconscious fertilizer applications, product quantity and quality decrease and soil and environmental pollution may occur. Furthermore, disruption of the balance between plant nutrients reduces the use efficiency of nutrients. Therefore, the use of fertilizers of the appropriate variety and dosage is the most important approach in maintaining-improving soil fertility with increasing the amount and quality of crops to be obtained from plants (Kataki et al. 2016; Moore et al. 2017).

The amount of plant nutrients is important in the development of fenugreek, seed quality, and the formation of primary and secondary metabolites. Depending on cultivation conditions, climatic conditions, soil properties and fertilizer management system, plant nutrient uptake and accumulation of fenugreek may differ. Even among different genotypes of fenugreek plants, differences can be seen in terms of plant nutrient elements (Naula et al. 2018).

In different studies on this subject, especially nitrogen, phosphorus, and potassium fertilizer applications were applied to fenugreek plants and these fertilizers were obtained from different sources organically and inorganically. Somdutt et al. (2019) stated that 30 kg N + 30 kg P ha^{-1} and 2.5 t ha^{-1} vermicompost + Rhizobium + PSB (phosphate soluble bacteria) application is the most effective fertilizer application dose in fenugreek plants. As a result of this study, they stated that, in addition to chemical fertilizers, it is necessary to use organic fertilizers and bio fertilizers in terms of high seed yield and medicinal content of fenugreek plants. However, when the climatic region and soil characteristics of fenugreek plants are changed, the highest yield and quality are obtained with 60–50–50 NPK kg ha^{-1} fertilizer dose 75% + 3.3 tons ha^{-1} vermicompost + Rhizobium + PSB (phosphate soluble bacteria) application (Naidu et al. 2016).

The effectiveness of the applied fertilizers can be affected by soil properties. Therefore, foliar applications can be preferred to increase fertilizer use efficiency (Liu and Lal 2015). As a result of foliar application, morpho-physiological properties, grain yield, and protein percentage of fenugreek can be increased (Amirnia et al. 2018). Foliar fertilizer applications may vary depending on the nutrient requirement of the plant or the nutrient element. Effective results in microelement applications can be obtained from once-twice or more application (Pariari et al. 2009). It is stated that depending on cultivation conditions, soil properties, climate factors and genotypic differences, yield and quality parameters increased in

Table 4.6 Nutrient requirement of fenugreek plants

Fertilization	Doses	References
Organic fertilization	30 kg N + 30 kg P ha^{-1} and 2.5 t ha^{-1} vermicompost + rhizobium + PSB 45–37-37 NPK kg ha^{-1} + 3.3 ton ha^{-1} vermicompost + rhizobium + PSB	Somdutt et al. (2019), Naidu et al. (2016)
N	60–90 kg ha^{-1}	Tunçtürk et al. (2011), Datta et al. (2017), Singh et al. (2018)
P_2O_5	30–80 kg ha^{-1}	Kan et al. (2007), Tunçtürk et al. (2011), Datta et al. (2017)
K_2O	40 kg ha^{-1}	Datta et al. (2017)
S	20–40 kg ha^{-1}	Tunçtürk et al. (2011)
Fe	7.5% as $FeSO_4.7H_2O$	Chhibba et al. (2007)
Cu	30 kg ha^{-1}	Shams et al. (2014)
B	0.1% (twice from leaves) 25 kg ha^{-1} as borax from soil	Pariari et al. (2009), Datta et al. (2017)
Zn	0.2% twice from leaves 1–4 kg ha^{-1} from soil	Pariari et al. (2009), Kan et al. (2007)

fenugreek when plant nutrients are given as indicated in Table 4.6 (Pariari et al. 2009; Mehrafarin et al. 2011; Naidu et al. 2016; Somdutt et al. 2019).

Although there are significant increases in yield and quality depending on the application of plant nutrients, there is no significant effect on the yield and quality of plant nutrients depending on the amount in the soil. In a study where 50 kg Zn,–250 kg K ha^{-1} was applied, it was observed that fertilizer applications did not have a statistically significant effect (Seghatoleslami et al. 2013). Fertilizer applications also cause an increase in the amount of antioxidant and phenolic compounds in fenugreek. The source of the applied nutrient element may have more impact on this increase. Especially compared to chemical fertilizer application, more antioxidant and flavonoid amounts can be obtained in organic fertilizer applications (Salehi et al. 2019). However, the use of microbial fertilizers may also be required to increase the efficiency of the fertilizers to be applied and to increase the plant nutrient parts that can be taken by the plant (Chaichi et al. 2015).

4.4.3 Stress Applications

As a result of global climate change, biotic and abiotic stress factors cause significant yield and quality losses in agricultural production (Stefanelli et al. 2010). The fact that herbal products have high nutritional values and health components is becoming more and more important day by day. The low level of environmental stress conditions during the plant vegetation period, without causing any loss in product amounts, increases the amount of nutrients and some phytochemicals in the products. This situation may cause a significant increase in the quality of the products (Stagnari et al. 2016). Environmental biotic (pathogens such as fungi,

bacteria, and nematodes) and abiotic (salinity, drought, heavy metal, and temperature) stress conditions are important factors limiting agricultural production (Gull et al. 2019).

Drought, salinity, temperature, heavy metal, nutrient, and pathogen levels affect various biochemical activities in the plant. Under stress conditions, some plant metabolites increase while others show decrease. The effects of stress on plants may vary depending on the plant type and genetic characteristics. Different stress conditions may cause significant effects on fenugreek seeds and significant decreases in its product quantity and quality. Fenugreek is sensitive to water stress during the development period. Low amount of water causes a decrease in growth parameters such as plant weight and total leaf area that significantly affect quality (Acharya et al. 2006; Hussein and Zaki 2013). Sugar components such as protein and monosaccharide of fenugreek are increased under water stress conditions (Alhadi et al. 1999). These changes may differ according to the varieties. Due to water stress, the amount of chlorophyll A and B increased while the amount of carotenoids decreased in some varieties (Hussein and Zaki 2013). Photosynthesis and photosynthesis products depending on the CO_2 fixation in fenugreek decrease due to low water amount or single irrigation during development period (Kurdali et al. 2013; Ahmed et al. 2015; Sadak 2016). Fenugreek contains trigonelline, which acts as an osmo-regulator, especially in abiotic stress. In water stress conditions, trigonelline, proline, and antioxidant enzyme activity increase in fenugreek to increase resistance to stress conditions (Zamani et al. 2019).

Water stress that occurs in different growth stages of the plant can cause different metabolic functions in the plant. Water stress that will occur during and after flowering in fenugreek may cause an increase in the oil content of the plant (Saxena et al. 2019). By selecting genotypes that are resistant to water stress conditions, fenugreek can be grown in areas where water is low or water stress occurs. In this case, the amount of product and oil may increase (Basu et al. 2009; Ahari et al. 2009). On the other hand, water stress that may occurs during vegetative, pod formation and pod filling periods of the fenugreek, it can be negatively affected yield, quality and harvest index values (Ahmed et al. 2018).

Soil properties on which fenugreek plants are grown have a significant impact on quality. Stress conditions that occur depending on soil properties may negatively affect yield and quality. In stress conditions such as salinity, the germination and growth parameters of fenugreek can decrease. Proline, chlorophyll, and photosynthetic activity decrease, and important problems can be experienced in quality (Haroun 2002; Al-Saady et al. 2012). However, when the amount of salt in the soil is in certain amounts, fenugreek can be considered as medium resistant to salinity due to it can germinate in medium salinity soil conditions (Ghorbanpour et al. 2011).

In fenugreek, which is a very sensitive plant under high salt conditions, antioxidant enzyme shows different amounts depending on salt stress. With the increase of catalase enzyme activity, peroxidase enzyme activity and lipid peroxidase ratio also increases (Kapoor et al. 2013). In addition, depending on the amount of salt, seed nutritional value, development parameters, and product quality decreases (Abdelmoumen and El Idrissi 2009; Tunçtürk 2011; Pour et al. 2013).

Another stress condition that affects quality in plants is heavy metal. Depending on the amount of heavy metal, germination and seed quality are negatively affected in the plant. Increasing the amount of chromium rather than zinc and copper causes more stress. The increase in the amount of copper causes more decrease in seed germination and quality of fenugreek compared to the amount of zinc (Menon et al. 2016). In the presence of heavy metals such as mercury and cadmium, antioxidant enzyme activity and quality of product decreases (Karmakar et al. 2013; Patel and Patel 2013). However, in case of toxicity of some heavy metals (such as Cu, Zn, Fe), antioxidant activity increases even though the amount of product decreases (Sinha et al. 2007).

4.4.4 Bacterial Inoculation Applications

Bacterial inoculation of the legume family members is common in terms of increased yield. In some studies, it has been reported that bacterial inoculation is an important application in regions with low soil nitrogen content (Abdelgani et al. 1999). Legume plants increase the nitrogen of the soil by benefiting from the atmosphere nitrogen thanks to the *Rhizobium* bacteria that live in their roots. Thus, since this causes less commercial nitrogen usage, it provides benefits both economically and ecologically (Kılıç 2014).

Fenugreek is an important member of the legume family with its pods. Fenugreek uses atmospheric nitrogen for growth and development thanks to *Rhizobium meliloti*, a symbiotic bacterial strain (Poi et al. 1991). Many studies have reported that bacterial inoculation has effects on seed yield and quality characteristics of fenugreek (Poi et al. 1991; Abdelgani et al. 1999; Tunçtürk and Çiftçi 2011). In some of these studies; Abdelgani et al. (1999) reported that bacterial inoculation in fenugreek increases the content of oil, fiber, and protein, and that a proper inoculation could improve seed composition and quality. Wierzbowska and Żuk-Gołaszewska (2014) determined the effect of nitrogen fertilization and *Rhizobium* inoculation on the yield and quality of fenugreek. Researchers were reported that bacterial inoculation showed a high correlation with seed quality and decreased crude oil content, and increased phosphorus, calcium, and sodium concentrations. Żuk-Gołaszewska et al. (2015) investigated the effects of potassium fertilization, *Rhizobium* inoculation, and water restriction on yield and quality of fenugreek seed. In this study, it was reported that bacterial inoculation increased protein content in the seed and slightly decreasing the oil content in fenugreek. In another study, Tunçtürk and Çiftçi (2011) examined the effects of different fertilizer sources, sowing time, and bacterial inoculation on yield and quality characteristics of fenugreek. In this study, it was reported that bacterial inoculation had a positive effect in terms of many characters examined according to control plots. All these studies show that bacterial inoculation is effective on the quality parameters of fenugreek and it may be an alternative agricultural practice, especially in plant growing.

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Agronomic Practices in Fenugreek

5

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Abstract

Fenugreek is a medicinal and aromatic herb that is used in many areas. It is known that fenugreek has been used medically since ancient times. Its seeds contain various important components. Applications of different agricultural system can increase the fenugreek yield and phytochemical properties. There are various agricultural practices that affect the quality characteristics in every stage of the plant, from sowing to harvesting. The crop has also a wide adaptability and is successfully cultivated both in the tropics as well as temperate regions. Application of fertilizers has a beneficial effect on the enhancement of vegetative growth and resulted in higher dry matter production of fenugreek. Fenugreek contains numerous good varieties suitable for different agro-climate regions. Varieties selection to any region depends primarily on its adaptation to the soil and local climatic conditions and preferably should have resistance / tolerance to pests and diseases prevailing in that region. In this chapter, we have reviewed different aspects including botany, genetics, geographical distribution, varieties, diseases and their management as well as various scientific strategies for the cultivation of fenugreek.

Keywords

Botany · Diseases · Insect-pests · Fertilizers and manures · Weed management · Agronomical practices

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5.1 Introduction

Trigonella foenum-graecum L. (English name: fenugreek) is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family Fabaceae. The genus name Trigonella means 'tri-angled', may be because of' triangular shape of its flowers, whereas the species name *foenum-graecum* means 'Greek hay' (Petropoulos, 2002).

5.2 Importance and Uses

Fenugreek is a multipurpose crop in which the tender leaves (Fig. 5.1) and pods are eaten as fresh or fried vegetable and dried seeds (Fig. 5.2) as spice in Indian culture.

It also has immense medicinal value which is mainly attributed to bioactive secondary metabolites such as alkaloids, flavonoids, steroids and saponins. In particular, a steroidal saponin diosgenin, known for its medicinal uses and is

Fig. 5.1 Fenugreek plant leaves

Fig. 5.2 Fenugreek seeds

reported as source of raw material for the production of steroidal hormones and hormones such as testosterone, norethisterone, glucocorticoids and progesterone. Diosgenin also exhibited anticancer and anti-aging activities, as well as cardio-protective and contraceptive properties (Liu et al. 1993; Qin et al. 1997; Dias et al. 2007; Lee et al. 2007; Tada et al. 2009; Yan et al. 2009; Gong et al. 2010; Agarwal et al. 2015). It encompasses renowned culinary and medicinal uses in the history of old civilizations. Egyptians used fenugreek for embalming their prestigious majestic dead bodies, while Romans and Greek were found to use it as cattle fodder. The fenugreek leaves were used to produce the Egyptian incense Kuphi, a holy smoke used in fumigation and embalming rites (Rosengarten 1969). Fenugreek uses are well documented date back to fifteenth century. Melius compiled the details of this plant species in the well-known Kolozsvar Herbarium, in 1578 (Petropoulos 2003). Hidvegi et al. (1984) mentioned some uses of fenugreek, in sixteenth century. Fenugreek seed powder was used to expel placenta after child birth during seventeenth century. (Howard 1987).

5.3 Botany

The plant is an annual herb, erect or branched and, generally, grows to a height of about 30–60 cm, depending on the variety. It has trifoliate, pinnate leaves; roots bearing nodules; white to yellow flowers, flowering 30–40 days after sowing; 3–15 cm long, thin pointed, hoop-like pods; golden yellow seeds (Basch et al. 2003; Acharya et al. 2008; Moradi and Moradi 2013). The genus *Trigonella* consists of 50 species, most of which have an oriental origin in the Iranian Indian region. Of these, 11 species occur in India, out of which *Trigonella foenum-graecum* L. (common fenugreek) and *Trigonella corniculata* L. (*Kasuri* type fenugreek) are cultivated in India. These two differ in their growth habit and yield. The latter one is a slow growing type and remains in rosette condition during most of its vegetative growth period.

5.4 Origin and Distribution

It is believed that fenugreek was native from Europe, though De Candolle mentioned that it could be a plant from Indian origin. If fenugreek was from European origin, then, it should be common in Europe, but this is not the case. Fazli and Hardman (1968) reported that fenugreek was native from Punjab and Kashmir, Mesopotamia desert, Persia and some European countries as Greece, Italy and Spain (Linnaeus 1753; De Candolle 1886). Even though the centre of origin of fenugreek is South-Europe, Mediterranean area and Western Asia, India is also to be a native of fenugreek and found growing wild in Kashmir, Punjab and upper Gangetic plains. Major fenugreek producing countries are India, Argentina, Egypt, France, Spain, Turkey, Morocco, China and Afghanistan. India is the largest producer of fenugreek in the World. In India, Rajasthan state alone grows about 84% of nation's fenugreek.

The other major fenugreek producing states of India are Gujarat, Madhya Pradesh, Uttaranchal, Uttar Pradesh, Haryana, Punjab and Tamil Nadu. It is largely exported to the countries like Saudi Arabia, Japan, Sri Lanka, Korea and UK.

5.5 Genetics

There are few data available on fenugreek karyotype and genome that have identified the somatic chromosome numbers of *Trigonella* taxa as 2n = 14, 16, 30 and 46 along with B chromosomes (Martin et al. 2011; Vaidya et al. 2013). The C-value of fenugreek was found to be 0.7, which is 1.5 fold higher than the values of model legumes *Lotus corniculatus* L. var. japonicus Regel [syn. *Lotus japonicus* (Regel) K. Larsen] and barrel (*Medicago truncatula* Gaertn.); genome size of both these species is around 470 Mbp, whereas the genome size of fenugreek is around 685 Mbp (Young et al. 2003; Vaidya et al. 2013). The transcriptome contains 42 million high quality reads, with 18,333 transcripts functionally annotated and 6775 transcripts related to metabolic pathways including genes of diosgenin biosynthesis (Vaidya et al. 2013).

5.6 Varieties

Fenugreek contains numerous good varieties suitable for different agro-climate regions. Varieties selection to any region depends primarily on its adaptation to the soil and local climatic conditions and preferably should have resistance/tolerance to pests and diseases prevailing in that region. There are many varieties released for cultivation to different areas.

Pusa Early Bunching It was developed at IARI, New Delhi. It matures in 100–125 days and is quick growing variety with upright shoots having bold seeds. It is suitable for seed as well as leaf cut and gives average seed yield of 12 q/ha.

Pusa Kasuri It is small seeded *kasuri* type variety, mainly cultivated for leaf purpose and not for seeds IARI, New Delhi. It is late flowering variety with rosette type leaves and 5–7 cutting may be taken. It is heavy yielder of green leaves with special fragrance. It gives average seed yield of 5–7 q/ha and the crop grown exclusively for greens produces an average yield of about 80 q/ha.

RMt–1 It was developed at SKN College of agriculture, R.A.U., Jobner through pure line selection from local collection. The plants are semi-erect, tall and moderately branched, bold, typically containing yellow coloured grains. It is suitable for Gujarat and Rajasthan states and matures in 140–150 days and gives an average yield of 14.7 q/ha. It is moderately resistant to root rot and tolerant to powdery mildew.

RMt–143 It was developed at SKN College of Agriculture (R.A.U.), Jobner through pure line selection from Jodhpur region and identified for release in 1997. It is suitable for Rajasthan and give an average yield of 16 q/ha. It is suitable for Bhilwara, Jhalawar and Jodhpur areas of Rajasthan.

RMt–305 It was also developed at SKN college of Agriculture, (R.A.U.), Jobner. This was developed through mutation breeding in RMt-1 and is the first determinate type of fenugreek, yielding 1300 kg/ha. The plant is dwarf, determinate, multipoded, early in maturity, resistant to powdery mildew and root knot nematode and suitable for all fenugreek growing areas.

Ajmer Methi 1 (AM–1) The variety has been developed through pure line selection from local germplasm at NRCSS, Ajmer. The seeds are bold and large. The number of seeds per pod ranges from 17–20 with 17–20 g test weight. The crop takes 137 days to mature and gives seed yield of 27.2 q/ha. The crop grown exclusively for leaf purpose yields 76 q/ha green leaves from three cuttings.

Ajmer Methi 2 (AM–2) The variety has also been developed through pure line selection from local germplasm at NRCSS, Ajmer. The seeds are small in size. The number of seeds per pod ranges from 16–18. The crop takes 138 days to mature and gives seed yield of 18.1 q/ha. The crop grown exclusively for leaf purpose yield 72 q/ha green leaves from three cuttings.

GM–1 This variety was developed by Gujarat Agriculture University, Jagudan. Its plants are dwarf and having average yield 18.6 q/ha. This variety is suitable for Gujarat region.

CO–1 It was developed by Tamil Nadu Agriculture University through selection from TG 2336 of IARI and released in 1982. The plants are short and green with medium sized brownish orange seeds. It is tolerant to root rot. It matures in 95 days and gives an average yield of 6.80 q/ha.

Rajendra Kranti It was developed by Rajendra Agricultural University, Pusa through mass selection from Raghunathpur germplasm and released in 1987. The plants are bushy green with medium sized golden yellow seeds. It is moderately resistant to powdery mildew, caterpillars and aphids. It matures in 120 days, and gives an average yield of 12.50 q/ha.

Lam Selection–1 It was developed by Andhra Pradesh Agriculture University, Guntur through selection from germplasm collected from M.P. and released in 1992. The plants are bushy in nature with medium sized golden yellow seeds. It is tolerant to root rot, powdery mildew, caterpillars and aphids. It matures in 90 days and gives an average yield of 7.40 q/ha.

Hisar Sonali It was developed by CCS, Haryana Agriculture University, Hisar through pure line selection from local germplasm and identified in 1983. The plants are bushy, semi-erect with bold yellow attractive seed (13–15 g/1000 seeds). It is moderately resistant to leaf spot and root rot disease. It matures in 140–150 days and gives an average yield of 19.0 q/ha.

Hisar Suvarna This variety was developed through pure line selection from local germplasm by CCS Haryana Agriculture University, Hisar. This is a dual-purpose cultivar and suitable for Haryana, Rajasthan and Gujarat. This variety is resistant to powdery mildew and moderately resistant to downy mildew. It gives an average yield of 19–20 q/ha.

Hisar Mukta This variety was also developed at CCS Haryana Agriculture University, Hisar. It is a natural green seed coat mutant, selected from IL-335-1 germplasm line, a collection from U.P. Yield is around 20–23 q/ha. It is resistant to downy mildew and moderately resistant to powdery mildew. It is suitable for cultivation under all fenugreek growing states of North India.

Hisar Madhavi (HM–350) It was developed by CCS Haryana Agriculture University, Hisar through single plant selection from the line PLME 46–1, a pure line selection from collection from U.P. It is medium in maturity and having average yield 19–20 q/ha. This variety is resistant to powdery mildew and moderately resistant to downy mildew.

Hisar Manohar (HM-444) A fenugreek variety with high yield of 20.6 q/ha and unique green seed colour developed by Chowdhary Charan Singh Haryana Agricultural University, Hisar, Haryana.

Pant Ragini This variety was developed by GB Pant University of Agriculture & Technology, Pantnagar. It is a dual-purpose variety means can be grown for leaf as well as seed purpose. Plants are tall and bushy type. The variety is resistant to downy mildew and root tot. The variety matures in 170–175 days.

LAM METHI-2 (LFC-84) It is a high yielding variety developed by Dr. YSR Horticultural University, Venkataramannagudem, A.P. The plant grows up to 50 cm with profuse bearing. It is a medium duration which comes to maturity in 80–90 days with an average yield of 7–9 quintals per hectare under rainfed conditions and 12.0–15.0 quintals under irrigated conditions. It has higher diosgenin content (0.45–0.83%). Grains are flat, rectangular shaped with attractive brown colour having better market acceptance. The variety tolerates terminal moisture stress. The variety has yield advantage of 30–35% over the existing Lam Selection-1 variety.

LAM METHI-3 (LFC-103) It is a high yielding variety released from Dr. YSR Horticultural University, Venkataramannagudem, A.P. It grows up to 50 cm with profuse bearing. It is a medium duration which comes to maturity in 90–95 days. The

variety is identified for release at national level. It is suitable for Andhra Pradesh, Telangana, Madhya Pradesh and Bihar. It gives an average yield of 7–9 quintals per hectare under rainfed conditions and 12.0–19.0 quintals under irrigated conditions. It has higher diosgenin content (0.72%). Grains with attractive brown colour having better market acceptance. The variety suitable for both rainfed and irrigated cultivation. It tolerates dry root rot in field conditions. The variety has yield advantage of 30–35 % over the existing Lam Selection-1 variety.

5.7 Climate and Soil

The crop has a wide adaptability and is successfully cultivated both in the tropics as well as temperate regions. The crop can be grown successfully both in tropical and temperate regions up to an altitude of 2000 m MSL. It is tolerant to frost and freezing weather. It does well in places receiving moderate or low rainfall areas but not in heavy rainfall area. *Kasuri* type varieties require preferably extra cool weather for longer duration and thus raised more successfully in northern states during winter than the southern states of India. Fenugreek can be grown on a wide variety of soils but clayey loam is relatively better. For rainfed cultivation, black cotton soils are best suited. Although the crop is tolerant to salinity up to 8.4 soil pH, for higher yields with better quality of leaves the optimum pH soil should be 6.0–7.0.

5.8 Land Preparation

The land should be well prepared for better germination of seeds and growth of plant. A total of 3–4 ploughings are required. The first ploughing should be done by soil turning plough followed by 2–3 ploughing with harrow to bring the soil to a fine tilth. At the time of sowing there should be good moisture in the soil for better germination of seed.

5.9 Sowing Time

Fenugreek, being cool season crop, is sown from October and November in northern plains, whereas, in hilly tracts, it is sown from March to May, depending on altitude. In areas with mild climate, fenugreek for fresh greens may be grown round the year except extreme hot months of summer and rainy season. In southern states of India, particularly Karnataka, Andhra Pradesh and Tamil Nadu, fenugreek is sown twice, once in *rabi* (September–December) and again in *Kharif* season (June–July). For higher yield, it is better that the sowing time should be so adjusted that the pod development and seed maturity phase may coincide with a dry and rain free period. First fortnight of November is the best time for sowing of fenugreek (Anonymous 1988).

5.10 Seed Rate

Seed rate requirement is fenugreek is 20–25 kg/ha and for *Kasuri* type 10–12 kg/ha. The quantity of seed depends on the purpose for which the crop is sown. To raise a healthy crop and to obtain better yield with quality produce, proper seed rate should be maintained. A seed rate of 20-24 kg/ha with row spacing 40 cm was found to be best (Kumar et al. 2018).

5.11 Seed Treatment

Being a legume crop, it fixes nitrogen about 283 kg/ha/year from the atmosphere. The role of *Rhizobium* in fenugreek production is well established, and thus inoculation of seed before sowing has proved beneficial in getting higher seed yield. Seeds should be treated with *Rhizobium meliloti* local culture prior to sowing, especially when the crop is sown in new field. Treating the seeds with *Rhizoctonia meliloti* culture is also recommended. The seed should be treated with bavistin or captan or thiram @ 2 g/kg seed for the control of fungal diseases during initial stages of crop growth.

5.12 Sowing Method

Fenugreek can be sown either in lines or by broadcasting seeds in well-prepared flat seedbeds and raking the bed surface prudently; however, sowing in lines is comparatively better than the broadcasting since it facilitates the intercultural operations, like hoeing and weeding. Line to line spacing of 25–30 cm is required and later the plants are thinned to maintain 10–15 cm spacing within the lines. The seed germinates in about 5–7 days of sowing. Although the depth of the sowing seeds depends on soil type and soil moisture at the time of sowing, but being small size, the seeds of common fenugreek are usually sown at a depth of 2–3 cm and *kasuri* fenugreek at 1.0–1.5 cm. Seed yields were highest at spacing of 20 cm and without harvesting leaves**,** in rows 22.5 cm apart using 30 kg seeds/ha (Pandita and Randhawa 1994). Rana et al. (2015) found that row spacing of 45 cm with one leaf cutting at 60 DAS resulted in a seed yield of 9.10 q/ha and leaf yield of 48.96 q/ha and also recorded highest net returns per hectare (Rs. 34,065/−) and B:C ratio (1.41).

5.13 Manures and Fertilizers

Application of FYM (10 t/ha) has a beneficial effect on the enhancement of vegetative growth and resulted in higher dry matter production of fenugreek. Chaudhary (1999) studied response of fenugreek to N, P and *Rhizobium* inoculation. Application of 40 kg N/ha significantly increased the mean plant height, branches and pods/

plant, pod length, test weight and straw yield compared with 0 and 20 kg N/ha. Seeds/pod and seed yield increased significantly with the application of 40 kg N/ha. Compared with the control, P at 40 kg/ha produced significantly higher mean number of branches, pods/plant, test weight, straw and seed yields than 20 kg P/ha. Rhizobium inoculation resulted in significant increase in number of branches per plant, pod length and test weight and improved yield compared with the uninoculated control. Dayanand et al. (1999) studied the influence of phosphorus and sulphur on nutrient uptake and quality seed production of fenugreek cv. RMt-1. Basal application of phosphorus @ 40 kg/ha was significantly improved total N uptake by the crop and sulphur @ 50 kg/ha also increased N and protein content of the seed along with N and P uptake.

5.14 Irrigation

First irrigation should be applied very soon after sowing and should be followed by another light irrigation on third day to facilitate rapid and uniform germination. Subsequent irrigations are given at 12–15 days interval, depending on soil type, season, rainfall, and other temporary weather conditions. The early growth period and seed setting are the critical stages for irrigation in the crop grown exclusively for grain or seed purpose. Too much irrigation is also as harmful as the scarcity of moisture, since excessive moisture in any form and at any stage increases the incidence of root rot and especially at flowering powdery mildew. Normally 6–7 irrigations are required in light soil and 4–5 irrigations are needed in heavy soils.

5.15 Weed Management

Weeds emerging 30–40 DAS of fenugreek plant caused relatively little reduction in plant growth and yield. First 30–40 days were identified as a critical period with respect to crop weed competition in fenugreek. Fenugreek, being leguminous crop, needs proper soil aeration for the development of root system, thus, hoeing and weeding during early stages of plant growth are very essential to make the soil loose around the roots and to control the weeds, since weeds due to slow growth of fenugreek seedlings may pose problem in initial stages, however, in later stages, when the crop canopy is fully developed, weeding is not at all required as the crop itself suppresses the weeds.

Generally, 2–3 hand weedings are required to keep the crop weed free and reducing the crop weed competition for growth resources like nutrients, moisture, light and others. The first hoeing and weeding is done at the time when the plants are 5 cm tall. Integrated weed management using pre-sowing application of pendimethalin @ 1 kg/ha, or pre-sowing application of fluchloralin @ 0.75 kg/hg in 500–600 L of water, with the one hand weeding at 50 DAS is very effective method of weed control for realizing higher yield and benefit in fenugreek cultivation. Among the different herbicide treatments pendimethalin at 0.75 kg/ha and

fluchloralin at 1.0 kg/ha were found superior. Meena et al. (2009) reported that the application of Oxadiargyl @ 0.075 kg/ha just after sowing with the one hand weeding at 45 DAS was found to be best integrated weed management practice in fenugreek crop as it resulted in better plant growth, development, easily flowering pod formation and higher yield under Ajmer (Rajasthan) conditions. Herbicidal application as well as manual weeding reduce the nutrient removal by weeds and enhance the nutrient uptake by the crop at all stages of growth.

5.16 Harvesting and Yield

The common fenugreek becomes ready for cutting fresh green leaves and young shoots in about 20 days after sowing, while *kasuri* type fenugreek is ready in 25–30 days after sowing and subsequent cuttings may be taken at an interval of 15–20 days. The crop when grown for dual purposes after taking one cutting, which does not affect the seed yield, is left for seed production. The crop after harvest is bunched and marketed. The cutting is usually done with sharp knife by leaving stubs 3–4 cm above the ground level and after taking 4–5 cutting the crop grown exclusively for green leaves is uprooted. Common fenugreek can be harvested by clipping the young plants from the base and the clipped plants are allowed to grow further and their tops are nipped periodically until flowering. If fenugreek is harvested late, its leaves develop a bitter taste.

Depending upon variety and season of growing the crop grown for grain takes about 80–165 days from sowing to harvesting. The entire plant is either pulled out of cut from the base with sickle when 70% of the pods turn yellow, and made into small bundles for drying them in sun. Seeds are separated manually or by thresher winnowing. The grain/seed is dried up to 7–8% moisture, cleaned, graded and packed in different type of packages. Under irrigated conditions, the common type fenugreek varieties normally give a fresh green leaf and seed yield of 70–80 and 15–20 q/ha, respectively, and *kasuri* type 80–100 q green leaves per hectare.

5.17 Packaging and Storage

Green leaves are very perishable in nature; therefore, they are marketed soon after harvesting. However, well dried leaves can be stored for about 10–12 months. The leaves can be stored only for about 24 hours after harvesting under ambient conditions, however, in cold stores at 0 °C temperature and 90–95% RH the storage period for leaves can be extended up to 10 days. Seeds are packed in gunny bags lined with polythene film plastic bags, vacuum packages, CAP/MAP, etc. Vacuum gravity separator is used for cleaning fenugreek seeds. The properly cleaned fenugreek seeds are stored with an initial moisture level of 7–8% and at an equilibrium relative humidity of 40%. Fenugreek seeds well packed is stored in ventilated dry and cool place under ordinary conditions till sowing of next season crop.

5.18 Major Insect-Pests

Aphids (*Acyrthosiphon pisum*, Henis. *Myzus persicae*, Sulzer and *Aphis craccivora*, Koch.)**:** The most common insect that attacks the fenugreek crop is aphid and found in colonies, sometimes very serious in patches on tender leaves, stem and inflorescence. Both nymph and adult are sucking the sap from the tender leaves, flower, etc. The severe infestation affects the yield and quality of leaves badly. With the attack of aphids the plants turn yellow and result in shriveling of seeds and reduction in seed yield as well as quality. Excess application of nitrogenous fertilizer like urea, ammonium sulphate and irrigation makes plant succulent and subsequently higher insect population build up. In severe condition two spray of imidachlorprid (0.005%) or dimethoate (0.33%) after 10 days interval help in management.

Leaf Eating Caterpillar (*Spilarctia oblique* and *Spodoptera litura* Fabricius) The large numbers of caterpillar appear and destroy the leaves. The eggs are laid in clusters and young larvae gregariously feed on leaves. They scrap out the green matter from the leaves and give the appearance of papery white structure. The last instars of larvae feed voraciously causing defoliation of plants and thereby causing considerable losses in yield and quality of greens. Destroy the egg mass and gregarious young larvae by hand picking and killing. Spray NSKE (5%) or neem oil 2% in the early stage of larvae development. Use of nuclear polyhedrosis virus @ 250 LE/ha and *Beauveria bassiana* @ 1010 spores/ml is an effective biological control.

Pod Borer (*Helicoverpa armigera Hubner*) The pest is found to feed on leaves, flowers and pods. Eggs are laid on young leaves, which are later damaged by young larvae. The first instars of larvae bore the fruits and damage them, causing 10–90% losses, if left unprotected. Spray of Endosulphan (0.05%) or quinalphos (0.05%) when population reaches at higher level.

Jassids (*Empoasca spp.*) Jassids attack on fenugreek crop at early stage. Nymph and adult suck the leaf sap causing browning of leaves. Foliar spray of endosulphan (0.05%) for effective control of jassids is used.

White Fly (*Bemisia tabaci*) White fly is serious pest of fenugreek in some areas. It attacks the crop at early stage and continues up to pod formation stage. Nymph and adult suck the sap of plant causes yellowing of plant and subsequently plant dies. Spraying of imidachlorprid (0.005%) at initial stage of infestation by using 500 L of solution with water is sufficient to cover 1 ha area.

Leaf Minor (*Empoasca spp.*) Excess mining of leaves at early stage causes stunting growth of plant. Severely infested crop gives less production. Make solution of insecticide (endosulphan 0.05%) in 400–500 L of water to spray in 1 ha area is sufficient. Effective control of mites by foliar spray of phosphomidon (0.03%) using 400–500 L of water in 1 ha.

5.19 Major Diseases

Root Rot (*Rhizoctonia solani* Kuhn) Root rot of fenugreek is caused by *Alternaria alternata* (Fr.). This is a soil-borne disease a problem in major fenugreek growing areas and drastically reduces the yield. Young plants are relatively more susceptible to this disease. Stunting of infected plants is observed and plants are easily detached at soil level when pulled out (Chattopadhyay and Maiti 1990). The symptoms include varying degrees of rotting of the roots leading of foliage yellowing generally in 30–45 day old plants. The affected plants wither and dry up later on.

Control Deep summer ploughing of field and adoption of crop rotation can minimize disease incidence. This disease can be controlled by seed treatment with thiram or captain @ 2.5 g/kg of seeds. Seed pelleting with antagonistics like *Trichoderma viride, T. harizianum* (talk bases formulatin 4 g/kg of seed) followed by soil application of neem cake 150 kg/ha was recommended for disease management (Ravindran et al. 2001). Drenching twice with carbendazim (0.1%), first at initial appearance and second after one month with carbendazim or brassical (0.1%).

Powdery Mildew (*Erysiphe polygoni* D.C. and *Leveillula taurica* Lev) Powdery mildew of fenugreek is caused by *Erysiphe polygoni* DC and *Leveillula taurica* (Lev.) Am. *Erysiphe polygoni* produces white floury patches on both the surface of leaves and other aerial parts of the plants while *Leveillula taurica* produces mycelia patches on the leaves (Agrawal 2001). Cleistothecia appear at a later stage as black specks immersed in the mycelia mats. Pathogen *Leveillula taurica* is an obligate parasite, which perennates through cleistothecia present on the debris of the previous cropping season was reported. The disease generally appears late in the season and is of minor importance.

Control In early sown (October 20 to October 30) crop the powdery mildew disease intensity was found to be comparatively less than that in late sown crop (November 20 to November 30) (Sharma and Sharma 1999).

Powdery mildew can be controlled by dusting 30 mesh sulphur (25 kg/ha) or spraying wettable sulphur or Dinocap (0.25%) or Karathane (0.1%) twice at flowering stage and after 15 days of first spray @ 400–500 L solution/ha (Ravindran et al. 2001).

Downy Mildew (*Peronospora trigonella* Gaum) Downy mildew of fenugreek is caused by *Rhizoctonia trigonella* Gaum. The lesions caused by pathogen are mostly found at collar region. Water soaked lesions ultimately cause damage by rotting of the epidermal or vertical tissues at collar region and result in damping off (Agrawal 2001). Symptoms include the presence of yellow patches on the upper surface in the corresponding areas. In the advance stage of infection, the leaves turn yellow and shed, thus plant growth is checked adversely. Spraying of Bordeaux mixture (1%) as restricted use has proved beneficial regarding control of fenugreek downy mildew.

Two sprays of zineb (3 g/L) at 15 days interval are also recommended for the control of this disease.

Damping Off (*Pythium aphanidermatum*) Damping off is caused by *Rhizoctonia solani*. Water soaked lesions cause damage by rotting of the epidermal or vertical tissues at collar region and results in seedlings topple down. As the disease advances, the stem becomes constricted at the base and the plants collapse. The sclerotic fungus can survive in the soil for several months depending upon the temperature and moisture. Initial infection starts from the pre-infected soil. The mycelium grows inside the tissues in all directions, initiating secondary infection and producing sclerotic on the diseased parts. The infected seedlings appear water soaked, discoloured and soft, emitting a bad odour.

Control Crop rotation and removal of diseased plants are effective to minimize the source of infection. Seed treatment with Carbendazim (3 g/kg seed) is very effective (Ravindran et al. 2001).

Leaf Spot (*Cercospora traversiana*) The causal organism of this disease is *Cercospora traversiana*. This disease is internally seed borne. Seed germination is not affected by internal seed borne inoculums but heavy post-emergence losses are caused and the inoculums are transmitted from seed to seedlings (Rastogi et al. 1998). Initially symptoms of this disease appear as large spots on leaves and branches during and after anthesis (Agrawal 2001). The spots are white at the centre and brown at the periphery. The affected leaves drop-off prematurely. Young pods are soon infected and the fungus invades the immature seeds. The occurrence of disease adversely affects the market quality of green leaves.

Control To eradicate the internal seed borne infection seed treatment with systemic fungicides should be done (Agrawal 2001). Two sprays of mancozeb (0.02%) are useful (Ravindran et al. 2001). Follow at least 2 years crop rotation in infested areas and use of healthy seed at the time of sowing.

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6

Exogenous Gibberellic Acid Supplementation Renders Growth and Yield Protection Against Salinity Induced Oxidative Damage Through Upregulating Antioxidant Metabolism in Fenugreek (*Trigonella foenum-graceum* L.)

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Abstract

Gibberellic acid (GA) is a well-established group of phytohormones with growth eliciting properties. Considering the substantial damage by salt stress, we investigated whether foliar sprays of 10^{-6} M GA_3 could reverse salinity implicated constraints in fenugreek plants and up to what extent. Our study suggested that exogenous GA_3 could significantly ($p \leq 0.05$) mitigate the effects of salinity in the fenugreek plants. This treatment maximised the growth and yield variables, as well. The activities of various assimilatory enzymes, such as carbonic anhydrase and nitrogen reductase, observed an increment of about 17% each over salt-stressed plants (50 mg L^{-1}). Further metabolomic analyses revealed an upregulated antioxidant defence system with increased activities of superoxide dismutase (18%), catalase (13%), and ascorbate peroxidase (15%). The enhanced proline content (19%) in tandem with upregulated antioxidant enzymes minimised cellular damage through restricting TBARS and H_2O_2 contents by about 16% and 14%, respectively. Thus, in the light of sufficient data, we are convinced that foliar sprays of 10^{-6} M GA_3 could be used for minimising the salinity induced growth and yield constraints in the fenugreek crop.

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Keywords

Abiotic stress · Antioxidant metabolism · Gibberellin · Fenugreek · Phytohormone · Salt tolerance

6.1 Introduction

Fenugreek (*Trigonella foenum-graceum* L.) is an aromatic leguminous plant belonging to the family Fabaceae (Beyzi 2020). The plant is exploited for the broad spectrum of its medicinal properties, which includes antidiabetic, anticancer, analgesic, antimicrobial, antioxidant, hypocholesterolemic, along with lactation and appetite enhancing attributes (Ouzir et al. 2016; Choudhary et al. 2021a). These therapeutic characteristics can be attributed to the high concentration of bioactive phytochemicals present in the fenugreek, such as flavonoids, alkaloids, amino acids, vitamins, saponins, and fibres (Ouzir et al. 2016; Bitarafan et al. 2019). However, the concentration of these secondary metabolites, as well as the overall growth and development of the fenugreek plant, can be influenced by various environmental stimuli (Bitarafan et al. 2019; Mickky et al. 2019).

Soil salinity is one of the leading environmental stresses that hamper crop growth and physiology, causing a reduction in plant productivity (Van Zelm et al. 2020). The salt stress affects plants in two ways: first limiting the plant water uptake and subsequently building an ion excess in the plant system (Munns and Tester 2008). The first phase creates oxidative stress, while the later one contributes to the ionic stress (Shabala and Cuin 2008). Most crops, including fenugreek, are glycophytes and cannot grow well in high salt concentrations, and thus, depending on the severity, salinity can regulate seed germination, cell expansion, stomatal conductance, photosynthesis, and other metabolic and development pathways in such plants (Shabala and Cuin 2008; Mickky et al. 2019; Van Zelm et al. 2020). An essential aspect of salinity induced damage lies in the overproduction of reactive oxygen species (ROS) by salt stress (Zhu 2001). Although ROS acts as a secondary messenger for various vital physiological processes, their accumulation can cause oxidative damage to proteins, lipids, and nucleic acids (Apel and Hirt 2004; Foyer 2018; Mukarram et al. 2021a). As a counter mechanism, plants possess an efficient antioxidant defence system to regulate the ROS build-up (Gill and Tuteja 2010; Mukarram et al. 2021b, c). Principal ROS scavenging antioxidants are superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), and the harmony among their activities to adjust superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2) can determine the plant tolerance level to the stress (Mittler 2002; Corpas et al. 2017; Zehra et al. 2020; Choudhary et al. 2021b).

Phytohormones are chemical messengers that interact with various physiological processes to ensure normal growth, development, and productivity of the plants (Javid et al. 2011). Gibberellin (GA) is a well-known plant hormone that promotes a wide range of phenomena pertaining to plant growth and development, such as seed germination, cell expansion, stem and leaf elongation, flower induction, and pollen development (Ueguchi-Tanaka et al. 2007; Choudhary et al. 2021c). Although

more than 130 types of GAs have been discovered by far in different organisms, only a few of them can impart the aforementioned effects (Davière and Achard 2013). Nevertheless, external stimuli such as salt stress can influence the endogenous level of gibberellin and its biosynthesis through regulating plant water relation and membrane permeability (Tuna et al. 2008). Given salinity causes damage by reducing gibberellin content in the fenugreek plants, the present study hypothesised that these effects could be minimised if additional GA is provided to the plants. To test our hypothesis, we exogenously applied GA_3 to salt-stressed fenugreek plants as foliar treatments. Data analyses suggested that GA_3 can assist in the reversal of salt-induced constraints and oxidative damage through enzymatic enhancements and upregulating antioxidant metabolism in fenugreek, and thus can ensure enhanced plant growth and productivity.

6.2 Materials and Methods

6.2.1 Growth Conditions and Experimental Setup

Fenugreek (*Trigonella foenum-graceum* L.) plant was used as the plant material for the present study. The authorised seeds from the Indian Agricultural Research Institute (IARI), New Delhi, India, were used for propagating fenugreek plants. Subsequent surface sterilisation of the seeds was performed with 0.2% $HgCl_2$ for 5 min and repeated washing with deionised water. Sterilised seeds were sown in the earthen pots (25 cm × 25 cm) in the semi-automatic net house at the Department of Botany, Aligarh Muslim University, Aligarh (27°52′ N latitude, 78°51′ E longitude, and 187.45 m altitude). Each pot of the 5 kg capacity was filled with a mixture of soil and organic manure in a 5:1 ratio (w/w). A temperature range of 17–25 °C (±4 °C) and the relative humidity [68 ± 5%] were recorded during the experimental period. Soil analysis at the IARI, New Delhi of the random samples collected from different pots revealed the following soil attributes: texture- sandy loam, pH (1:2): 7.6, and electrical conductivity (1:2): 0.52 m mhos cm^{-1}. Available nitrogen, phosphorous, and potassium content in the soil was recorded as 94.7, 8.8, and 136.6 mg kg^{-1} of the soil, respectively. The pots were arranged according to a simple randomised block design with five replicates, and the plants were irrigated daily to keep the soil hydrated. The effects of the salinity and gibberellic acid were assessed in terms of the modulation observed in the growth, physiology, and productivity of the 90 days old fenugreek plant.

6.2.2 Induction of Salt Stress

Fenugreek plants were maintained under two different NaCl concentrations (50 and 100 mg L^{-1}). These concentrations were supplied in the form of 300 mL of modified full-strength Hoagland's nutrient solution on alternate days starting from 10 days

after the seed germination, while the control group was supplied with 300 mL nutrient solution only.

6.2.3 Supplementation of Gibberellic Acid

Exogenous application of gibberellic acid was carried in the form of foliar sprays for the amelioration for salinity induced constraints. Based on the literature available, a concentration of 10^{-6} M of GA_3 was supplemented to the fenugreek plants (Miceli et al. 2019a, b). In total, five foliar sprays (50 mL each) were applied every week starting from 30 days after seed germination using a battery sprayer. The control group was supplied with the sprays of deionised water only.

6.2.4 Determination of the Growth Attributes

Growth parameters were evaluated in terms of plant length and weight. Five plants from each treatment were uprooted after 90 days of seed sowing and were cleansed carefully with deionised water in order to remove stuck foreign particles. The plant surface was dried with blotting paper. The length of the shoot and root was measured using a metric scale and was expressed in cm. Subsequent shoot and root fresh weights were measured using an electric balance. Thereafter, the plants were dried for 40 h in an oven at 80 °C to attain a dry state. Separate weights of the shoot and root were calculated using the electric balance. All the weight measurements (fresh and dry) were expressed in g. The leaf area index was determined using the graph paper sheet by following the procedure of Watson (1958). The average number of leaves for each treatment was counted accordingly.

6.2.5 Determination of the Total Chlorophyll Content

The total chlorophyll content in the fresh fenugreek leaves was estimated using the methods developed by Lichtenthaler and Buschmann (2001). Fresh tissue from the interveinal leaf area was ground with acetone solution (80%) with the help of a mortar and pestle. The optical density of the chlorophyll extract was recorded at 662 nm for chlorophyll *a* content and at 645 nm for chlorophyll *b* content with a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). Total chlorophyll content was estimated by adding chlorophyll *a* and *b* content and was expressed in mg g^{-1} FW.

6.2.6 Determination of the Nitrate Reductase (NR) Activity

The intact tissue assay method of Jaworski (1971) was adapted for nitrate reductase (E.C. 1.7.1.1) activity in the fenugreek leaves. 0.2 g of fresh leaves were chopped

and transferred into the test tubes. Each test tube contained a mixture of 2.5 mL of phosphate buffer (pH 7.5) with 0.1 M, 0.5 mL of potassium nitrate (0.2 M) and 2.5 mL of isopropanol (5%). The reaction mixture was incubated at 30 °C for 2 h. Thereafter, 0.4 mL of the aliquot was transferred to the test tube containing 0.3 mL of sulfanilamide (1%) and 0.3 mL of NED-HCl (N-1-naphthyl ethylenediamine dihydrochloride) (0.02%) for nitrite generation after azocoupling with sulfanilamide and NED-HCl. The reaction mixture was incubated again at 30 °C for 20 min for maximised colour development. Distilled water was used for subsequent dilution to reach a final volume of 5 mL. The absorbance of the solution was recorded at 540 nm using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan), and the enzyme activity was expressed as the nmol of nitrite generated per gram of fresh weight of the leaf tissue per hour (nmol NO_2^{-1} g^{-1} FW h^{-1}).

6.2.7 Determination of Carbonic Anhydrase (CA) Activity

Considering the crucial role in stomatal conductance, carboxylation, and conversion of CO_2 into bicarbonates, the activity of carbonic anhydrase (E.C. 4.2.1.1) was determined. CA activity was measured in the fenugreek leaves by following the procedure of Dwivedi and Randhawa (1974). 0.2 g of fresh leaves were chopped and transferred to the petri dishes, followed by dipping them in the 10 mL of cysteine hydrochloride solution (0.2 M). After leaving the setup was at 4 °C for 20 min, 4 mL of sodium bicarbonate solution (0.2 M) and 0.2 mL of bromothymol blue dye (0.022%) was added to each petri dish. Finally, the reaction mixture was titrated using methyl red as an indicator against 0.05 N HCl. The enzyme activity was expressed in μmol CO_2 kg^{-1} leaf FW s^{-1}.

6.2.8 Determination of H_2O_2 Content

Hydrogen peroxide (H_2O_2) content was determined by a peroxidase dependent assay adopting the method of Okuda et al. (1991). Peroxidase was added to initiate the reaction at 25 °C, and the increase in absorbance was observed at 590 nm spectrophotometrically for 3 min. The H_2O_2 content was expressed as μmol H_2O_2 g^{-1} FW.

6.2.9 Quantification of Lipid Peroxidation

The method of Cakmak and Horst (1991) was adapted to quantify lipid peroxidation in fenugreek leaves through estimating total thiobarbituric acid reactive substances (TBARS) content. TBARS content was determined as malondialdehyde (MDA) equivalents and expressed in nmol MDA g^{-1} FW. Conclusively, 0.5 g of fresh fenugreek leaves were crushed in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The mixture was centrifuged at 12,000 × g for 5 min. Thereafter, 0.5% (w/v) tetrabutylammonium (4 mL) in 20% (w/v) TCA was mixed with an aliquot of

1 mL of the supernatant. The setup was left for incubation for 30 min at 90 °C. Subsequent termination of the reaction was carried out in an ice bath. The mixture was centrifuged again at 10,000 × g for 5 min. Spectrophotometric analysis (Shimadzu UV-1700, Tokyo, Japan) of the supernatant was performed at 532 nm, and the values were corrected for non-specific turbidity by subtracting the absorbance at 600 nm.

6.2.10 Determination of Proline Content

The proline content was estimated according to the methods described by Bates et al. (1973). 0.25 g of the fresh leaves were ground in the 2.5 mL aqueous solution of sulfosalicylic acid (3%). The mixture was centrifuged for 10 min at 10,000 × g. The aliquot (2 mL) of the supernatant collected afterwards was transferred to a test tube containing sulfosalicylic acid (2.5 mL), glacial acetic acid (1 mL), and acid ninhydrin solution (1 mL). The test tube was boiled at 100 °C for 1 h using a hot water bath. Termination of the reaction was performed using an ice bath while extraction was carried by adding toluene (3 mL) and subsequent rousing of the mixture for 20–25 s. The reaction mixture was left for some time to separate the aqueous portion from the toluene layer. The optical density of the toluene aspired layer possessing chromophore was recorded at 520 nm using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The proline content was calculated from against a standard curve and was expressed in mg g^{-1} FW using the following equation:

$$[(\mu g\ \text{proline/ml} \times \text{ml toluene})/115.5\ \mu g/\mu\text{mol} - 1]/[(\text{g sample})/5] = \mu\text{moles proline/fresh weight of the material (g)}.$$

6.3 Quantification of the Enzymatic Antioxidant Defence System

6.3.1 Preparation of Enzyme Extract

For the enzymatic assays, 0.2 g of fresh fenugreek leaves was ground in liquid N_2 using a mortar and pestle at 4 °C. The resulting coarse powder (0.5 g) was transferred to 5 ml (w/v) of chilled extraction medium containing potassium phosphate buffer (100 mM and pH 7.8), 1% (w/v) polyvinylpyrrolidone and 0.5% (v/v) Triton-X-100. Homogenates were centrifuged at 15,000 × g for 5 min at 4 °C. The supernatants acquired after centrifugation was used for the determination of enzymatic antioxidants activity (Kuo et al. 1982).

6.3.2 Superoxide Dismutase (SOD) Activity

The estimation of SOD (E.C. 1.15.1.1) activity was done according to the procedure of Beauchamp and Fridovich (1971). Riboflavin (1 mM), methionine (9.9 mM), nitro blue tetrazolium (55 mM), EDTA (2 mM), and Triton-X-100 (0.02%) was added to the 40 mL of freshly prepared enzyme extract and illuminated and maintained for one hour at 30 °C. The reaction mixture was analysed by a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan), and absorbance was recorded at 560 nm. One SOD unit is the amount of the enzyme needed for half inhibition of nitro blue tetrazolium reaction at the set wavelength.

6.3.3 Catalase (CAT) Activity

The activity of CAT (E.C. 1.11.1.6) was determined with the methods of Chandlee and Scandalios (1984) with slight modification. In the 0.04 mL of the enzyme extract, 2.6 mL of potassium phosphate buffer (50 mM with pH 7) and 0.4 mL of H_2O_2 (15 mM) was added. The solution was centrifuged afterwards at 12,500 × g for 20 min at 4 °C. Enzyme activity was measured by determining the disappearance of H_2O_2 at 240 nm for 2 minutes with 5 seconds interval.

6.3.4 Ascorbate Peroxidase (APX) Activity

Enzyme activity for APX (E.C. 1.11.1.11) was measured according to Nakano and Asada (1981). A reaction mixture was prepared with enzyme extract containing phosphate buffer (50 mM with pH 7), EDTA (0.1 mM), ascorbate (0.5 mM), and H_2O_2 (0.1 mM). Enzyme activity was measured by determining the reduction in the substrate absorbance at 290 nm using the extinction coefficient of 2.8 mM^{-1} cm^{-1}. One APX unit is the amount required per min for the decomposition of 1μmol substrate at 25 °C.

6.3.5 Determination of Yield and Quality Variables

The yield parameters in the fenugreek plant were quantified in terms of seed attributes. The total number of seeds per pod and pods per plant was calculated accordingly. The weight of 1000 seeds and seed yield were weighed using an electric balance and were expressed in g while the length of the pods was expressed in cm using a metric scale.

The quality of fenugreek seeds was determined in terms of alkaloid content. Seed alkaloid content was estimated by grounding 1 g of seed powder with methanol (80%) and magnesium oxide using a mortar and pestle. The mixture was incubated for 30 min at 60 °C, followed by centrifugation. The supernatant collected was

allowed to dry and then transferred to a flask. The Seed alkaloid content was calculated using the following equation:

$$\text{Seed alkaloid content } (\%) = [\text{weight of petri dish after evaporation } (\text{g}) - \text{weight of empty flask } (\text{g})]/\text{weight of seed powder } (\text{g}) \times 100.$$

6.3.6 Statistical Analyses and Graphics

SPSS-25.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis of the data. The standard errors were calculated, and analysis of variance (ANOVA) was performed on the data with five replicates to determine the least significant difference (LSD) between treatment means with the level of significance at $p \leq 0.05$. The graphs presented in the study were generated using SigmaPlot 12 (Systat Software Inc., California, USA).

6.4 Results

6.4.1 Exogenous GA_3 Supplementation Protected Growth and Development in Fenugreek Under Salinity

We observed that salinity reduced growth and development modules to a minimum in a dose-dependent manner. Nevertheless, plants supplied with 10^{-6} M GA_3 exhibited better vegetative growth over the control group, even under salt stress. Although gibberellin mitigated growth and development in both NaCl 50 mg L^{-1} and NaCl 100 mg L^{-1} stressed fenugreek, the optimum amelioration was observed with NaCl 50 mg L^{-1}. Plants under this concentration exhibited mitigation in shoot length (24.89%), root length (25.38%), shoot fresh weight (28.21%), shoot dry weight (15.70%), fresh root weight (20.18%), and root dry weight (16.22%) with the GA_3 application. Similarly, the average number of leaves $plant^{-1}$ and leaf area was increased with 10^{-6} M GA_3 sprays by 23.36% and 8.64%, respectively, over its salt-stressed counterpart (NaCl 50 mg L^{-1}) (Table 6.1).

6.4.2 GA_3 Treated Plants Exhibited Upregulated Photosynthetic Pigment and Assimilatory Enzymes Activities

Foliar sprays of 10^{-6} M GA_3 boosted photosynthetic pigment and the activities of enzymes pertaining to carbon, nitrogen, and sulphur assimilation. This concentration also assuaged salt-induced constraints through upregulating chlorophyll content by 19.81% in plants exposed to NaCl 50 mg L^{-1}. Similarly, the best amelioration of enzymatic activities through gibberellin supplementation was observed in NaCl

Table 6.1 Effect of exogenous 10^{-6} M GA_3 application on growth variables of fenugreek under salt stress

Treatments	Control	10^{-6} M GA_3	50 mM NaCl	100 mM NaCl	50 mM NaCl + 10^{-6} M GA_3	100 mM NaCl + 10^{-6} M GA_3
SL (cm)	34.23 ± 1.39b	47.87 ± 1.81a	27.12 ± 1.13c	22.92 ± 0.96d	32.87 ± 1.22b	26.56 ± 1.09cd
RL (cm)	15.60 ± 0.52b	20.76 ± 0.87a	11.98 ± 0.38cd	09.68 ± 0.22e	13.02 ± 0.51c	11.09 ± 0.29de
SFW (g)	07.50 ± 0.21b	11.09 ± 0.48a	05.21 ± 0.12c	04.15 ± 0.10d	05.68 ± 0.17c	05.01 ± 0.14c
SDW (g)	02.28 ± 0.07b	03.04 ± 0.09a	01.75 ± 0.06cd	01.56 ± 0.04d	01.89 ± 0.05c	01.69 ± 0.06d
RFW (g)	01.54 ± 0.12b	02.16 ± 0.24a	01.09 ± 0.09c	00.96 ± 0.05c	01.21 ± 0.10bc	01.07 ± 0.07c
RDW (g)	00.48 ± 0.02b	00.61 ± 0.03a	00.37 ± 0.02cd	00.31 ± 0.01e	00.41 ± 0.02c	00.35 ± 0.01de
ANL (per plant)	22.05 ± 1.69b	28.11 ± 1.95a	19.21 ± 1.23bc	14.06 ± 0.82d	21.43 ± 1.45b	16.66 ± 1.02cd
LA (cm2)	06.15 ± 0.18ab	06.61 ± 0.20a	05.79 ± 0.16bc	05.56 ± 0.15c	05.87 ± 0.16bc	05.69 ± 0.15bc

The table depicts a general eliciting trend of GA_3 on all the studied growth variables. The highest growth was observed with 10^{-6} M GA_3, while the maximised mitigation of salt stress was observed with its application on 50 mM NaCl treated plants. *SL* shoot length, *RL* root length, *SFW* shoot fresh weight, *SDW* shoot dry weight, *RFW* root fresh weight, *RDW* root dry weight, *ANL* average number of leaves, *LA* leaf area. Each value represents the mean ± SE ($n = 3$). Means followed by the same letter(s) do not differ by LSD test at 5% probability level ($p \leq 0.05$)

50 mg L^{-1}. The obtained data suggest an upregulation of 16.94% and 17.22%, in the activities of CA and NR, respectively, with exogenous gibberellin application in salt-stressed (NaCl 50 mg L^{-1}) fenugreek. Additionally, this degree of palliation was followed by gibberellin application in plants treated with NaCl 100 mg L^{-1} concentration (Fig. 6.1).

6.4.3 GA_3 Application Reduced Lipid Peroxidation and Electrolyte Leakage Through Upregulating Antioxidant Defence

Salinity-imposed substantial membrane damage through lipid peroxidation in a dose-dependent manner. The maximum membrane damage was observed with NaCl 100 mg L^{-1}, followed by NaCl 50 mg L^{-1} concentration. Salt stress-induced electrolyte leakage also followed a similar pattern through H_2O_2 overproduction. Exogenous application of 10^{-6} M GA_3 minimised the lipid peroxidation and electrolyte leakage in both NaCl concentrations. The optimum mitigation in TBARS content (16.36%) and H_2O_2 content (14.11%) was observed foliar application of GA_3 in salt-stressed (50 mg L^{-1}) fenugreek plants.

The antioxidant defence system in fenugreek plants was found hyperactivated in both stressful (with NaCl treatments) and assuaging environment (with GA_3 treatments). Plants under the highest salt concentration (100 mg L^{-1}) possessed the maximum activity for antioxidant enzymes to counter the induced damage. Nevertheless, the best amelioration in the activities of key antioxidant enzymes such as SOD (17.96%), CAT (13.02%), and APX (15.38%) was observed after the GA_3 application in NaCl 50 mg L^{-1} stressed plants. The content of proline, a key osmolyte, followed the analogous trend with the antioxidant enzymes. The proline content was increased by 18.88% with the 10^{-6} M GA_3 application compared to its salt-stressed (NaCl 50 mg L^{-1}) counterpart (Fig. 6.2).

6.4.4 Foliar Sprays of GA_3 Mitigated Salinity Induced Yield Constraints in Fenugreek

The fenugreek plants supplied with 10^{-6} M GA_3 treatments experienced maximum production. Although both concentrations of NaCl (50 and 100 mg L^{-1}) restricted crop yield significantly ($p \leq 0.05$), the best placation of this reduction was observed in 50 mg L^{-1} NaCl-stressed plants with foliar GA_3 supplementation. This treatment exhibited an increment of 62.90%, 38.27%, and 30.92% in seed yield, alkaloid content, and pod length, respectively. Moreover, a similar enhancement was observed in seeds pod^{-1} (11.75%) and pods $plant^{-1}$ (25.25%) (Table 6.2).

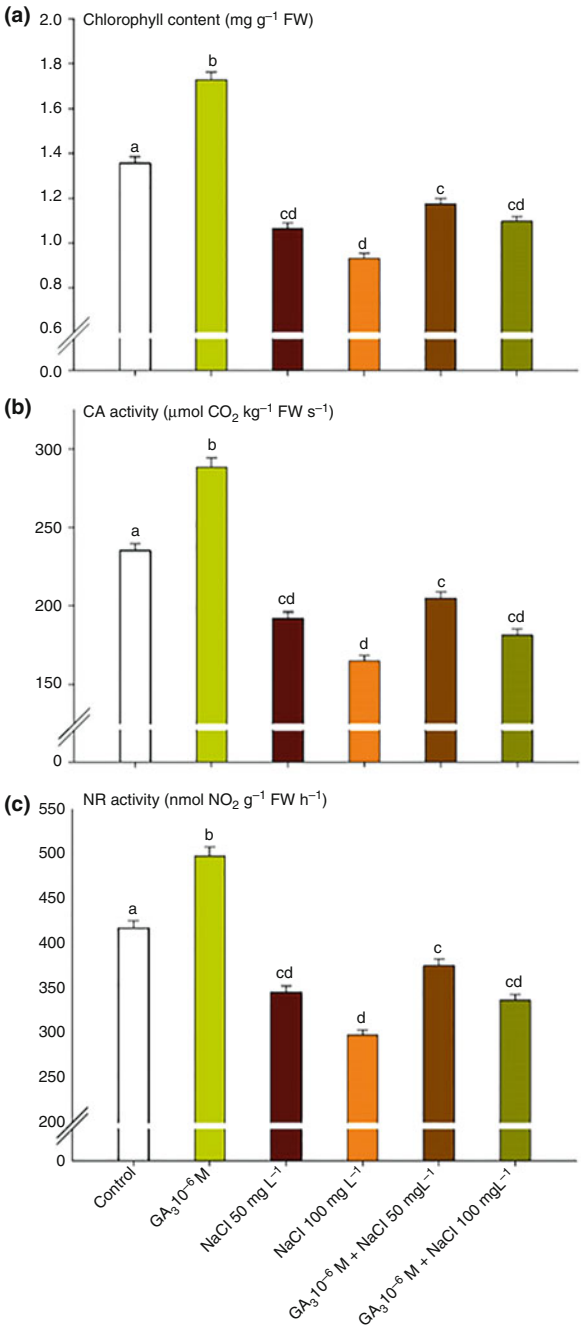

Fig. 6.1 Foliar sprays of 10^{-6} M GA_3 enhanced photosynthetic pigment and enzymatic activities in fenugreek plants under salinity stress. Although both the salt concentrations restricted photosynthetic pigment content and activities of CA and NR, the maximum reduction was observed with 100 mg L^{-1} NaCl treatment. 10^{-6} M GA_3 upregulated chlorophyll content as well as the enzymatic activities in fenugreek. Foliar sprays of GA_3 also mitigated salinity induced restrictions in chlorophyll content (**a**) and the activities of CA (**b**) and NR (**c**). *CA* carbonic anhydrase, *NR* nitrate reductase. Each bar represents the mean ± SE ($n = 3$). Means followed by the same letter (s) do not differ by LSD test at 5% probability level ($p \leq 0.05$)

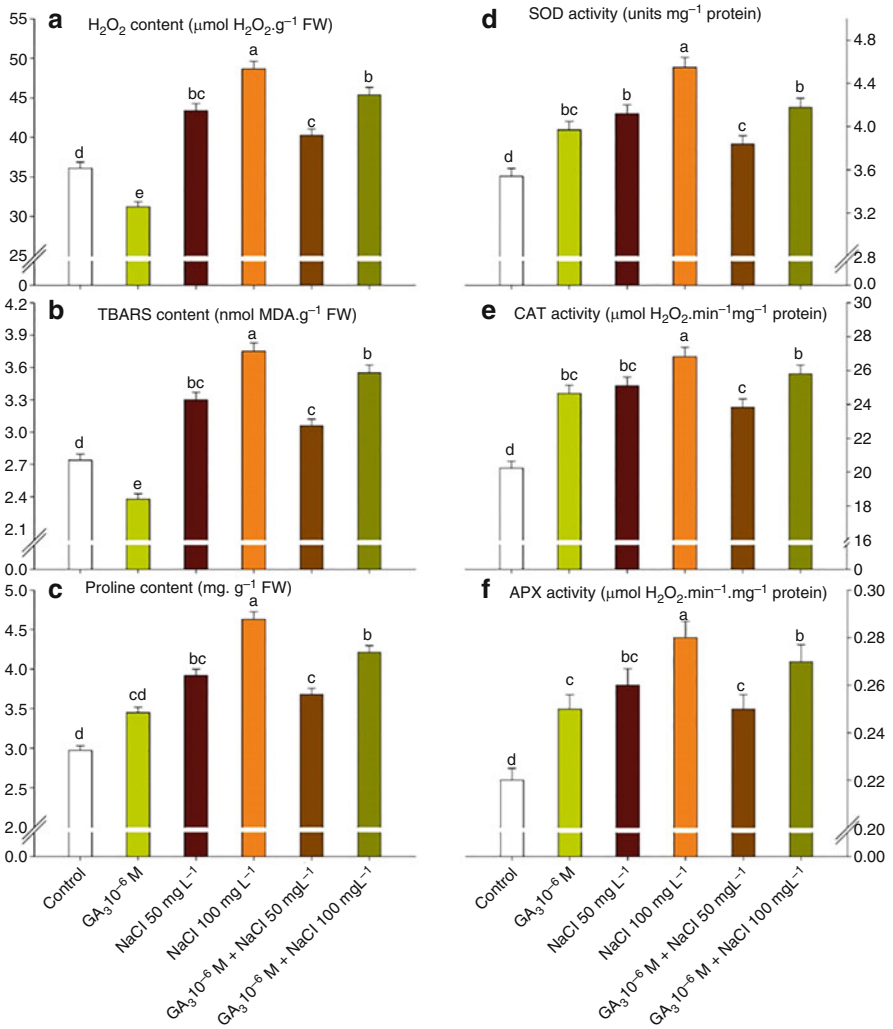

Fig. 6.2 Effect of 10^{-6} M GA_3 application on the oxidative damage marker and antioxidant metabolism in the fenugreek plants under salinity stress. The marker for oxidative damage, i.e., H_2O_2 (**a**) and TBARS (**b**) contents were significantly ($p \leq 0.05$) reduced with 10^{-6} M GA_3 application. Moreover, this treatment also enhanced osmoprotectant content, i.e., proline (**c**) and the activities of antioxidant enzymes, e.g., CAT (**d**), SOD (**e**), and APX (**f**) when applied alone. Additionally, the same treatment substantially ameliorated salinity induced oxidative stress and helped maintain cellular homeostasis. *H_2O_2* hydrogen peroxide, *TBARS* thiobarbituric acid reactive substances, *SOD* superoxide dismutase, *CAT* catalase, *APX* ascorbate peroxidase. Each bar represents the mean ± SE ($n = 3$). Means followed by the same letter(s) do not differ by LSD test at 5% probability level ($p \leq 0.05$)

Table 6.2 The effect of exogenous GA_3 application on production modules of fenugreek under salt stress

Treatments	Control	10^{-6} M GA_3	50 mM NaCl	100 mM NaCl	50 mM NaCl +10^{-6} M GA_3	100 mM NaCl +10^{-6} M GA_3
Seed yield (g)	01.12 ± 0.08^{b}	01.67 ± 0.13^{a}	00.62 ± 0.05^{c}	00.43 ± 0.03^{c}	01.01 ± 0.08^{b}	00.59 ± 0.05^{c}
No. of seeds plant^{-1}	128.49 ± 1.53^{b}	187.26 ± 2.18^{a}	105.66 ± 1.23^{c}	90.32 ± 1.07^{d}	125.32 ± 1.48^{b}	102.27 ± 1.17^{c}
No. of pods plant^{-1}	09.12 ± 0.36^{b}	11.55 ± 0.41^{a}	07.88 ± 0.27^{c}	06.55 ± 0.23^{d}	09.87 ± 0.33^{b}	08.33 ± 0.29^{bc}
Pod length (cm)	09.07 ± 0.27^{b}	12.33 ± 0.29^{a}	06.92 ± 0.26^{d}	05.19 ± 0.25^{e}	08.86 ± 0.27^{b}	07.79 ± 0.26^{c}
Seed alkaloid content (%)	05.36 ± 0.24^{b}	07.11 ± 0.31^{a}	03.92 ± 0.17^{c}	03.21 ± 0.15^{d}	05.42 ± 0.21^{b}	04.17 ± 0.21^{b}

The mean values suggest promoting effects of 10^{-6} M GA_3 on all the production modules that were evaluated during the present study. This treatment substantially reversed salinity induced yield constraints in the fenugreek. Each value represents the mean ± SE ($n = 3$). Means followed by the same letter(s) do not differ by LSD test at 5% probability level ($p \leq 0.05$)

6.5 Discussion

Soil salinity is one of the leading abiotic stresses that can restrict plant growth and yield to a minimum (Zelm et al. 2020). Salt stress can cause damage depending on certain variables, including salt concentration and plant adaptability (Munns and Tester 2008). Fenugreek being a salt-sensitive crop afflicts severe oxidative damage on the advent of salinity (Mickky et al. 2019). In the present study, different salt concentrations (50 and 100 mg L^{-1}) reduced growth variables related to plant length, weight, and leaves. A similar impact was observed in yield variables of fenugreek plants. Seed production, pod length, and alkaloid content were all restricted with increasing salinity concentration. These alterations are understandable as salinity limits water uptake, stomatal conductance, and mineral uptake that negatively influence plant water relation and source-sink potential (Vetrano et al. 2020). As a result, plants experience retarded growth and yield, as were observed in our study. Notably, salinity could reduce GA_3 biosynthesis, and improving plant GA_3 status through exogenous gibberellin supplementation could have upregulated cell expansion, leaf area, and stem length, resulting in improved growth and productivity (Wang et al. 2019). Additionally, GA_3 has also been attributed as a growth elicitor for various crops (Khan et al. 2006; Vetrano et al. 2020). We perceived similar promoting activity of GA_3 in the fenugreek as well. Exogenous application of GA_3 could reverse salinity drawn growth and production constraints through improving water and ion uptake besides stomatal adjustments (Javid et al. 2011). Additionally, GA_3 might promote leaf expansion and shoot elongation, contributing to the growth and yield enhancements in fenugreek (Ueguchi-Tanaka et al. 2007).

In the present study, we observed limited chlorophyll content in salt-stressed plants. The higher salt concentration had a more severe effect on chlorophyll synthesis. Salinity can achieve such an effect by destabilising the chlorophyll-protein complex through chlorophyll oxidation or damaging the enzymes that synthesise chlorophyll (Wang et al. 2019). Additionally, salinity could induce chlorophyllase synthesis in the mesophyll cells. Chlorophyllase is a proteolytic enzyme with chlorophyll digesting potential and could also disrupt the photosynthetic machinery and, thus, regulates the chlorophyll content (Tuna et al. 2008). This view is also shared by other plant physiologists who made similar observations in different crops during salt stress. Salinity induced Na^+ accumulation increases the osmotic potential that could disrupt the photosynthetic electron transport system and damage the chloroplast. Salt stress could restrict photosynthetic enzymes, gaseous exchange, and poses structural and functional threats to the thylakoid membrane (Hendawey 2015). The low chlorophyll content is generally considered as a disadvantage, but interestingly, plants could utilise the same phenomenon to protect the photosynthetic electron transport system from over reduction, and thus, reducing the ROS production (Belmecheri-Cherifi et al. 2019). Gibberellin could enhance the ultra-structural morphogenesis of plastids and chlorophyll retention (Ahmad 2010).

Salt stress influenced the activities of CA and NR in a dose-dependent manner causing significant reduction at the highest salt concentration. Carbonic anhydrase

(CA) is a metal-containing ubiquitous enzyme that catalyses the reversible conversion reaction of CO_2 and H_2O to bicarbonate ions and plays an essential role in stomatal conductance and carboxylation (Naeem et al. 2020). The salinity reduces CA activity by restricting stomatal conductance and CO_2 fixation and subsequently reducing CO_2 availability for CA (Singh et al. 2016). Additionally, salinity induced ion excess could make it harder for the plant to uptake other ions and minerals. In this context, salinity could downregulate CA activity by reducing Zn uptake, which is a crucial component of CA (Chakraborty et al. 2016; Singh et al. 2016). Exogenous application of GA_3 overcame the constraints in CA activity under salt stress. This upregulation could be because of the positive influence of GA_3 on stomatal conductance and CO_2 metabolism (Ribeiro et al. 2012). Moreover, GA_3 is also attributed to improve plant water relations and minerals uptake (Ueguchi-Tanaka et al. 2007; Wang et al. 2019). As a result, we observed enhancement in CA activity with GA_3 supplementation. Several studies are in harmony with our observation, where GA_3 application enhanced CA activity in different plants under salt stress (Afroz et al. 2006; Siddiqui et al. 2008; Tuna et al. 2008).

Salt stress also inhibited the NR activity, a crucial enzyme associated with nitrate metabolism, facilitating protein synthesis at various stages of plant growth (Afroz et al. 2006). Given the salinity interfere with the mineral uptake and assimilation, decreased NR activity could be an outcome of salt stress-induced restriction in the nitrogen and sulphur uptake (Nazar et al. 2011; Chakraborty et al. 2016). Moreover, salt stress might also enhance the activity of DNase, RNase, and protease enzymes that could affect NR activity negatively (Siddiqui et al. 2008). However, the GA_3 application curbed the salt-induced effects on NR in the present study and enhanced its activity. Previous reports favour these correlations, where the similar eliciting pattern in NR activity was observed under salt stress with phytohormones action (Eleiwa et al. 2011; Iqbal et al. 2014).

The ROS overproduction and ion accumulation are the chariots that enable salinity to interfere with plant metabolism (Munns and Tester 2008). While ROS poses an osmo-oxidative threat in the fenugreek system, ion accumulation could regulate membrane permeability, stomatal conductance, and the uptake of other ions (Belmecheri-Cherifi et al. 2019). Fenugreek has antioxidant and osmolyte defence to counter such physiological complications to a certain extent (Mickky et al. 2019). Foliar sprays of GA_3 were noticed to enhance antioxidant generation in fenugreek plants compared to the control treatment. In this context, Maggio et al. (2010) suggested that GA_3-induced protein synthesis could have increased antioxidant enzymes production and upregulated their activities. However, salt severity might exceed the threshold defence capacity and cause severe damage at higher concentrations, as was observed in our study with 100 mg L^{-1} of NaCl. It could be noticed that although at this dose, the activities of SOD, CAT, and APX were increased, the natural biochemical defence of the plant was failing to tolerate salinity effectively. Nevertheless, the exogenous application of GA_3 might have ameliorated cellular stress by relieving antioxidant hyperproduction, as suggested by comparing salt-stressed fenugreek with GA_3 supplied plants under salt stress. This could

probably be the reason for GA_3 induced reduction in antioxidant content in salt-stressed fenugreek.

High TBARS and H_2O_2 contents directly intoxicate the plasma membrane and negatively influence cytosolic metabolism through their cell structure breaking tendency (Wang et al. 2019). Thus, from the data, we can conceive an increased lipid peroxidation and electrolyte leakage in salt-stressed fenugreek plants. Nevertheless, the foliar supplementation of GA_3 ameliorated salinity-imposed lipid peroxidation and electrolyte leakage, as indicated by lower TBARS and H_2O_2 contents. The antioxidant defence could likely have scavenged H_2O_2, while the osmolytes such as proline might have helped maintain membrane permeability through osmotic adjustments. In this context, it is noteworthy that plants can accumulate a very high concentration of organic and inorganic osmolytes and use them as a defencive measure against various stress conditions. The present study offered the same analogy with proline, where the highest proline content was found with the most severe salt stress. Apart from being an osmolyte, proline could also act as a storage house offering carbon and nitrogen for plant defence and development in dire situations (Matysik et al. 2002). Different studies have made similar observations where fenugreek accumulated proline besides other osmolytes in response to salinity exposure (Nair et al. 2017; Mickky et al. 2019). Additionally, proline can also assist the plant in cell expansion, pH homeostasis, and membrane stabilisation (Mickky et al. 2019). Interestingly, gibberellin seems to increase as well as decrease the proline content depending on the stress presence (Tuna et al. 2008). In the absence of stress, gibberellin enhances proline content while in stressed plants, it reduces proline content, probably by inhibiting lipid peroxidation (Tuna et al. 2008; Javid et al. 2011).

6.6 Conclusion and Future Perspectives

Fenugreek is an aromatic plant with substantial pharmacological potential. However, the growth, development, and yield of fenugreek crop are greatly restricted under salt stress. Increasing soil salinity is posing an imminent threat to fenugreek along with other agronomic crops. In the present study, we suggested an alternative for the salinity reversal through the exogenous supplementation of 10^{-6} GA_3 in the fenugreek crop. This treatment was proved beneficial to a broad spectrum of morpho-physiological parameters in the fenugreek plants. Plant length and weight were elicited with this treatment as well as the seed number and yield were also enhanced. Plant sprayed with GA_3 experienced better tolerance against salinity, and thus, salt concentration was unable to pose more significant damage in such plants than those without GA_3. Moreover, such plants exhibited an upregulated antioxidant defense and osmoprotection. Overall, the present investigation suggested that crop enhancement in fenugreek could be conferred through exogenous application of GA_3 in the plants under salinity stress. Considering the eliciting effects of GA_3 in this study, similar treatments could also be instigated for conferring stress tolerance in other related crops with agronomical potential.

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Various Diseases Incidence on Fenugreek Crops and their Management

7

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Abstract

Fenugreek (*Trigonella foenum-graecum*) is an important not only as a vegetable but also for spice. However, fungal, bacterial, viral, nematodes and insect-pest diseases are major constraints to reduce crop yield of fenugreek. The production and quality of the fenugreek are seriously affected due these diseases. Various management strategies such as cultural, botanical, rhizobacteria and bio control agents, integrated pest management are applying across the world to combat the diseases. The indiscriminate use of chemicals to control fenugreek pathogens is not environment friendly. Use of new resistant varieties, advanced biotechnological methods and resistance inducers are utmost importance for better control of fenugreek diseases. The present chapter summarizes the current knowledge on various fungal, bacterial, viral, nematodes and insect-pest diseases incidence in fenugreek crop and its management strategies.

Keywords

Fenugreek · Diseases · Management · Crop yield

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7.1 Introduction

The plant diseases are of paramount importance to humans because they damage plants and plant products on which human depend for food, clothing, furniture, the environment and housing. The kinds and amounts of losses caused by plant diseases vary with the plant or plant product, the pathogen, the locality, the environment, the control measures practiced and combinations of these factures. The quantity of loss may range from slight to 100%. (Agrios 2005). The fenugreek is attacked by several fungi, bacteria, viruses and nematodes causing serious diseases resulting yields (Khare et al. 2014). Diseases are the major constraints to reduce crop yield of fenugreek. They attack at different stages of growing plant. Fenugreek (*Trigonella foenum-graecum)* is an herbaceous annual plant belongs to the family Fabaceae. It is widely cultivated for its leaves as vegetable and seeds as condiments in India, China, northern and eastern Africa, parts of Mediterranean Europe, Argentina and Australia (Acharya et al. 2006). In India, approximately 90,000 metric tons of fenugreek is produced from a total 66,000 cultivated hectares annually (Rani and Hegde 2017). The average yield of fenugreek crop is, however, low due to its susceptibility to various fungal, bacterial, viral, aphids, insect-pest diseases and lack of new resistant varieties and advanced biotechnological methods. There is world-wide trend to search for eco-friendly alternative strategies or the management of fungal, bacterial, viral, nematodes and insect-pest diseases for better productivity of fenugreek crop. Besides using cultural, botanical, rhizobacteria and biocontrol agents in crop protection, use of plant resistant inducers such methyl jasmonate, salicylic acid, chitosan are gaining importance as the substitutes to chemical fungicides and also their ability to stimulate the plant's own resistance mechanism. Plant resistant inducers do not have any pesticidal and antibiotic activity and are better options in fenugreek disease management.

7.2 Methodology

The information on various fungal, bacterial, viral, nematodes, insect-pests and their causal organisms, symptoms, disease incidence and various disease managements strategies of fenugreek crop are collected and compiled from the available literature.

7.2.1 Fenugreek Diseases and Their Management Strategies

Generally, fenugreek diseases are classified into biological and physiological diseases on basis of their pathogenicity. The mineral deficiencies are responsible for physiological diseases. For example, chlorosis of fenugreek plants is associated with the deficiencies of boron, magnesium, manganese or potassium. The fungal, bacterial, viral diseases and insect-pest infections are the biological diseases. All these diseases have been reported to be responsible for damaging the forage and ultimately lowering seed yield in fenugreek. This is a serious concern for fenugreek

production. The knowledge on various diseases incidence on fenugreek crops and management strategies are utmost importance for increasing productivity of fenugreek.

7.3 Diseases

7.3.1 Fungal Disease

Cercospora Leaf Spot

Causative Agent *Cercospora traversiana* is a member of Ascomycetes. It is a serious disease causes considerable heavy loss. This disease has been reported in India, Australia, European countries, North America and South America. Khare et al. (1981) have described a new species of *C. foeneum graceum* causing fenugreek disease.

Symptoms The spots are developed on leaves which are circular, white and sunken. The necrotic areas with encircled with chlorotic halos spot. Spots are seen on stems and pods. Young leaves wilted and die in severe infection. The high severity of disease causes fallen leaves leading to a reduction of the number of leaves. Infection at flowering and fruit setting stage, damages of the pod, and reduction of seed number, finally affect the yield. Disease spreads through infected seeds.

Management Prasad et al. (2014) have identified L3717 and P1138687 resistant and moderately resistant (F86 and L3698) accessions for breeding programs to develop Cercospora leaf spot resistant cultivars, use of certified seeds or pre-treatment of seeds with fungicides reduced the disease severity. The crop rotation program can be used for reduction of pathogen inoculum and severity in the field.

Charcoal Rot

Causative Agent *Macrophomina phaseolina* It is an ascomycetes (Botryosphaeriaceae) fungus. It has wide host range and affects beans, tobacco, soybean, pigeon pea and many other crops. The disease is primarily spread through microsclerotia.

Symptoms Wilting and drooping of leaves from plant, black discoloration of stem, root roting, development of plenty of small black sclerotia (fruiting bodies) in infected parts.

Management Organic soil amendments such as neem cake or manure could be used for the reduction of pathogen inoculum in the soil. Amending soil with well-aged compost and neem meal or cake might lower and suppress the development of the pathogen to some extent.

Powdery Mildew

It is one of the serious diseases of fenugreek affecting both biomass and yield under moist agro-climatic conditions in North America, such as Creston in British Columbia (Canada) and Vermont (USA). Also, commonly found in hot and humid tropical, sub-tropical, temperate to subtemperate regions. Powdery mildew caused by *E. polygoni* found in Israel, India, Ethiopia and England. Whereas, *L. taurica* found in Israel and *Oidiopsis* sp. in Israel, Ethiopia and England as pathogens.

Causative Agent *Erysiphe polygoni*, *Leveillula taurica.*

Symptoms White to grey powdery spots or patches are formed on the leaves. These powdery spots or patches contain numerous conidia and seen on both the sides of leaf. In case of severe condition, spots or patches coalesce (Fig. 7.1). The symptoms seen on all the plant parts except root. Due to disease, seed yield greatly reduced. On the young leaves, *Oidiopsis* sp. infections exhibit blister like areas which later on covered with white to grey powdery mass. In case of *Leveillula taurica* fungus, powdery mildew is identified by small black cleistothecia along with mycelia mass formed on leaves at crop maturity. Disease is favoured by dry weather conditions.

Fig. 7.1 Image showing powdery mildew disease incidence on fenugreek leaf

Management Penconazole fungicide is most effective against powdery mildew (Dhruj et al. 1996); Hissar Suvarna, Hissar Madhuri and Rmt-305 are tolerant; Rmt-1 and Lam selection-1 are tolerant to powdery mildew disease (Malhotra and Vashishtha 2008), organic amendment of soil with neem cake reduced powdery mildew; use of *Pseudomonas fluorescens,* spray of neem seed kernel extract and neem cake to manage the disease (Chhata and Verma 2010), non-systemic fungicide wettable sulphur, systemic fungicide propiconazole and ready-mix of fungicide tebuconazole + trifloxystrobin found best in vitro against powdery mildew (Marakna et al. 2020)

Downy Mildew

Causative Agent *Peronospora trigonellae.*

Downey mildew of fenugreek was reported from Algeria, India, Pakistan and the United Kingdom. The first report of downy mildew occurrence was from California and the USA and India. Generally, disease occurs during February and March.

Symptoms The upper surfaces of leaves showed yellow patches and greyish violet growth on the corresponding lower surfaces. The greyish cottony mycelia growth known as downy growth. During flowering and pod formation, downy mildew infection is severe. The plants also showed stunted growth due to disease (Fig. 7.2).

Management For Management of this disease, the use of resistant cultivars can help to prevent the incidence and damage the crop example Hisar Mukta and Hisar Suvarna are resistant and moderately resistant cultivars used to control downy mildew in-field disease (Malhotra and Vashishtha 2008), The foliar application of biocontrol agent *Pseudomonas fluorescence* or extract of neem seed kernel for manage this disease (Chhata and Verma 2010).

Collar Rot/Root Rot/Foot Rot

Fusarium root rot

Ramteke et al. (2019a) have first reported *Fusarium solani* causing root rot of fenugreek in India.

Causative Agent *Fusarium solani.*

Symptoms The infected plants showed gradual yellowing and defoliation of the lower leaves, wilting and stunted growth, the infected roots are brown with decayed cortical tissues that shrivelled and resemble a thread (Fig. 7.3).

Management Chitosan @ 15 mg/ml completely inhibited and methyl jasmonate @ 20 mM reduced *F. solani* in vitro growth (Ramteke 2019), root rot severity caused by *F. solani* reduced due to salicylic acid @ 5 mM concentration in pot condition (Ramteke et al. 2019b).

Rhizoctonia Root Rot

It belongs to Basidiomycetes. It has been reported throughout India and causes economic loss to fenugreek.

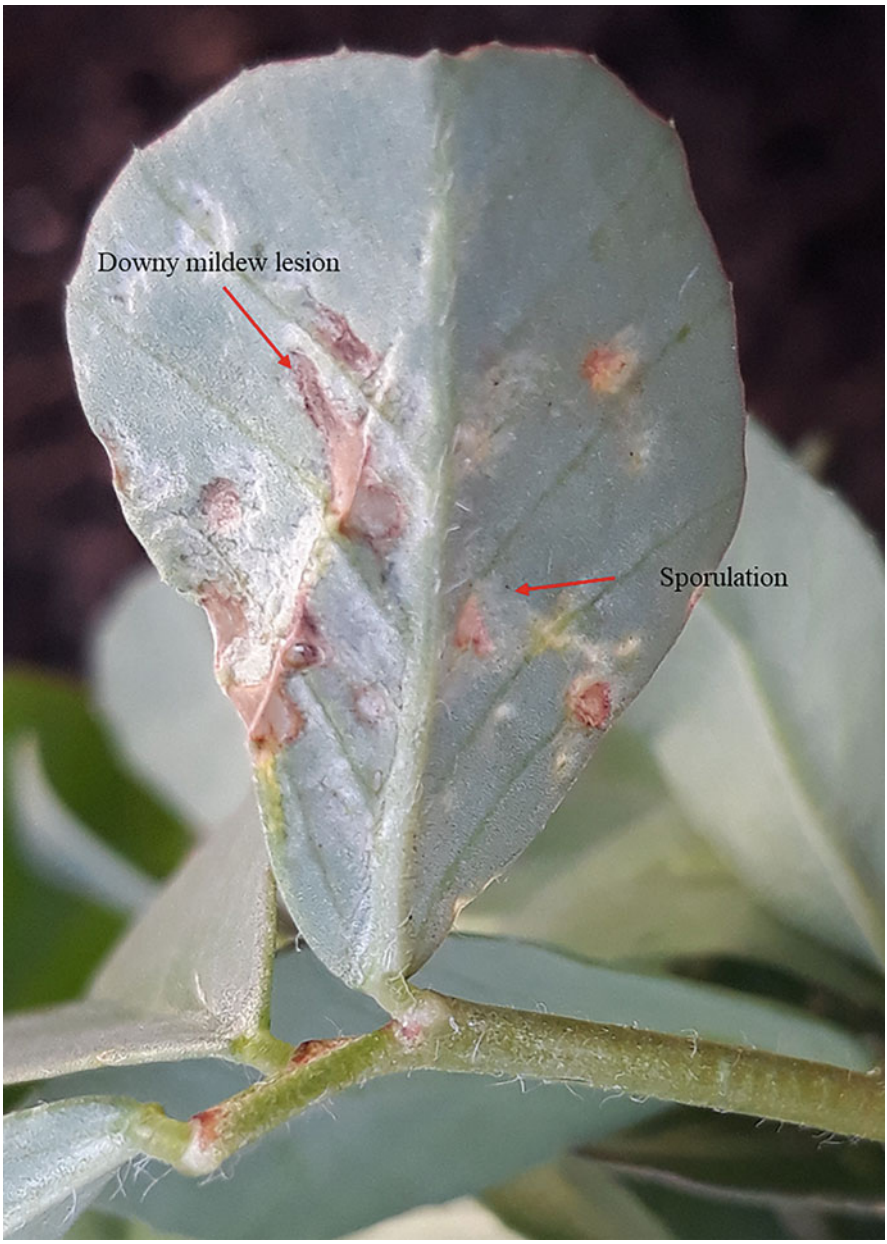

Fig. 7.2 Image showing downy mildew disease incidence on fenugreek leaf

Fig. 7.3 Image showing root rot disease of Fenugreek. Left side disease incidence in field study and right side in growth of *Fusarium solani* on radicle of germinated fenugreek seeds

Causative Agent *Rhizoctonia solani.*

Symptoms The infected plants showed yellowing and drying of foliage, discolouration and rotting of roots, infected roots easily removed, death of entire plants occur in severe condition, also infected to seed and hypocotyls.

Management Seed treatment with *Trichoderma viride* @ 4 g/kg + soil application of *T. viride* @ 5 kg/ha is a cost effective, eco-friendly management against *Rhizoctonia* root rot (Muthulaxmi et al. 2010), seed treatment with carbendazim is effective for managing root rot (Singh and Rao 2015), two fenugreek varieties, viz. Azad methi and AM-2 are highly resistant against *Rhizoctonia* root rot (Meena et al. 2020)

Spring Black Stem and Leaf Spot

This disease was first reported by Bretag and Cunnington (2005) from Rupanyup in the Wimmera region of Victoria, Australia.

Causative Agent *Phoma pinodella.*

Symptoms Infected leaves, petioles and stems showed numerous small, irregular, dark brown to black spots surrounded by small yellow zones, leaves turn completely

yellow in colour in severe infection, stunted growth of plant, tap roots also develop black lesions.

Management No perfect control measures are available. However, use of resistant varieties minimized the incidence.

Fusarium Wilt

Causative Agent *Fusarium oxysporum.*

Fusarium wilt is one of the most important diseases causing moderate to extensive damage to fenugreek plants. It is a member of ascomycetes and occurs in soil and in seeds of fenugreek. This disease was first reported by Shivpuri and Bansal (1987) from Rajasthan, India.

Symptoms Infected plants showed yellowing of lower leaves, defoliation, stunted growth, drooping and wilting of mature leaves, plants wilted and die at seedling stage upon infection, browning of vascular tissue occurs when wilted plants split longitudinally.

Management Plant growth promoting rhizobia obtained from root nodules of fenugreek strongly inhibited the growth *of F. oxysporum*, a wilt pathogen of fenugreek (Kumar et al. 2011), seed treatment with Carbendazim 50 WP @ 1.5 g/kg seed and neem leaf extract @ 5 ml/10 are effective against *F. oxysporum* infection (Khokhar et al. 2012). The combination of carbendazim 12% + mancozeb 63% was found superior against *Fusarium* wilt under pot condition (Bhimani et al. 2018), garlic extract and Jeevamrutha (slurry) very effective in vitro and also carbendazim and combination of carbendazim 25% + mancozeb 50% in vivo managing disease completely (Rani and Hegde 2016), four genotypes DFC-3, DFC-8, DFC-27 and DFC-29 are moderately resistant against *Fusarium* wilt (Rani et al. 2017).

Pod Spot

This disease was first investigated and described by Petropoulos (1973) in fenugreek.

Causative Agent *Heterosporium medicaginis.*

Symptoms Disease visible at the third stage of pod development as dark brown to black spots and spread to produce a dark olive, velvet like cover; at initial stage spots infection elongate transversely to the pod axis and then spread over pod surface as rounded to oblong lesions, the spots also visible on the stems but rarely on the leaves.

Management Use of resistant varieties, hot water treatment of seeds effective before sowing as pathogen enter into seeds through threshing.

Ascochyta Leaf Spot

It is a serious seed borne disease of fenugreek. Ascochyta leaf spot is a serious disease afflicting fenugreek. It is one of the important seed-borne diseases in the fenugreek crop.

Causative Agent The fungal pathogen is found out to be *Ascochyta* sp. a member of the Ascomycetes.

Symptoms The infected leaves show irregular brown to black spots with distinct margins. As the disease progresses, the leaves on the plant may die and fall off. Infected seeds show round, dark brown lesions. The seedlings from infected seeds start rotting from the point of seed attachment (Fig. 7.4). The rotting advances towards the stem and taproot; followed by the death of the young seedlings. The pathogen attacks the leaves, stems and pods of fenugreek, reducing both yield and quality of the crop severely. The fungus overwinters in soil, infected seed and on remnant crop residues. The dissemination of the pathogen occurs by both wind and rain splash. Cool, moist weather is favourable for rapid dissemination and vigorous growth of the fungus.

RUST

Rust is a minor disease of fenugreek belongs to subdivision Basidiomycotina.

Fig. 7.4 Image showing leaf spot disease of fenugreek

Causative Agent *Uromyces trigonellae.*

Symptoms Rust appears as brown coloured small pustules (uredia) on the ventral surface of the leaves and petioles, during early summer yellowish to dark brown coloured telia developed on the leaves, growth of plant stunted.

Management Crop rotation, removal of infected leaves and crop debris, avoid the much use of nitrogen for management of this disease.

Damping-Off

Causative Agent ***Pythium aphanidermatum.***

Symptoms The infected seedling stem appeared water-soaked lesions, rotting of stem at collar region and seedling collapse down (Fig. 7.5).

Management Removal of disease plants should be done to minimize the source of infection, seed treatment with carbendazim is effective to manage disease, use of resistant varieties also effective.

7.3.2 Bacterial Disease: List of Bacterial Diseases

Bacterial Leaf Blight/Spot

A bacterial leaf blight disease was first reported by Mc Cormik and Hollaway (1999) from Australia and leaf spot disease by Fogg et al. (2000) from New Jersey, USA.

Pathogen *Pseudomonas syringae* pv. *syringae.*

Symptoms Angular to circular spots with yellow uniform halos (Fig. 7.6).

Management Seed treatment with chitosan, bleaching powder and foliar application of Streptomycin, and copper oxychloride.

7.3.3 Viral Disease: List of Virus Diseases

Mosaic

Mosaic wilt virus in fenugreek was described by Bhaskar and Summanwar (1982). Alfalfa mosaic virus, bean mosaic virus, clover vein mosaic virus cow pea mosaic virus, pea mosaic virus, potato virus A and Y and soybean mosaic virus have also been reported in fenugreek (Petropoulos 2002). Beet western yellows virus infecting fenugreek was first reported by Kumari et al. (2005) from Yemen. Leaf curl disease on fenugreek was first reported by Swarnalatha et al. (2019) from India.

Fig. 7.5 Damping-off disease of fenugreek

Fenugreek Yellows Disease

Pathogen Beet western yellows virus.

Symptoms Yellowing and mottling of the leaves, the growth of the plant stunted

Leaf Curl Disease

Pathogen Ageratum enation virus.

Symptoms Curling and yellowing of the leaves.

Mosaic Wilt Virus Disease

Symptoms Vein clearing, shedding of leaves, flowers and fruits, curling of the leaves at the margin, reduction in leaf size, disease is transmitted by aphid.

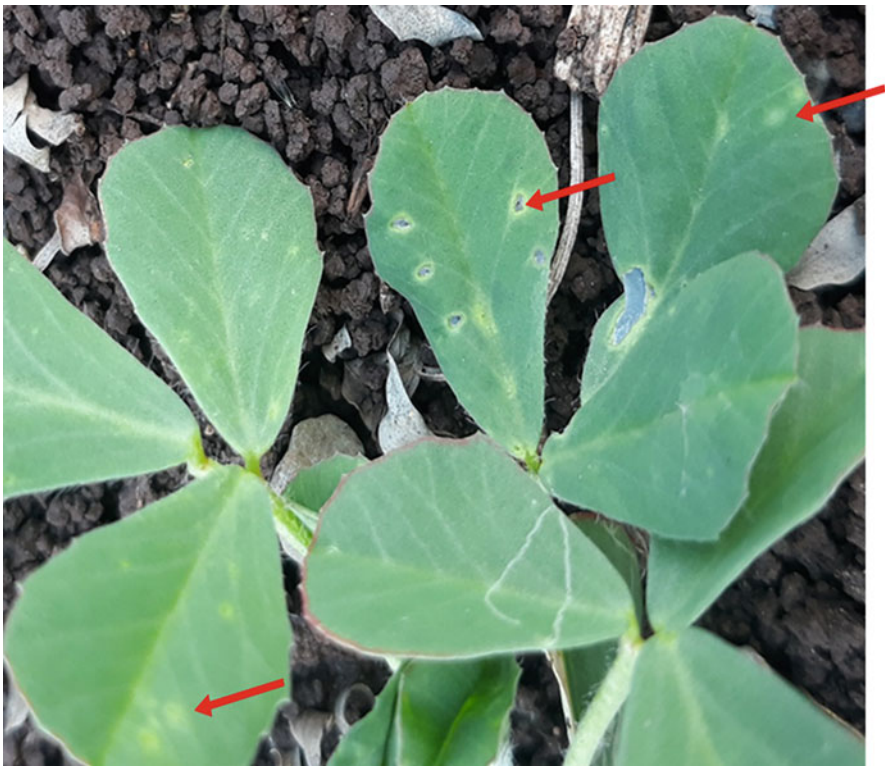

Fig. 7.6 Bacterial leaf spot of fenugreek

D. Nematode. The several parasitic nematodes such as *Meloidogyne incognita*, *M. javanica, M. hapla, M. arenaria Rotylenchulus reniformis*, *Helicotylenchus indicus* and *Tylenchorhynchus brassicae* are attacked the fenugreek crops.

Causative Agent *Meloidogyne incognita*, *M. javanica, M. hapla, M. arenaria Rotylenchulus reniformis*, *Helicotylenchus indicus* and *Tylenchorhynchus brassicae.*

Symptoms The presence of galls and rootknot on infected plants, stunted growth, wilting and death of immature plants. Due to nematode infection, induction of susceptibility of plant to other pathogens.

Management Use of resistant varieties such as Rmt- 305 (Malhotra and Vashishtha 2008) and UM-72, UM-178 (Moh Tariq et al. 2016) reduced the nematode infections. Application of *Argemone mexicana*, *Calotropis procera*, *Solanum xanthocarpum* and *Eicchornia echinulata* botanicals also controlled the populations of nematodes (Tiyagi et al. 2010).

Fig. 7.7 Image showing aphids attack on fenugreek leaf

7.3.4 Insect-Pest

Aphids The fenugreek is attacked by different species of aphids, viz. *Aphis craccivora* and *Acyrthosiphon pisum.*

Symptoms The aphids secreted a sticky, sugary substance called honeydew which helps the growth of sooty mould on the fenugreek. In severe attack of aphids causes distortion, yellowing, necrosis of leaves and finally stunted the growth. The aphids generally attack the fenugreek crop at flowering stage and suck the sap from tender leaves and flowers affecting the growth of plant (Figs. 7.7, 7.8, and 7.9).

Management Use of tolerance varieties; in severe infections, insecticides are used, insecticidal soaps or neem/canola oil are generally the best method of aphids control,

Fig. 7.8 Image showing leaf minor pest attack on leaf of fenugreek

use of *Pseudomonas fluorescens,* spray of neem seed kernel extract and neem cake to manage the disease (Chhata and Verma 2010),

Insects Fenugreek was prone to attack of 37 insect species of Lepidoptera, Coleoptera, Orthoptera and Hemiptera orders, of which 35 were new records on fenugreek. The insects *Agrotis segetum, Utetheisa pulchelloides, Amata passalis, Acanthodelta janata Euproctis* sp. and four bugs on species, viz., *Chrysocoris stollii, Dysdercus similis, Eysarcoris ventralis* and *Bagrada hilaris* were recorded for the first time on fenugreek. *Brumoides suturalis, Cheilomeness exmaculata, Illeis cincta* and *Coccinella transversalis* lady bird beetle species noticed on fenugreek (Manjula et al. 2015).

Management Emamectin benzoate 5SG @ 0.25 g/L, indoxacarb 15.8 EC @ 0.25 ml/ L and fipronil 5 Sc @ 1 ml/L were found to be effective in reducing larval population and foliar damage of fenugreek plants (Manjula and Kotikal 2018).

Fig. 7.9 Caterpillar attack on fenugreek

7.4 Conclusion

Fenugreek is cultivated in different countries as vegetable and spice as well. The diseases are adversely affecting fenugreek crop results in low productivity. Book chapter provides current knowledge on all fungal, bacterial, viral, aphids and insect-pests diseases of fenugreek with special reference to their causal organisms, symptoms and management strategies. The cultural, botanical, rhizobacteria, bio control agents, integrated pest management are applying across the world to combat the diseases. In addition to these, use of new resistant varieties, advanced biotechnological methods and resistance inducers as fungicide alternatives is utmost importance for better control of fenugreek diseases.

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8

Various Mitigation Approaches Applied to Confer Abiotic Stress Tolerance in Fenugreek (*Trigonella foenum-graecum* L.): A Review

Rukhsar Parwez, Aarifa Nabi, Mohammad Mukarram, Tariq Aftab, M. Masroor A. Khan, and M. Naeem

Abstract

Trigonella foenum graecum L. contains several nutritionally and pharmaceutically important bioactive constituents particularly trigonelline and diosgenin and imparts immense economic value to the plant making it a promising medicinal legume. Fenugreek bears anti-diabetic, anticancerous, anti-inflammatory, analgesic, emollient, laxative, antihypercholesterolemic, and anti-spasmodic properties. A large number of its medicinal properties are still under clinical trials. Fenugreek being a sensitive crop faces a number of environmental stresses in its natural habitat, and due to its inability to survive transplantation, it further faces survival threats. Fenugreek (*Trigonella foenum graecum* L.) being an immense medicinal plant in the modern world has drawn the concern of various scientists regarding its overall crop improvement to enhance its production and sustainable development. This review focuses on the general description, medicinal uses, agronomical strategies, and various scientific strategies like exploring the potential of diverse PGRs, nanoparticles, elicitors, mineral nutrients, as well as various biotechnological approaches for crop improvement and secondary metabolites production in fenugreek under stress as well as non-stress conditions. It also covers the crop responses towards abiotic stresses such as salinity, drought, heavy metals, temperature, and UV-radiation.

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Keywords

Active constituents · Environmental stresses · PGRs · Mineral nutrients · Nanoparticles · Crop productivity

8.1 Introduction

Cancer and diabetes are the two most prominent human health challenges faced by the current world. According to the recent statistical reports, about 18.1 million new cancer cases and 9.6 million cancer deaths are reported worldwide in 2018. Diabetes is a major cause of morbidity and mortality, increasing prevalence and the fastest growing disease worldwide. According to a WHO report, this is also a growing menace in India with an estimated 8.7% diabetic population between the age group of 20 and 70. There are two most vital factors, namely obesity and overweight, which increase the risk of type 2 diabetes (Kumar et al. 2014; Gaddam et al., 2015; Brandão and Cardoso 2018). World scientists are making unstinted efforts to explore the cure for diabetes by extended research to improve the understanding of its biology and the development of more effective diabetic treatment. Many drugs are commercially available for use in the management of diabetes. However, their side effects and high costs underscore the need for alternative herbal drugs. In fact, fenugreek (*Trigonella foenum-graecum* L.) contributes significantly in the alternative systems of medicine and on-going dynamic efforts worldwide in the research and development in curing such deadly diseases (Geberemeskel et al. 2019). Oral administration of the herb was traditionally used in digestive, respiratory problems and to ease labour and improve lactation in females (Shawahna et al. 2018). Recent research has provided evidence favouring fenugreek in lowering blood sugar levels, lowering lipid content, boosting testosterone, reducing cholesterol levels, and lowering inflammation (Meghwal and Goswami 2012; Pundarikakshudu et al. 2016), and helping in appetite control to fight obesity. Besides, it bears various immense medicinal properties, and their benefits have also been well recognised (Ashihara et al. 2015).

Trigonella foenum-graecum L. is a therapeutic legume commonly known as fenugreek, belongs to family Fabaceae. It is a widely grown rabi crop in West Asia and South-Eastern Europe. In India, fenugreek is grown mainly in Rajasthan, Madhya Pradesh, Gujarat, Uttar Pradesh, Maharashtra, and Punjab with Rajasthan being the leading producer followed by Gujarat and Haryana (Spices Board 2015). Besides India, the crop is usually grown and used in Middle East Asia, North Africa, Russia, Mediterranean Europe, United Kingdom, Australia, Canada, the USA, and some parts of West Asia (Kumar and Meena 2012) as depicted in Table 8.1. The two most notable fenugreek species in India are *Trigonella foenum-graecum* (methi) and *Trigonella corniculata* (Kasuri methi). The plant *T. foenum graecum* has multipurpose uses. Due to the presence of diverse range of biochemical constituents, the plant bears a wide range of applications in medicinal, nutraceutical, pharmaceutical, cosmetic, and bakery industries. The tender stem and trifoliate leaves are used as

Table 8.1 Common names of fenugreek in different parts of the world

Country	Common name of plant	Plant part used	Used for/as	References
Africa	Fenegriek	Seeds	Roasted grain used as coffee-substitute	Moradi and Moradi (2013)
Arab	Hulba, Hilbeh	Leaves	Oral treatment with herbal extracts used for curing kidney stones as it is believed to dissolve calcium oxalate crystals	http://www.indianetzone.com/1/fenugreek.htm
Burma	Penantazi	Leaves	Used in curries and as a spice for flatbreads	https://burmaspice.com/product/fenugreek-leaves/
Canada	Fenugrec, Senegre, Trigonelle	Whole plant	It is being developed as a high quality hay crop. Fenugreek is also grown as a green manure and cover crop	https://uses.plantnet-project.org/en/Trigonella_foenum-graecum_(PROSEA)
China	K'u - Tou	Seeds Leaves	For treating many afflictions, including abdominal pain, nephrosis, hernia, and arthritis. Used to boost physique, to treat weakness of body, and gout	Bahmani et al. (2016) https://uses.plantnet-project.org/en/Trigonella_foenum-graecum_(PROSEA)
Egypt	Hilba	Leaves Seed	Hilba tea to alleviate menstrual pains and sedating tummy problems. Seed powder is mixed with flour for making flatbread. Fenugreek seeds are added with maize to their pita bread to produce aish merahrah, a staple of their diet	Moradi and Moradi (2013) Bahmani et al. (2016)
Ethiopia	Abesh (or Abish)	Seeds	Used in preparing 'Hilbet', a soft, delicious white food. Used to make tea, flatbread,	Gall and Shenkute (2009)

(continued)

Table 8.1 (continued)

Country	Common name of plant	Plant part used	Used for/as	References
			'Enjera', as a spice in milk and in traditional herbal medicine for the treatment of diabetes	
France	Fenugrec, Senegre, Trigonelle	Seeds	Seed kernels are ground and mixed with water they greatly expand; hot spices, turmeric, and lemon juice are added to produce a frothy relish called hilbeh eaten with a sop	https://en.wikipedia.org/wiki/Fenugreek
Germany	Bockshornklee, Griechisch Heu	Seeds	Used externally in form of an aqueous paste for poultices to reduce inflammation. Occasionally it is used internally as a component of cholagogue and gastrointestinal remedy compounds	Bisset (1994), Khan and Abourashed (2011)
India	Methi, Methika	Seeds Leaves	In powdered form in preparation of pickles and as whole in vegetable dishes, dal and Sambhar. Seeds important constituent of spice mixes such as panch phoran. The oleoresin extracted from the seed is used in perfumery, cosmetics, and hair tonics. Grounded dried herbs or boiled fresh herbs in water in form of paste applied to injured or inflamed skin	Branch (2013), https://blog.prepscholar.com/fenugreek-benefits-side-effects, https://uses.plantnet-project.org/en/Trigonella_foenum-graecum_(PROSEA)

(continued)

Table 8.1 (continued)

Country	Common name of plant	Plant part used	Used for/as	References
Iran	–	Leaves Whole plant seeds	Incorporated into the herb stew ghormeh sabzi, the herb frittata kuku sabzi and a soup known as eshkeneh. In boiled form used for treating red spot of eye and cough. Used as tonic and blood sugar lowering	Hajimehdipoor et al. (2010), https://en.wikipedia.org/wiki/Fenugreek
Japan	Koruha, Fenuguriku	Seeds	Forms essential part in the flavour of curry powder. Its bitter and sweet flavour is used in soups, dals, bean and vegetable dishes, and fish and seafood dishes	http://www.airgreen.co.jp/fenugreek/index_e.html
Middle East	Hulba, Hilbeh	Stem and leaves	Used as a folk remedy for abdominal cramps associated with both menstrual pain and diarrhoea or gastroenteritis	Moradi and Moradi 2013
Nepal	Methiyam, mi	Seeds Leaves	Soaked and sprouted seeds used in salads. Powdered or whole form used in pickles. Used as leafy vegetable, cattle feed, and various other ways	http://tasteofnepal.blogspot.com/2011/12/fenugreek-seeds-methi-ko-geda.html, http://gernot-katzers-spice-pages.com/engl/Trig_foe.html
Netherland	Fenegriek	Seeds	Used to impart nutty flavour to Dutch goat cheese	http://amsterdamcheesecompany.webshopapp.com/en/dutch-goat-blends-fenugreek.html
Persia	Shanbalileh	Fresh leaves	Used to flavour stews, make soups and salads	https://www.linsfood.com/methi-leaves-fenugreek-leaves/

(continued)

Table 8.1 (continued)

Country	Common name of plant	Plant part used	Used for/as	References
Switzerland	Molotru, Molotru comun, Schinduf	Seeds	Used to flavour Swiss cheese	Moradi and Moradi (2013)
United States	Fenogreco	Seeds	Extract used as component of cholagogue and gastrointestinal remedy compounds and also in traditional galactagogue preparations	Duke (1997)

vegetable and seeds are popular condiment and spice. The seeds contain proteins, carbohydrates (in the form of galactomannans), fixed oils, pyridine, alkaloids (trigonelline), flavonoids, free amino acids, saponins, glucosides, steroidal diosgenin, calcium and iron, vitamins A, B_1, C, and nicotinic acid (Mehrafarin et al. 2010; Yadav et al. 2011; Brar et al. 2013). The leaves and seeds are extensively used for medicinal purposes and are prescribed in the treatment of dropsy, chronic cough, colic troubles, and enlargement of liver and spleen (Bahmani et al. 2016). Seeds of fenugreek are particularly rich in folic acid and dietary fibres such as galactomannans and other major bioactive compounds (polyphenols), such as rhaponticin and isovitexin (He et al., 2015). The therapeutic uses of fenugreek due to presence of these biochemical constituents are briefly described in Fig. 8.1.

Fenugreek seeds contain three important biochemical constituents: trigonelline, diosgenin, and alpha hydroxyisoleucine responsible for anti-diabetic properties of the plant. Trigonelline is a pyridine alkaloid biosynthesised from methylation of nicotinic acid. Trigonelline bears glucose-lowering properties (Olthof et al. 2011) due to the hypocholesterolemic agent (Khorshidian et al. 2016). Trigonelline improves insulin sensitivity by modulating pancreatic β-cells regeneration and stimulating glucose catabolising enzymes, thus lowering blood glucose levels aiding in control of type 2 diabetes (non-insulin-dependent diabetes) (Naicker et al. 2016; Aldakinah et al. 2017).

Diosgenin (25R)-5-spirosten-3H-ol) with molecular formula $C_{27}H_{42}O_3$ is another major secondary metabolite present majorly in young leaves and oily endosperm of the fenugreek seeds and its lower levels are detected in stem and roots of *T. foenum-graecum* L. (Ortuno et al. 1998). It is a steroidal saponin, a class of glycosylated triterpenes biosynthesised from cholesterol as the precursor molecule. However, the Asian fenugreek seeds contain approximately 5–6% diosgenin. Researchers have

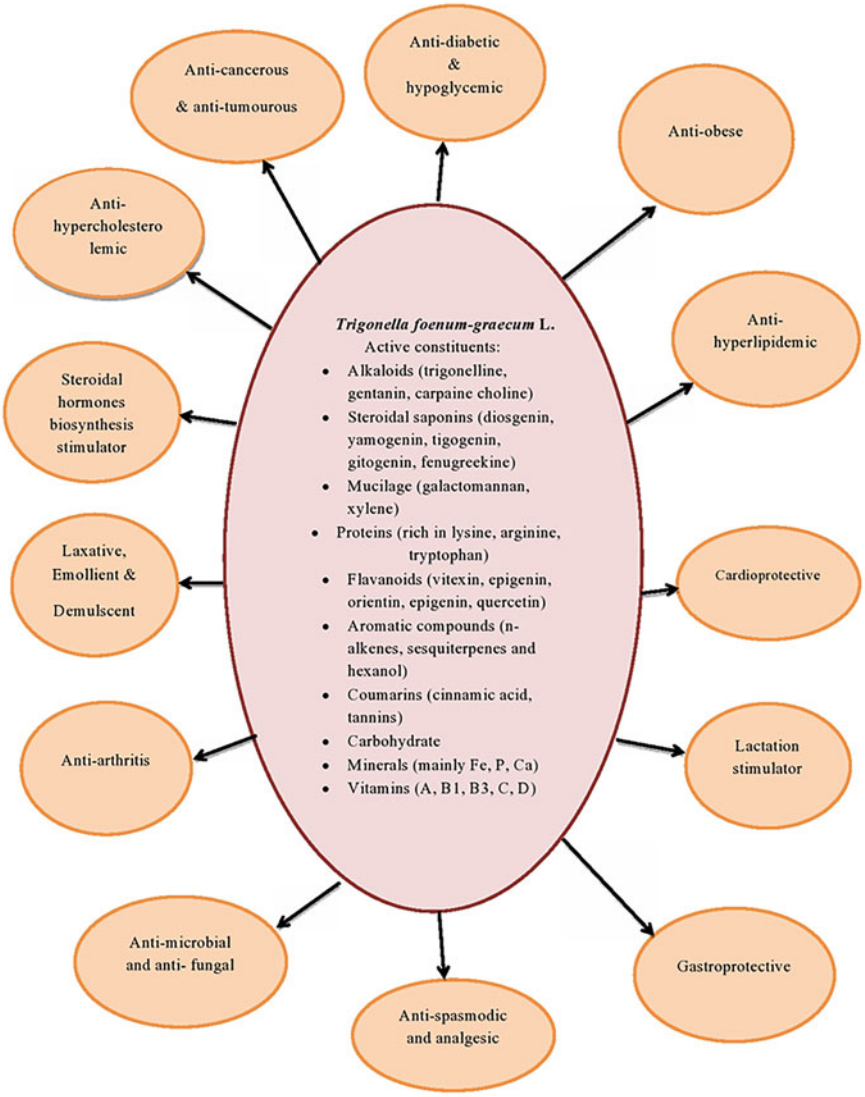

Fig. 8.1 Biochemical constituents and therapeutic uses of fenugreek

reported that diosgenin compound to be effective against a number of disorders such as diabetes, hypercholesterolemia, hyperlipidaemia, cancer (Chen et al. 2011; Tong et al. 2012), cardiovascular disease, oxidative stress, and inflammation (Jung et al. 2010). Anti-diabetic effects of diosgenin are mainly attributed to its efficiency in restoring pancreatic β-cells, downregulating hepatic gluconeogenesis and glucose export enzymes, and upregulating hepatic glucokinase, hepatoprotective, and anti-oxidant enzymes (Kalailingam et al. 2014). These secondary metabolites

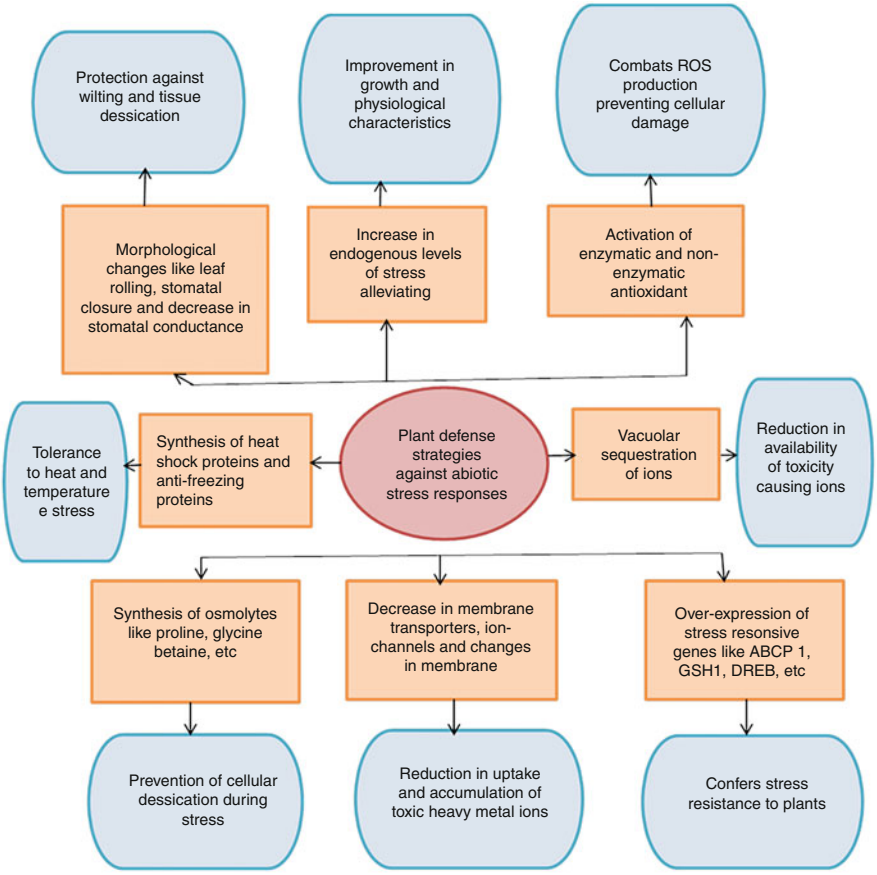

Fig. 8.2 Plant defence strategies against various abiotic stresses

(trigonelline and diosgenin) are involved in altering plant physiology to control both biotic and abiotic stress responses (Jasim et al. 2017). Thus, various biotic and abiotic stresses serve as elicitors for their increased biosynthesis. Keeping in view the enormous medicinal importance of this plant, we summarise information regarding medicinal uses of the plant, impact of abiotic stress factors and involvement of potent PGRs, nanoparticles, serving as elicitors and other scientific strategies for the overall development including secondary metabolites production of fenugreek under normal and stress conditions in this review article (Fig. 8.2).

8.2 Responses of *Trigonella foenum graecum* L. Towards Abiotic Stress

Abiotic stress in plants refers to the harmful effect of non-living factors on growth, physiology, and plants development. These include heavy metal stress, nutrient stress, salinity stress, water stress, heat stress, temperature stress, and UV stress. There are various ways by which these abiotic stresses act on plants. Heavy metals (HMs) stress is the most common stress the modern world faces, due to increasing human interference with the environment such as industrialisation and mining. The contamination due to HMs harms vital metabolic processes such as seed germination (Sethy and Ghosh 2013), photosynthesis, mineral nutrition, and interactions with water (Aggarwal et al. 2012; Rucińska-Sobkowiak 2016; Ozyigit et al. 2018). Moreover, nutrient stress arises from any deviation from the optimum nutrient level (either excess or deficiency) of particular soil nutrients. As each nutrient has its own physiological and metabolic role (Taiz and Zeiger 2006), their stress causes deficiency or toxicity symptoms in the plant. There are various abiotic stresses challenged by the *T. foenum graecum* during its lifespan. In this regard, the responses of the crop towards different levels of micronutrients and heavy metals are briefly described in Table 8.2. Specific responses of fenugreek to various other abiotic stresses are described below:

8.3 Salinity Stress in *Trigonella foenum graecum* L.

Salinity stress arises from an excess of Na^+ or Cl^- in the soil, harming plant growth (Munns 2005). In *T. foenum graecum* L. germination was not affected up to 140 mM NaCl. Although the plant survived up to 175 mM of NaCl, various phenotypic alterations such as short length of stems, a small number of leaves, reduction in fresh weight and number of nodules hamper overall yield and productivity of the crop under high levels of NaCl (Abdelmoumen and Idrissi 2009). These changes are a result of the modification in osmotic potential gradient which inhibits various cellular processes and results in reduced nutrient uptake and accumulation (Rogers et al. 2003; Hu and Schmidhalter 2005) and limitation of electron flow from H_2O to P_{680} in Photosystem II reaction centre (Lu and Vonshak 2002; Mittal et al. 2012) caused by high salinity levels. Salinity stress at the level of 200 Mm causes a marked reduction in various studied parameters viz. germination percentage, seedling vigour index, shoot and root lengths, shoot-root ratio, number of leaves and branches, fresh and dry weights, moisture content, relative water content, and photosynthetic pigments in *T. foenum graecum*. Impact of salinity was more severe on shoots than roots (Kapoor and Pande 2015). There was marked accumulation in proline content with increasing salinity levels in fenugreek (Al-Saady et al. 2012). Also at this concentration of NaCl (200 mM), a considerable reduction was observed in K^+/Na^+, and Ca^{2+}/Na^+ ratios in all organs showing salinity hamper the uptake of essential macronutrients like K^+ and Ca^{2+} in fenugreek plants causing their

Table 8.2 Impact of different micronutrient and heavy metals on morphological and physiological characteristics of *Trigonella foenum graecum* L. (↑ represents increase while ↓ represents decrease in the referred parameter)

Treatment	Optimum concentration	Growth and yield characteristics	Biochemical characteristics	References
Boron (B) (deficiency symptoms occurs below 13 μg/g of dry weight of plant)	5 $kgha^{-1}$ (soil application)	Plant height ↑, number of pods↑, root nodules number ↑, seed yield ↑, delayed seed germination, early crop maturity	Uptake of N ↑, K↑, P↑, Fe↑, cu↑, B↑ and Mn↑	Molgaard and Hardman (1980), Aishwath et al. (2018)
Chromium (Cr VI) (highly toxic >50 ppm)	10 ppm (soil application) 5 ppm to 50 ppm (soil application)	Early seedling growth, germination % ↑, seedling vigour index (SVI) ↑. Radicle emergence ↓, more brownish root colouration, shoot biomass ↓, dry weight of roots ↓	–	Jontey et al. (2013)
Cobalt (co) (toxic >12 ppm)	12 ppm (soil application)	Plant height ↑, number of branches/plant ↑, leaves number/ plant ↑, leaf area index(LAI)↑, fresh weight of shoots and roots↑, biomass of shoot/ plant↑ biomass of root/plant↑, pods/ plant↑, seeds wt./ plant↑, seed oil percentage↑, seed oil/plant↑	Nitrogenase activity↑, content of N↑, P↑, K↑, Mn↑, Zn↑, co↑ and cu↑ in seeds but Fe content↓. Total carbohydrate↑, total protein↑, vitamin C↑, L-ascorbic acid↑, vitamin A↑ carotenoids content↑	Gad and Abdul-Moez (2015)
Copper (cu) (highly toxic above 50 ppm)	50 ppm (soil application)	Total germination percentage↑, shoot length↑, root length↓, seedling vigour index↓, fresh weight and dry biomass of whole plant↑	Chlorophyll content↓, flavonoids↑, phenols↑	Menon et al. (2016), Noorul Ain et al. (2017)
Iron (Fe) (toxic ≥75 μg/ml concentration)	10 $mgha^{-1}$ (soil application)	Early seed germination, plant height↑, number of pods per plant↑, root nodulation rate ↑, seed yield ↑ and early crop maturity	Uptake of N↑ and K↑, chlorophyll a↑, chlorophyll b↑ and total carotenoids↑	Mabood and Ahmad (2017), Aishwath et al. (2018)

(continued)

Table 8.2 (continued)

Treatment	Optimum concentration	Growth and yield characteristics	Biochemical characteristics	References
Manganese (Mn)	10 $kgha^{-1}$ (soil application)	Hastened seed germination, plant height↑, number of pods per plant↑, root nodulation rate ↑, early crop maturity and high seed yield	Chlorophyll a↑, total carotenoids↑, uptake of N↑, K↑, cu↑, B↑ and Mn↑	Aishwath et al. (2018)
Molybdenum (Mo)	0.5 $kgha^{-1}$ (soil application)	Plant height↑, branches number/ plant↑, nodules number per plant↑, LAI (leaf area index)↑, number of pods and seed yield per plant↑, seed and oil yield per hectare↑	Chlorophyll content↑, protein↑, carbohydrate↑, seed NPK content↑	Meena et al. (2018)
Nickel (Ni) (toxic ≥40 $mgkg^{-1}$)	20 $mgkg^{-1}$ (soil application)	Fresh weight of Stover and root ↑, dry biomass of shoot and root ↑	Uptake and accumulation of Fe ↑ and Zn↓	Parinda et al. (2003)
Zinc (Zn) (toxic above 50 ppm; best concentration in terms of plant growth is 75 µg/ml and in terms of seed yield is 7.5 $kgha^{1)}$	0.6% (foliar application)	Number of nodules/plant↑, number of pods/ plant↑, seed yield↑, seed number ↑ and early initiation of flowering	Chlorophyll content↑, carotenoids↑, trigonelline yield↑	Sammauria and Yadav (2010), Lal et al. (2015)
Arsenic (as) (toxic at ≥30 $mgkg^{-1}$)	20 $mgkg^{-1}$ (soil application)	Shoot dry weight ↑, pods number/ plant ↑, 100 seed weight, seed yield per plant↑	Leaf photosynthetic rate↑, chlorophyll (chl.) a↑, chl. a/b ratio↑	Talukdar (2013)
Cadmium (cd) (toxic at ≥0.1 mM)	0.5 mM (soil application)	Germination % ↓, seedling growth↓, shoot biomass ↓, root biomass↓	Amylase activity ↓, chl. a↓, chl. b↓, proline↓, protein content↓, phenolic content ↑, flavonoid content ↑, MAD content↑, SOD↑, CAT↑ and APX activity↑	Zayneb et al. (2015)

(continued)

Table 8.2 (continued)

Treatment	Optimum concentration	Growth and yield characteristics	Biochemical characteristics	References
Lead (Pb) (highly toxic at and above 75 μgL^{-1})	10 ppm (soil application)	Germination rate↑, no. of leaves/ plant↑, total area of leaf↑, single leaf area↑, dry biomass↑, root and shoot length↑	Chlorophyll content↑, total free amino acids↑, insoluble carbohydrate↑	Mukherjee (2015)
Mercury (hg)	10 ppm (soil application)	Germination percentage 100%, shoot length↓, root length↓, fresh and dry weight of shoot and root ↓. Roots became thick and highly twisted and thickening of primary leaves	MAD levels ↑, SOD activity↑, POD activity ↑, CAT activity ↓, proline levels ↑, phenols↑	Karmarkar et al. (2014)

deficiency in the plant (Hasni et al. 2009). Higher salinity stress significantly reduced germination per cent and seedling growth of fenugreek (Hojjat and Kamyab 2017).

8.4 Mitigation of Salinity Stress

There are various following ways through which salinity stress can be mitigated in fenugreek plants.

8.4.1 Plant Growth Regulators (PGRs) Application

The PGRs are effective in salinity stress as it improves endogenous level of growth regulators, which control plant water balance. Seed priming with 20 mg L^{-1} solution of gibberellic acid (GA_3), 6-furfuryladenine (Kinetin), and benzyladenine (BA) significantly improved toxicity symptoms in plants raised under saline conditions for 45 days through the improved accumulation of protein and photosynthetic pigments and decreasing the level of stress indicators like proline and sugars (Ratnakar and Rai 2014). Pretreatment of fenugreek seeds through soaking in the solution of salicylic acid, citric acid, and proline significantly improved salt tolerance ability of the plant up to 3000 ppm of NaCl which is inferred from the proteomic analysis which shows the generation of new isoenzyme bands which may serve as a good indicator for salt tolerance mechanism in *T. foenum-graecum.* Further, there was an increase in activities of antioxidant enzymes like catalase, proline dehydrogenase, and ascorbate oxidase improving studied plant growth parameters (Behairy

et al. 2017). Exogenous foliar application of salicylic acid alone is reported to overcome the reduction in gas-exchange parameters (net CO_2 assimilation rate, substomatal CO_2 concentration, transpiration rate, and stomatal conductance) caused as a result of salinity stress improving overall biomass production in *T. foenum-graecum* (Babar et al. 2014).

8.4.2 Nanoparticles Application

Silicon dioxide (SiO_2) nanoparticles can ameliorate the adverse effects of salinity on the shoot and root lengths and dry weight of the seedlings in fenugreek plants subjected to various concentrations of salinity levels up to 150 mM. Nanosized SiO_2 applied at 50 ppm was much more effective than 100 ppm of SiO_2 and other bulk SiO_2 concentrations (Ivani et al. 2018). In another study, application of AgNPs was beneficial in enhancing salinity tolerance in the fenugreek seedling and stimulated the defence mechanisms of plants against salt stress (Hojjat and Kamyab 2017).

8.5 Nutritional Management

Nutritional management of the soil also serves as an aid in mitigating salinity stress in fenugreek plants. It was reported that a combination of Mn, B, and Zn applied as a foliar spray in a ratio of 1:1:1 and in concentrations of 2.5 and 5 ppm showed a remarkable increase in all overall growth of fenugreek suggesting that this combination to be a potent ameliorator of salinity stress (Ibrahim and Faryal 2014). Foliar application of boron at 0.1% and zinc at 0.2% twice was found effective in enhancing most of the yield attributes and seed yield of fenugreek (Pariari et al. 2009). A combination of two micronutrients (Zn and Mo) and vermicompost was the most effective treatment for the studied parameters (Karmakar et al. 2014). Further, a study was carried out the effect of five micronutrients, viz. Fe, Zn, Mn, Cu, and B, on the growth, yield, nutritional, and physiological parameters of fenugreek (Aishwath et al. 2018). They concluded that Fe and Mn were found superior among all the applied micronutrients. However, the role of B was magical for delaying seed germination and hastening maturity in the plants. Four different concentrations of foliar boron (H_3BO_3), viz. control, 100, 200, 400, and 800 mg L^{-1}) were applied to fenugreek plants (Beyzi and Gürbüz 2019). The authors reported that the most significant biological and seed yields were recorded with 800 mg L^{-1} of boron treatments.

8.6 Biotechnological Approach

Salinity adversely affects *Rhizobium tibeticum*-fenugreek symbiosis lowering nitrogen fixation and nodulation in the legume by affecting the nod gene expression. To study the effect of salinity stress on nod gene in bacterium, the nod gene of *R. tibeticum* was fused with lacZ gene of *E. coli* expressing β-galactosidase and changes in nod gene expression under salinity were measured. There was a significant decrease in β-galactosidase activity under soil salinity. Further, it was reported that preincubation of *R. tibeticum* with a combination of hesperetin (7.5 μM) and apigenin (7.5 μM) enhanced β-galactosidase activity alleviating salinity stress in fenugreek (Abd-Alla et al. 2014a). One of the approaches involves applying *Arbuscular mycorrhiza,* which improves nutrient uptake, water use efficiency, antioxidant metabolism, and ionic homeostasis curbing toxic effects of salinity (Evelin et al. 2019). Mycorrhizal (*Glomus intraradices*) associated plants, when exposed to increasing levels of salinity showed reduced leaf senescence, reduction in lipid peroxidation in membranes, improved root nodulation and increased the uptake of NPK in case of fenugreek. All these changes are attributed due to *A. mycorrhizal* fungi ability to prevent excess uptake of Na^+, regulated translocation of Na^+ in tissues and maintenance of optimum concentration of nutrients (Ca^{2+}, K^+, Cu^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}) in mycorrhizal plants, thus contributing positively in better plant growth (Evelin et al. 2012).

8.7 Drought Stress in *Trigonella foenum-graecum* L.

Another common stress faced by fenugreek is water stress or drought stress due to irregular and erratic rainfall pattern. Fenugreek plants irrigated with lower WHC (water holding capacity) resulted in a decrease in all growth parameters, photosynthetic pigments, and yield components including carbohydrate and protein content; however, there was an increase in phenolic and flavonoid contents during drought stress (Sadak 2016). A similar trend was reported in temperature stress. In another study, the transmission electron micrographs of heat-stressed fenugreek leaves (35 °C) showed some cellular abnormalities in chloroplasts, nucleus, and intercellular spaces (Osman et al. 2015).

8.8 Ultraviolet (UV) Stress in *Trigonella foenum graecum* L.

Ultraviolet (UV) stress is a rare form of stress experienced by *T. foenum graecum* in its natural habitat. These rays can induce the plant culinary and medicinal properties through altering its physiological and biochemical profile (Kumari and Prasad 2013, 2014). Nonetheless, when plants are exposed above the sub-lethal dose for a maximum period it retards plant growth and development. UV-B exposure of 8.0 hr. decreased plant growth, chlorophyll and carotenoid levels in *T. foenum graecum*. There was also a significant decrease in PAL (phenylalanine lyase) activity

which reduced secondary metabolite production in fenugreek lowering its medicinal value (Sebastian et al. 2018).

8.9 Heavy Metals and their Impact on Growth, Physiology, and Yield of *Trigonella foenum graecum* L.

Nowadays, heavy metal contamination has become a cause of great concern resulting from rapid industrialisation and anthropogenic activities. These heavy metals are non-biodegradable and persist indefinitely in soil interfering with the physiological and biochemical processes in plants hampering their growth and development. Some heavy metals are essential for plant metabolic pathways in low doses and become toxic only at higher concentration while some are non-essential and toxic even at low doses (Tchounwou et al. 2012). Heavy metals are essential for plant physiological processes that include Cu, Zn, Mo, Ni, Mn, and Fe while Pb, Hg, Cd are considered non-essential heavy metals (Tchounwou et al. 2012). Heavy metals also cause cell toxicity through the generation of oxidative stress resulting in overproduction of reactive oxygen species (ROS). The generated ROS such as hydroxyl, hydrogen peroxide, or superoxide ions impairs antioxidant defence system and leads to cellular damage and disturbance of cellular ionic homeostasis. It also damages biological molecules including lipids, DNA, and proteins. Thus, high concentrations of heavy metals generate abiotic stress, affecting plants from molecular to physiological levels (Panchal et al. 2013).

There are several heavy metals which act as essential nutrient elements for plant growth and development at low concentration. These are Zn, Cu, Mo, Co, Cr, Ni, Fe, and Mn.

8.9.1 Zinc

Zinc (Zn) plays an important role in physiological processes of plants in small quantity. It takes part in the biosynthesis of chlorophyll and some carbohydrates, conversion of starches to sugars, and is also essential in forming auxins. Zn deficiency often leads to interveinal chlorosis, necrotic spot formation in leaves, reduced internodal expansion exhibiting rosette appearance and reduced budding, resulting in poor flowering and reduced seed yield. Application of Zn (ZnO and $ZnSO_4 \cdot H_2O$) or ($ZnSO_4 \cdot 7H_2O$) recovers the deficiency of Zn in soils (Mortvedt 1992; Rashid and Ryan 2004; Alloway 2009). Foliar application of Zn at the rate of 0.6% improved number of pods per plant and enhanced plant yield by improving seed number per pod in *Trigonella* (Lal et al. 2015). This concentration further increased chlorophyll and carotene production and trigonelline content of the plant. The combination of 0.2% of Zn with NPK resulted in the highest number of pods and seed yield in fenugreek (Pariari et al. 2009). Zinc applied through the soil as $ZnSO_4$ at the rate of 7.5 kg ha^{-1} of soil resulting in high returns and high benefits: cost ratio. Further researchers have suggested that increasing concentration of Zn up to 50 ppm

increased seed germination, root and shoot growths, however, concentration higher than 100 ppm showed inhibitory effects and reduced seed germination percentage, seedling growth and fresh and dry weights of seedling (Menon et al. 2016). Toxicity of nanosized ZnO particles causes a significant decline in root nodule number and biomass in fenugreek (Siani et al. 2017).

8.9.2 Copper

Copper (Cu) is an essential micronutrient required by plants for a number of redox reactions. It is component of ascorbic acid oxidase, cytochrome oxidase, tyrosinase, uricase, monoamine oxidase, phenolase, laccase, and plastocyanin. The contamination of Cu mainly occurs in the agricultural environment through excessive use of fungicides like Bordeaux mixture, runoff from mines and industrial settings. In small concentration, it promotes photosynthesis and respiration processes in plants, but in excess, it causes toxicity symptoms in plants in the form of chlorosis, lodging, and decreasing plant yield (Yruela 2005). In case of *Trigonella*, Cu^{2+} increased root growth, amylase activity, phenol and flavonoid content only up to 1 mM and above 10 mM there was a prominent decrease in root and shoot growth, germination percentage, chlorophyll content and increase in H_2O_2, lipid peroxidation and ascorbate peroxidase activity indicating oxidative stress in *Trigonella* plants (Elleuch et al. 2013). Another experiment showed that Cu toxicity severely increased above 50 ppm in *T. foenum graecum* marked by a decrease in germination and growth parameters (Menon et al. 2016). Soil application of *Nostoc muscorum* (2 g/kg) is known to effectively alleviate Cu toxicity symptoms in fenugreek (Dewedar et al. 2013).

8.9.3 Molybdenum

Molybdenum (Mo) contamination rarely reported in Indian soils. However, in the case of legumes, Mo requirement is often higher in contrast to other plants. Mo performs several physiological functions in plants with particular importance in legumes. Mo forms part of 'FeMoCo' cofactor which regulates nitrogenase activity catalysing biological nitrogen fixation. It also enhances nitrate reductase activity (Manuel et al. 2018). It also affects the viability of pollens and development of anthers. Mo′s deficiency results in a decrease in nitrogen fixation and reduction in nodule biomass impairing the plant's overall development. Thus, application of molybdenum in the form of soil fertilisers as sodium molybdate or ammonium molybdate often contaminates the soil and results in toxicity symptoms. Molybdenum application up to 20 μM increases productivity and nodulation in fenugreek (Paudyal et al. 2007). Foliar application of 30 ppm of Mo as ammonium molybdate increased plant growth characters, yield components, chlorophyll content, protein and carbohydrate levels in seed and nitrogen and potassium percentage in seeds improving its nutritional value (Gendy 2013). It was also reported that soil

application of Mo at the rate of 1 kg ha^{-1} produced significantly higher seed yields and net economic returns in *T. foenum-graecum* (Choudhary et al. 2015). Soil application of 0.5 kg ha^{-1} Mo significantly enhanced the various parameters, viz. plant height, number of branches per plant, dry matter accumulation, pods number per plant, pod length, number of seeds per pod, seed and straw yield, N, P, Mo content and total uptake in seed and straw of fenugreek over their respective control (Meena et al. 2018).

8.9.4 Cobalt

Cobalt (Co) is also an important micronutrient required, especially for leguminous plants. Cobalt forms the structural component of cobalamin required for activity of several enzymes and co-enzymes responsible for the formation of leghaemoglobin involved in nitrogen fixation in root nodules of leguminous plants (Jayakumar et al. 2009). On average, cobalt concentration varies from 0.1 to 70 mg kg^{-1} soil worldwide, but the ideal concentration of Co to support plant growth lies in the range 1–2 mg kg^{-1} soil. It is reported that optimum concentration (12 ppm) of Co was found suitable for the best growth and biochemical parameters of *T. foenum-graecum*. This concentration enhanced the plant growth and yield characters, nodulation rate, nitrogenase activity, and biochemical constituents in fenugreek (Gad and Abdul-Moez 2015). In another study, foliar spray of 11.77 ppm of cobalt application significantly increased diosgenin content up to 14.77 mg g^{-1} seed dry weight basis (Dwivedi 2017).

8.9.5 Manganese

Manganese (Mn) is an essential plant mineral nutrient, plays a crucial role in several physiological processes. It also acts as a cofactor for a number of enzymes that catalyse redox, decarboxylation and hydrolytic reactions (Marschner 1995). Application of Mn at the rate of 10 kg ha^{-1} soil increased plant height, pod number, nodulation rate, early crop maturity, and high seed yield of *T. foenum-graecum*. This concentration also enhanced uptake of N, K. Cu and B from the soil and improved chlorophyll *a* and carotenoid content of the plant (Aishwath et al. 2018).

8.9.6 Nickel

Nickel (Ni) is responsible for activation of urease, an enzyme involved with nitrogen metabolism in plants. High level of urea accumulates in tissues leading to the formation of necrotic wounds. It also promotes nodulation by benefitting the growth of nitrogen-fixing bacteria through activation of hydrogenase enzyme in bacteria in case of leguminous plants (Seregin and Kozhevnikova 2006). It was reported that increasing concentration of Ni up to 20 mg kg^{-1} soil resulted in flourishing growth

and an increase in dry matter yield but at and above 40 mg kg^{-1} of soil toxicity symptoms were reported in the form of interveinal chlorosis and decrease in dry matter of *T. foenum graecum* L. Further, with the increasing Ni concentration, there was a decrease in Cu and Zn and an increase in Fe content in fenugreek plants (Parinda et al. 2003). Nickel toxicity in *T. foenum graecum* L. can be effectively treated by soil addition of *Rhizobium tibeticum* activated with a mixture of flavonoids (Abd-Alla et al. 2014b).

8.9.7 Iron

Iron (Fe) uptake takes place from the soil in the form of solubilised Fe^{3+} which gets reduced to Fe by a membrane-bound Fe^{3+} reductase oxidase (Jeong and Guerinot 2009) and finally transported into the root by an iron-regulated transporter. Iron is responsible for several essential redox reactions of photosynthesis and respiration in plants. It is essential for chlorophyll synthesis. Moreover, there is a high iron requirement in the case of leguminous plant as it is required for biosynthesis of various key enzymes of the nitrogenase complex and for some hydrogenase (Weisany et al. 2013). Culture studies revealed that Fe up to 25 μM positively supports *Rhizobia* growth and nodulation rate while at higher concentration, it has detrimental effects on the bacterial population (Paudyal et al. 2007). However, its higher amount is also required for the heme component of leghaemoglobin in legumes. Soil application of Fe at 10 mg ha^{-1} soil showed hastened seed germination, enhanced plant height, and increase in the number of pods, root nodulation, early crop maturity, and high seed yield with enhanced uptake of N and K and increase in photosynthetic pigment content in fenugreek (Aishwath et al. 2018). Other report suggested that Fe at the concentration of 75 μg mL^{-1} inhibited plant growth with lethal effects on physiological and biochemical characters in *T. foenum graecum* L. (Mabood and Ahmad 2017).

8.9.8 Chromium

It is reported that Cr (VI) is more toxic for plants compared to Cr (III) (Nibha and Aery 2000) *Trigonella* plants do not show toxicity up to 40 kg Cr (VI) ha^{-1}, but at 80 kg Cr (VI) ha^{-1} plant growth significantly decreases showing marked toxicity soil (Xanthate et al. 2012). Further experiment reveals that low concentration of Cr (VI) significantly improves germination parameters in terms of germination percentage and seedling vigour index but further increasing concentration up to 50 ppm there is gradual decrease in radical emergence, fresh and dry biomass of roots and shoots whereas above 50 ppm there is no radical emergence (Jontey et al. 2013). Another study showed that seed germination was 100%, but there was a reduction in radicle and plumule length up to 100 mg L^{-1} Cr (VI), but germination per cent decreased with this concentration (Menon et al. 2016). Another aspect of fenugreek grown in chromium contaminated soil focuses on its improvement of medical

efficacy through its substantial uptake in fenugreek seedlings as chromium is prescribed for diabetic control as it improves insulin sensitivity by increasing insulin binding, insulin receptor number, and insulin receptor phosphorylation (Xanthate et al. 2012).

8.9.9 Cadmium

Cadmium is a non-essential element, negatively affects overall growth and development of plants. Cadmium toxicity in plants is reflected as chlorosis, leaf rolls and stunting due to its interference with the uptake of essential micronutrients like Fe, Ca, Mg, P, and K (Das et al. 1997). Cadmium concentration up to 10 mM affected germination percentage, but there was a marked decrease in germination of fenugreek at higher concentration. However, low concentration (0.5 mM) of Cd showed toxicity symptoms in plants, such as decreased seedling growth and reduction in root biomass. Total phenolic and flavonoid content reached their highest values at 0.5 mM Cd, but with the increase in Cd concentration, there is a marked decrease in photosynthetic pigments and protein content (Zayneb et al. 2015).

There are various mechanisms that help plants overcome the mild level of abiotic stress by themselves. The negative impacts of the abiotic stresses, including heavy metals on plants could be successfully mitigated through scientific strategies by applying diverse PGRs, nanoparticles, and elicitors. These applied techniques may enhance the inbuilt pathways of plant defence strategies. Biotechnological approaches like transgenic production of stress tolerant species and species overexpressing stress mitigating enzymes also gain importance but are highly expensive and require skilled manpower. In this regard, an overview of these plant defence strategies is given in Fig. 8.1.

8.10 Role of PGRs in Physiological Performance of *T. foenum-graecum*

A plant growth regulator is a natural or artificially synthesised organic compound at low concentration that acts as chemical messenger coordinating intercellular communication and influencing the growth and differentiation of plant cells, tissues, and organs. It may either accelerate or delay the growth of the plant. Plant growth regulators (PGRs) include naturally produced phytohormones like auxin, gibberellic acids, cytokinins, abscisic acid, ethylene, salicylic acid, jasmonic acid, strigolactones, and brassinosteroids. Other potent PGRs are synthetically produced and include triacontanol, thidiazuron, etc. (Gasper et al. 1996). PGRs can alter various physiological and biochemical pathways of plant affecting their overall plant growth and development (Davies 2013) (Table 8.3).

There are various ways through which a PGR is applied to the plants. PGRs may be applied through foliar sprays (Sajjad et al. 2014), drenching (applying solution to the growing substrate), sprenches (high-volume sprays where the solution is applied

Table 8.3 Role of selective PGRs in alleviation of abiotic stresses in fenugreek through improvement of growth and biochemical characteristics

Treatment	Concentration	Growth characteristics	Biochemical characteristics	References
IAA (indole acetic acid)	10 µM and 100 µM IAA given to seedlings under 3 mg cd kg-1 soil and 9 mg cd kg-1 soil	Overcomes decrease in shoot length, root length, fresh and dry biomass of shoot and root.	IAA at both concentrations enhances the activity of antioxidant enzymes (SOD, CAT, POD, GST) as well as APX and DHAR only at 3 mg cd kg-1 soil stress alleviating cd toxicity at low levels.	Bashri and Prasad (2016)
SA (salicylic acid)	0.25 mM and 0.50 mM SA treatment given along with 0.075 mM cd stress in the form of $CdCl_2$ and 0.075 mM Pb stress in the form of $Pb(NO_3)_2$. Seed primed with 100 mg/L of salicylic acid grown under four levels of cadmium concentration (0, 10, 20, 30 mg/L) 0.2 mM salicylic acid added to seeds treated with 0, 3 mM of $Na_2HAsO_4.7H_2O$ and 5 mM of ZnSO4 for 72 h of germination	Improved plant height, fresh and dry biomass of root and shoot. Primed seeds with SA (100 mg/L) proved protection against cd stress and increased the germination percentage, root elongation, shoot elongation and dry weight of seedlings compared to the control treatment. Improvement in radicle and cotyledon growth	Improved total chlorophyll content, reduction in uptake and accumulation of cd and Pb, upregulation of SOD and CAT activity. Increase in chlorophyll and carotenoid contents, the accumulation of mineral nutrients (K, ca, and cu), soluble proteins and free amino acids in cotyledons, as well as the activities of hydrolytic enzymes (amylase and phosphatase). Upregulation of SOD, CAT, APX, and GR activities	Goel (2012), Espanany et al. (2015), Mabrouka et al. (2019)

(continued)

Table 8.3 (continued)

Treatment	Concentration	Growth characteristics	Biochemical characteristics	References
24- EBL (24-epibrassinolide)	Seeds soaked in 10 mM CdCl2 supplemented with 0.5 μM, 1.0 μM, and 2.0 μM EBL	Increase in fresh and dry biomass of seedlings, shoot length, seed germination otherwise suppressed by cd stress	Increase in biochemical parameters like CAT activity, APOX and GPOX activity, GR activity and decrease in POD activity, AAO activity and MDA levels	Swamy et al. (2011)
28- HBL (28-homobrassinolide)	Foliar application of 0.5 μM and 1.0 μM HBL on plants grown under 10 mM Pb2+	Significant increase in plant height, shoot length, root length, LAI, leaf number, fresh and dry biomass	Restoration of the biochemical parameters like chlorophyll content, carbohydrate levels, proteins and nucleic acids levels	Swamy et al. (2014)
Putrescine	Soaking seeds in 5 mM putrescine 2 hrs before temperature stress of 35 °C. Foliar spray of 100 mgL^{-1} putrescine under drought stress	Increase in shoot length, root length, fresh weight of root and shoot compared to stress exposed seedlings alone. Overcomes decrease in number of leaves/plant, number of branch/plant, dry weight/plant, leaf area/plant and LAI	Increase in chl a, chl b, carotenoid, total soluble protein and total carbohydrate content. Decrease in proline content, MDA content, peroxidase and cellulose activity and increase in catalase and SOD activity. Increase in chl a, chl b, total carbohydrate and total crude protein content. Increase in SOD and catalase activity	Osman et al. (2015), Shalaby et al. (2018)
Spermidine	Foliar spray of 100 mgL^{-1}	Increase in number of leaves/plant,	Increase in chl a, chl b, total carbohydrate	Shalaby et al. (2018)

(continued)

Table 8.3 (continued)

Treatment	Concentration	Growth characteristics	Biochemical characteristics	References
	spermidine under drought stress	number of branch/plant, dry weight/ plant, leaf area/plant and LAI	and total crude protein content. Increase in SOD and catalase activity	
Trehalose	Foliar spray of 500 μM trehalose at 30 and 35 days after sowing in seedlings exposed to drought stress grown in soil with 60% water holding capacity	Increase in shoot length, root length, number of pods/plant, number of seeds/plant, 1000 seeds weight/plant, seed index	Increase in chl a, chl b, carotenoids, carbohydrate content, protein levels, total phenolic content, flavonoid content and antioxidant activity	Sadak (2016)
Melatonin	Foliar spray of 50, 100, 300, and 500 μM melatonin (MEL) applied twice in two consecutive weeks and plants subjected to drought stress using 19.5% PEG (polyethylene glycol)	Fresh shoot weight increased at 100 and 300 μM MEL while 300 and 500 μM showed higher values in shoot length. No significant effect on fresh root weight	Increase in chl a, chl b and carotenoid at all concentrations of MEL. Increase in trigonelline content significant at 100 and 300 μM MEL concentrations. Increase in CAT, POD, and SOD activity	Zamani et al. (2019)

to both the foliage and the growing substrate), seed soaking, liner dips (partially submerging a tray of rooted liners in a PGR solution) (Schnelle and Barrett 2010), seed priming, bulb, tuber and rhizome dips or soaks (Whipker et al. 2005). Among these, foliar sprays and drenching are the most common methods used and found effective in most plants. Seed priming is also found very effective in case of legumes, particularly fenugreek. Effectiveness of a PGR in producing the physiological effects depends upon its mode of application, uptake by the plants, time of application, concentration of PGRs, plant species, and the prevailing environmental conditions (Wroblewska and Debicz 2013).

8.10.1 Auxins

Auxins plays a pivotal role in various physiological processes in plants including cell elongation, cell division, differentiation, vascular tissue, root initiation, apical dominance, leaf senescence, leaf and fruit abscission, fruit setting and flowering (Taiz and Zeiger 2006). Application of NAA at 50 ppm applied through pre-plant soaking and spraying at 20 days after planting significantly increased plant growth parameters, yield components, and alkaloid production except the number of leaves and SPAD value of *T. foenum-graecum* plantlets (Danesh-Talab et al. 2014). In vitro callus induction from leaf and SAM treated with 2,4-D at 1 mg L^{-1} increased fresh and dry weights of the callus at 15 and 45 days after culture and highest diosgenin production was noticed on day 45 in leaf culture (Rezaeian 2011). Auxin applications are also reported to alleviate heavy metal toxicity in plants up to some concentration (Yuan et al. 2013). Another research report suggested foliar application of even 10 μM of IAA on fenugreek seedlings is sufficient to counteract the oxidative stress caused by cadmium up to 3 mg kg^{-1} of soil by increasing level of antioxidants and upregulation of genes coding for enzymes of the ascorbate-glutathione cycle (Bashri and Prasad 2016).

8.10.2 Gibberellins

Gibberellins are mainly involved in controlling and promoting stem elongation, flowering, leaf expansion, and seed germination (Taiz and Zeiger 2006). Foliar sprays of gibberellic acid (GA_3) at 50 ppm thrice at 25, 45, and 65 days after sowing exhibited the best performance for studied growth parameters, however, early flower initiation and early maturity were observed with 75 ppm of GA_3 (Veni 2012). Moreover, seed treatment with GA_3 at 50 ppm concentration markedly improved the germination parameters in *T. foenum-graecum* by promoting the release of various hydrolytic enzymes like α-amylase from the aleurone layer (Jakhi et al. 2013). In another study, individual application of foliar spray of GA_3 (50 ppm) at day 20 or a combined application of 25 ppm of GA_3 at pre-plant soaking and foliar spray at 20 days after planting enhanced yield components of fenugreek by increasing number of pods per plant and alkaloid production (Danesh-Talab et al. 2014).

8.10.3 Cytokinins

Cytokinins (CK) belong to a class of plant growth substances that are chemically amino-purines (kinetin, zeatin, and 6-benzylaminopurine) or phenyl urea derivatives (diphenylurea and thidiazuron) that stimulate cell division, or cytokinesis, in plant system. Application of 10^{-5} M of benzyladenine in isolated embryos with excised axis was reported to partially overcome inhibition of galactomannan degradation (Spyropolous and Reid 1985). Benzylaminopurine (BAP) and zeatin at 0.1 mg L^{-1} in culture media resulted in the rapid production of green calli and further leafy

shoots from protoplast derived colonies in *Trigonella* sp. (Shekhawat and Galston 1983). Soaked seeds treated with 20 ppm solution of BAP showed higher accumulation of diosgenin in leaves and stem in 30 days old seedlings of fenugreek (Ortuno et al. 1998).

8.10.4 Salicylic Acid

Salicylic acid is a β-hydroxyl phenolic acid derived from phenylalanine via the phenylpropanoid route in the cytoplasm (Metraux 2002) and through the isochorismate pathway in the chloroplast. It is reported for the first time from leaves and bark of willow trees (*Salix*), therefore named salicylic acid. Its synthesis increases in plants subjected to environmental stress. It may occur in the plant as free salicylic acid or in the form of carboxylated esters and phenolic glycosides. Salicylic acid is involved in various physiological processes in plants such as seed germination, vegetative growth, photosynthesis, respiration, thermogenesis, flower formation, seed production, senescence, and defence against various biotic and abiotic stresses (Hayat et al. 2007; Hasanuzzaman et al. 2017). It also stimulates CO_2 assimilation, ribulose-1,5-bisphosphate carboxylase/oxygenase activity, stomatal conductance, and photosynthetic pigments in various plants resulting in increased photosynthesis under abiotic stress (Fariduddin et al. 2003; Thjib-ul-Arif et al. 2018). However, there are contradictory reports regarding the role of SA in seed germination in some plants as it inhibits germination causing oxidative stress. In contrast, in others, it increases seed vigour, but fenugreek seed priming with 2800 μM of SA significantly improved the germination percentage, seedling vigour index and other germination parameters (Moghaddam et al. 2018). Fenugreek seed primed with 100 mg L^{-1} of SA grown under cadmium (Cd) concentrations (0, 10, 20, 30 mg L^{-1}) protected against Cd stress and alleviated the negative effects of Cd on germination parameters (Espanany et al. 2015). Salicylic acid in a small concentration of 0.2 mM effectively restored the reduction in biochemical constituents of fenugreek caused due to arsenic and zinc toxicity (Mabrouka et al. 2019) proving itself to be potent ameliorator offering protection against heavy metal stress mainly through mechanisms involving ROS detoxification (Yuan et al. 2013). The alleviating effects of salicylic acid under heavy metal stress in fenugreek are well described in Table 8.3.

8.10.5 Methyl Jasmonate

Methyl jasmonate (MeJA) is a linolenic acid derivative produced via the octadecanoid pathway. It is reported to be a potent elicitor of secondary metabolites in various plants (Mathew and Sanker, 2012) and plays a regulatory role in several diverse physiological pathways. It promotes seed germination, root growth, flowering, fruit ripening, and senescence. The maximum increase in diosgenin content (10.5 fold) was found at 100 μL L^{-1} concentration of MeJA in fenugreek

(Debjani and Bratati 2010). Furthermore, application of 0.01% of MeJA on 12 days old *T. foenum-graecum* seedling was sufficient to increase diosgenin content via upregulation of enzymes 3-hydroxy-3-methylglutaryl CoA reductase and sterol-3-β-glucosyltransferase involved in diosgenin biosynthetic pathways (Chaudhary et al. 2015). Transcriptome analysis via RNA sequencing revealed that MeJA applied on fenugreek seedlings upregulated the expression of fatty acid ω-hydroxylase (CYP86A2) and steroid 22-alpha-hydroxylase (CYP90B1) genes, unspecific mono-oxygenase and 26-hydroxylase genes in plants resulting in the production of diosgenin content (Ciura et al. 2018) (Table 8.4).

8.10.6 Brassinosteroids

Brassinosteroids are a class of plant steroidal hormones which regulate physiological processes like cell expansion and elongation, photomorphogenesis, flowering, pollen development, seed germination, vascular differentiation, stomatal formation, and senescence in plants (Fariduddin et al. 2014; Ashraf et al. 2010). They also act as a defence against abiotic stresses like low and high temperatures (Sadura and Janeczko, 2018), heavy metals (Cao et al. 2005), salinity (Abbas et al. 2013), light and drought (Mahesh et al. 2013), as well as herbicides and pesticides (Xia et al. 2006; Sharma et al. 2013). Brassinolide applied at 1.0 ppm on plants showed a positive response in yield parameters in *T. foenum-graecum*, both under non-stress and water stress condition (Singh et al. 2018). Application of 24-epibrassinolide at 2.0 and 1.0 μM concentrations of 28-homobrassinolide found effective in mitigating Cd and Pd stress, respectively, in case of fenugreek (Swamy et al. 2011; 2014). Both PGRs effectively restored the growth, yield, and biochemical parameters in fenugreek seedlings by inducing ROS scavenging through enzymatic and non-enzymatic antioxidants, and mitigating oxidative stress. Also, BRs enhanced antioxidant enzymatic activities under zinc (Arora et al. 2010; Ramakrishna and Rao 2013), chromium (Sharma et al. 2011), and lead stress (Rady and Osman 2012).

8.10.7 Abscisic Acid

Abscisic acid (ABA) is a growth inhibitory isoprenoid derived plant hormone which exerts a significant inhibitory impact on cell extension and water uptake in plants. It controls several developmental processes in plants, including seed and bud dormancy (Nambara et al. 2010), stomatal closure, inhibition of fruit ripening, reserve accumulation, and response to environmental stresses (Tuteja 2007; Danquah et al. 2014). The studies on *T. foenum-graecum* showed that ABA hampered growth by lowering endogenous auxin levels and increasing the activity of auxin catabolising enzymes like IAA oxidase and peroxidase (Megha and Laloraya 1977). Abscisic acid is analogous to metabolic inhibitor found in seeds of *T. foenum-graecum* L. which suppresses the release of α-galactosidase and β-mannosidase activities

Table 8.4 Impact of selective PGRs and elicitors on growth and physiology of fenugreek

Treatment	Concentration	Effects	References
NAA (naphthalene acetic acid)	Foliar spray at 50 ppm NAA	Increase in shoot dry weight, 1000 seeds weight, number of seeds per pod, content of seed trigonelline, leaf area per plant, and also, plant height, stem diameter, number of pods per plant, content of seed mucilage, and root, stem, leaf, and pod dry weight	Danesh-Talab et al. (2014)
GA (gibberellic acid)	Foliar spray of 50 and 75 ppm GA_3 at 25, 45, and 65 DAS seeds soaked with 50 ppm GA	Increase in plant height, number of branches, number of flowers, number of pods, length of pod, number of seeds per pod, seed yield, fresh weight, and dry weight of fenugreek. Early flower initiation and early maturity was observed with application of GA3 at 75 ppm. Marked stimulation in the germination percentage, vigour index and also showed enhancement in shoot length and fresh and dry weights of root and shoot. Significant improvement in the amount of total carbohydrates, soluble protein, and activities of enzymes like amylase, protease and catalase	Veni, (2012), Jakhi et al. (2013)
6-benzylaminopurine	Seeds soaked in solution of 20 ppm 6-benzylaminopurine	No effect on growth parameters but increase in diosgenin content up to 47% in leaves 15 days after treatment. In 30 days old seedlings increase in diosgenin content in stem up to 113% compared to control was observed with no significant increase in diosgenin levels in leaves of the plant	Ortuno et al. (1998)
SA (salicyclic acid)	Seeds primed with 1700 and 2800 μM salicylic acid	At 2800 μM SA there is significant improvement in	Moghaddam et al. (2018)

(continued)

Table 8.4 (continued)

Treatment	Concentration	Effects	References
	and subjected to accelerated aging for 24 and 48 h	the mean germination percentage, seedling length vigour, seedling weight vigour, germination speed, coefficient of germination speed, mean daily germination, coefficient of germination uniformity, germination vigour, maximum mean daily germination, and germination percentage. Also increase in seedling length, plumule dry weight, and seedling dry weight was observed at same concentration	
MeJA (methyl jasmonate)	0.01% MeJA applied on 12 days old seedlings	Increase in diosgenin levels, increased expression of 3-hydroxy-3-methylglutaryl CoA reductase(HMG) and sterol −3-β- glucosyl transferase (STRL)	Chaudhary et al. (2015)
TRIA (triacontanol)	T1 = 0.5 ml/L at 25 DAS T2 = 0.5 ml/L at 25 and 45 DAS T3–0.5 ml/L at 25, 45 and 70 DAS. Two concentrations 500 ppm and 1000 ppm applied in three combinations each. Single foliar spray at 40 DAS, double spray at 40 and 60 DAS and triple spray at 40, 60, and 80 DAS	Maximum plant height, number of branches per plant, pod length, number of grains per pod, and yield were recorded by spraying TRIA at the rate of 0.5 ml/L at 25, 45, and 70 DAS in comparison with control. Significantly higher number of branches per plant, number of pods per plant, increase in test weight and gross returns were reported on application of 1000 ppm TRIA@ 40, 60, and 80 DAS than 500 ppm TRIA and control	Singh, (2010), Shivran et al. (2016)
Trehalose	Foliar spray of 0, 250 μM and 500 μM trehalose at 30 and 35 days after sowing	Increase in shoot length, root length and yield component, photosynthetic pigments, carbohydrate content, protein levels, total phenolic content, flavonoid content, and antioxidant activity	Sadak (2016)

(continued)

Table 8.4 (continued)

Treatment	Concentration	Effects	References
AgNPs (silver nanoparticles)	Five levels of silver nanoparticles (0, 10, 20, 30, and 40 μg mL^{-1}) were used. After germination, daily supply with 15 ml from each concentration was carried out for 12 days during plant growth. AgNPs at the rate of 0.2 mg per seedling applied. Foliar application of AgNPs at 40 mgL^{-1}	At low doses (10 μg/ml) seed there is increase in seed germination, early seedling growth, increase in fresh and dry weight of roots. At higher doses adverse effects are reported. Significant increase in shoot length, root length, leaf number, wet weight. Enhanced diosgenin production. Increase in shoot length, number of leaves/plant, and shoot dry weight, number of pods/plant, number of seeds/pod, weight of seeds/plant, and seed index. Increase in photosynthetic pigments, indole acetic acid (IAA) contents, carbohydrate%, protein%, phenolics, flavonoids, and tannins contents and antioxidant activity	Hojjat (2015), Jasim et al. (2017), Sadak (2019)
SiNPs (silicon nanoparticles)	Si was given in two forms as sodium silicate and as SiO_2 nanoparticles	Both treatments increased the uptake and accumulation of Si, xylem cell wall lignification, cell wall thickness, PAL activity and protein concentration in seedlings, while there was no effect on antioxidative enzyme activity	Nazaralian et al. (2017)
Silicon	Two levels of silicon (Si), 0 and 1/5 mM as Na_2SiO_3 supplemented at four levels of NaCl, i.e. 0, 60, 120, and 180 mM	Improvement in fresh and dry biomass of shoot and root, decrease in membrane permeability of leaves. Increase in chlorophyll a and b content, carotenoid content and leaf relative water content	Nasseri et al. (2012)
SiO_2 nanoparticles	50 ppm and 100 ppm SiO_2 nanoparticles applied at four NaCl levels 0, 50, 100, and 150 mM	Increase in shoot length, root length, shoot dry weight, root dry weight and seedling vigour index at 50 ppm nanosized SiO_2	Ivani et al. (2018)

(continued)

Table 8.4 (continued)

Treatment	Concentration	Effects	References
Gamma radiation	Seeds subjected to five doses of gamma radiation (25, 50, 100, 200, 400 Gy) prior to potting	Low doses up to 100 Gy showed increase in growth and yield characters, increase in soluble protein content of leaves, increase in phenolic and flavonoid content, ascorbic acid, α-tocopherol and retinol content	Hanafy and Akladious (2018)
Co- 60 gamma irradiated chitosan	Foliar spray of 40, 80 and 120 mg/L of irradiated chitosan individually as well as in combination with single dose of 40 kg/ha phosphorus	40 mg/L irradiated chitosan in combination with 40 kg/ha phosphorus showed significant increase in growth, yield and trigonelline content. Enhanced activity of nitrate reductase and carbonic anhydrase	Dar et al. (2015)
ISA (irradiated sodium alginate)	Foliar spray of 0, 40, 80, and 120 mg/L of ISA alone as well as in combination with single dose of 40 kg/ha phosphorus	At 80 mgL-1 ISA in combination with 40 kgha-1 phosphorus there is significant increase in seed yield, shoot length, root length, leaf area, shoot fresh and dry mass, photosynthetic pigments, trigonelline yield, trigonelline content and seed alkaloid content	Dar et al. (2016)

(Zambou et al. 1993), thus limits reserve mobilisation and water imbibition (Reid and Bewley 1979) by restraining galactomannan degradation.

8.10.8 Ethylene

Ethylene is a gaseous hormone which in low concentration up to 0.05 μL L^{-1} has growth promotion response in plants (Abeles et al. 1992) and at higher concentration, it negatively affects many physiological processes in plants. It is mainly involved in various processes like seed germination, root branching and elongation, the formation of root and shoot primordia, flowering, fruit ripening, cell wall lignification, anthocyanin production, leaf and flower senescence, abscission, etc. (Schaller 2012). In T. foenum graecum L., ethephon is reported to be a potent elicitor of diosgenin production in cell suspension cultures. Researches revealed that 5 ppm

concentration of ethephon was sufficient to increase diosgenin accumulation with an increase of 126% over control while at higher concentration (25 and 50 ppm) lowered the amount of sapogenin content. Additionally, molecular studies revealed that this fluctuation was observed due to changes in an important biosynthetic enzyme, isopentenyl diphosphate isomerase, activity which increased at 5 ppm and decreased at higher ethephon concentrations (Gomez et al. 2004). It was further revealed that there was a marked reduction in both fresh and dry biomass with increasing concentrations of ethephon, 5 days after treatment. Cytomorphological studies indicated that callus culture of *T. foenum graecum* treated with different concentrations of ethephon at the rate 5, 15, 25, 50, and 100 ppm increased cell diameter, decreased cell packing, increased cytoplasmatic density (25 ppm treatment) and alteration of the membrane structures using 50 and 100 ppm (Oncina et al. 2002). During abiotic stress conditions particularly during drought stress and a higher concentration of ethylene causes chlorophyll loss promoting senescence and it also works antagonistically to ABA causing failure of stomatal closure (Wilkinson and Davies 2009) worsening plant conditions during stress. So, it becomes necessary to lower ethylene concentration in plants in order to alleviate stress symptoms. As an aid to this problem soil is inoculated with 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing bacteria which effectively restores *T. foenum graecum* plants against injurious effects of drought stress by lowering endogenous levels of ethylene through its catabolism in plants (Barnawal et al. 2013).

8.10.9 Polyamines

Polyamines are small, positively charged, organic molecules with more than two amino groups. Besides their role in cell proliferation, growth and differentiation in plant tissue, flower and bud development, embryogenesis, fruit set and fruit ripening, they also play an important role as stress protectants (Ozturk and Demir 2003). Some naturally occurring polyamines are putrescine, spermidine, spermine. Thus, putrescine and spermidine have gained importance in plant science due to their anti-senescence and anti-stress activities, making them effective plant growth regulators (Velikova et al. 2000). Seeds soaking with putrescine (5 mM) for 2 h before exposing to temperature stress overcome its deleterious effects and the deformations occurred in nuclei and other cell organelles in plants exposed to temperature stress alone and over the control (Osman et al. 2015). Individual application of putrescine and spermidine has also been reported to enhance the fenugreek plant's growth during drought stress conditions. Foliar spray of 50 mg L^1 putrescine caused a significant increase in all growth parameters at 90 and 105 days after sowing date compared to control plants (treated with tap water). Further increasing putrescine concentration up to 75 and 100 mg L^{-1} exhibited a positive response on all growth parameters studied at 90 and 105 days after sowing. Similar results were also obtained with spermidine, but putrescine was more effective in enhancing Trigonella's growth over spermidine. However, putrescine and spermidine

concentration applied at 100 mg L^{-1} overcome the deleterious effects of drought stress in the plants by increasing the amount of photosynthetic pigments and decreasing proline and MDA content (Shalaby et al. 2018).

8.10.10 Trehalose

Trehalose is a non-reducing disaccharide with its ubiquitous distribution in biological systems ranging from insects and invertebrates, bacteria, yeast, fungi to higher plants (Elbein et al. 2003). Other names like tremalose or mycose know it. In plants, it is naturally in very low amounts so for its use as a potent PGR. Trehalose is highly efficient in legumes as it increases survival of nitrogen-fixing bacteria like *Bradyrhizobium* sp. in root nodules of the plant preventing their desiccation (Streeter 2003) during temperature and drought stress acting as an osmoprotectant (Lopez et al. 2008). Reports showed that application of trehalose under non-stress conditions significantly improves all growth, yield, and biochemical parameters of fenugreek seedling (as given in Table 8.1). A more straightforward approach of trehalose application in mitigating drought stress was studied in *T. foenum graecum*. Different trehalose concentrations (0, 250 and 500 μM) were sprayed on plants grown on soil with different water holding capacities at 30 and 35 days after sowing. Plants growth on soil with 60% water holding capacity showed significant stress symptoms and decreased the values of photosynthetic pigments, antioxidant activities, growth, seed yield, quantity and quality of seeds. However, the adverse effects of drought were successfully ameliorated using 500 μM of trehalose application (Sadak 2016).

8.10.11 Melatonin

Melatonin is a tryptophan derived indoleamine and a universal neurotransmitter in vertebrates. Melatonin is a newly discovered plant growth regulator (Kolar and Machackova 2001), and the mechanism of its action in plants is still imperfectly determined. Nonetheless, reports suggest that melatonin serves an important role in plant growth and development. Melatonin modulates reproductive development, controls root and shoot organogenesis, delays senescence, maintains plant tissues, and improves tolerance towards biotic and abiotic stresses (Paek et al. 2014; Hasan et al. 2015). Melatonin reversed the detrimental impacts caused by water stress in *T. foenum graecum* by significantly reducing chlorophyll degradation, lowering proline and MDA content and increasing the activities of ROS scavenging enzymes (Shi et al. 2015; Marta et al. 2016). Further it enhanced the anti-diabetic potential of the plant by increasing the trigonelline production (Zamani et al. 2019). Moreover, in another study, melatonin (50 μM) with glutathione (1 mM) and thiourea (3 mM) significantly alleviated the lead stress (1200 ppm) in *T. foenum graecum* and stimulated acid rain (pH 3.5) via stabilising membrane integrity, reducing ROS accumulation, lowering hydrogen peroxide and MDA levels, enhancing protein

accumulation, and upregulating gene expressions of catalase and superoxide dismutase (Xalxo and Keshavkant 2019).

8.11 Seaweed Extract

Seaweed is a term given to giant marine algae which belongs to phaeophyta (brown algae) or rhodophyta (red algae). Extracts of these seaweeds are considered to contain a large number of growth-promoting substances (Khan et al. 2009). The biochemistry of seaweeds makes them a potent enhancer of plants growth and development and confers stress tolerance against various environment stresses (Zhang et al. 2003; Zhang and Ervin 2008). They are also reported to increase nutrient status of soil (Turan and Kose 2004) and enhance antioxidant properties of the plant (Verkleij 1992). In a study, seaweed extracts of *Ulva fasciata, Sargassum ilicifolium,* and *Gracilaria corticata* compared with the Hoagland nutrient medium and untreated control were analysed in case of *T. foenum graecum,* and the results revealed that there was an increase in shoot growth and fresh biomass and photosynthetic pigments over the control but at par with the Hoagland solution treated plants. However, there was a significant increase in various biochemical parameters (carbohydrate, proteins, free amino acids, polyphenols, and nitrogen content) of seaweed treated plants over the control and Hoagland solution treated plants. The positive responses of fenugreek were more pronounced with *Ulva* extract over *Sargassum* and *Gracilaria* (Pise and Sabale 2010).

8.12 Radiation

Based on energy, radiations are classified into two types: ionising radiation and non-ionising radiation. Both ionising radiations and non-ionising radiation alter plant growth and development via different mechanism. Ionising radiations are reported to encourage plant growth at certain stages of development, induce early flowering, and stimulate lateral bud development (Caplin and Willey 2018). Recent research has established gamma rays to be potent elicitor of important medicinal metabolites like ginsenosides, saponins, camptothecin, shikonins, etc. in plants (Kim et al. 2012; Vardhan and Shukla 2017). The gamma rays at low doses enhance seed germination, plant growth, yield components and increase stress resistance (Melki and Marouani 2010; Marcu et al. 2013). However, it can induce mutations causing death of seedlings, sterility, and other deleterious effects at higher doses. Preliminary studies on germination parameters in fenugreek reveal that LD dose for fenugreek 50 is close to 350 Gy (Patel et al. 2017). In another variety of fenugreek, seed irradiation with low doses of gamma rays (at 100 Gy) before sowing significantly improved growth and yield parameters, leaf soluble protein, phenolic and flavonoids compound content, trigonelline and nicotinic acid content. While gamma rays at the higher dose (400 Gy) significantly decreased all the above parameters by causing alterations at the DNA level (Hanafy and Akladious 2018; Parchin et al. 2019).

Among non-ionising radiations, there are only a few reports regarding the impact of UV rays on fenugreek seedlings. Seedlings of fenugreek treated with UV-B rays retard plant growth by decreasing the levels of all photosynthetic pigments hampering photosynthesis; however, it turned out to be highly efficient elicitor of secondary metabolite production in fenugreek when applied for a short duration by increasing production of flavonoids, phenolics, and anthocyanin. Phenolic content was enhanced mainly due to an increase in activity of phenylalanine lyase enzyme with 4.0 h UV-B exposure but a longer exposure of 8.0 h even decreased the activity of these enzymes. Studies revealed that UV-B exposure promoted synthesis of short-chain fatty acids causing alterations in aromatic oil composition of *T. foenum-graecum* (Sebastian et al. 2018). The rays also increased the levels of antioxidant activities of POX and SOD in fenugreek, protecting the plant against oxidative stress (Sharma et al. 2019).

8.13 Radiation-Processed Natural Polysaccharides.

Some of the natural polysaccharides like chitosan, carrageenan, and sodium alginate in irradiated forms are reported to be powerful elicitors of secondary metabolites in medicinal plants. Exposure to gamma rays breaks the glycosidic bonds degrading these polymers into small-sized oligomers with low molecular weight. These oligomers act as plant growth promoters and enhance seed germination, plant biomass, root growth, flower production, alleviation of heavy metal stress, etc. (Aftab and Idrees 2014; Sadiq and Varshney 2017; Singh et al. 2017; Ahmad et al. 2019, 2020; Naeem et al. 2020a, b).

Sodium alginate ($NaC_6H_7O_6$) is a sodium salt of alginic acid extracted from cell walls of seaweeds mainly from *Laminaria* and *Sargassum* sp. Like PGRs, oligomers of sodium alginate improve the plant defence system in plants by acting as signalling molecules which might be similar with an endogenous growth elicitor that could function as signalling agent to stimulate gene expression and trigger the synthesis of different enzymes causing activation of various plant responses (Ma et al., 2010). Chitosan, another natural polysaccharides containing polymeric chain of ß-(1, 4)-glucosamine and N-acetyl-d-glucosamine, is recognised to be an active inducer for improving secondary metabolites in number of medicinal plants (Mukarram et al. 2021a). However, there is meagre information available regarding the effects of these oligomers of irradiated sodium alginate (ISA) and irradiated chitosan (ICH) in case of fenugreek. Foliar application of degraded oligomers of ISA at a concentration of 80 mg L^{-1} supplemented with optimum phosphorus concentration (40 kg ha^{-1} soil) was reported to be highly effective in fenugreek seedlings and found to increase seed yield by 131.0%, content and yield of trigonelline by 17.8% & 174.0%, content and yield of seed alkaloids by 32.9% & 208.6% over their respective control. Depolymerisation of chitosan brought about by gamma-irradiation using cobalt-60 is reported to enhance its plant growth-promoting activities (Hossain et al. 2013; Mukarram et al. 2021b). Supplementation with increasing concentration of chitosan up to 150 mg L^{-1} in hairy root culture of

T. foenum graecum has brought about fast growth and enhanced trigonelline production (Qaderi et al. 2016). In another study, seedlings of fenugreek treated with 40 mg L^{-1} of ICH in combination with phosphorus application (40 kg ha^{-1} of soil) proved to be optimum in increasing the total alkaloid content by 34.9%, seed yield by 125.4%, and trigonelline content by 17.8% (Dar et al. 2015). Also, application of ICH significantly enhanced photosynthetic pigments and activities of nitrate reductase and carbonic anhydrase enzymes.

8.14 Nanoparticles

Nanoparticles (NPs) are defined as materials which have at least one or two dimensions less than 100 nm in diameter (Auffan et al. 2009; Ball 2002). Because of its exclusive properties like small size, large surface area, high surface energy, easy attachment, fast mass transfer and quantum confinement, they have high penetration power which facilitates effective delivery of agrochemicals in plant tissues (Nel et al. 2006; Khodakovskaya et al. 2009; Ghormade et al. 2011; Mukarram et al. 2021c). NPs exhibited both positive and negative responses on seed germination, plant growth, and development depending on their size and concentration but smartly engineered nanoparticles can act as effective plant growth regulators by improving nutrient deficiency, acting as a carrier of plant growth hormones and inducing environmental stress tolerance in plants (Cheng et al. 2016; Santo Pereira et al. 2017).

Among different nanoparticles, the implication of silver and silicon nanoparticles has fetched researchers' attention in improving the growth and development of *T. foenum graecum*. The AgNPs ranging between 35–40 nm in size were found effective in enhancing the root and shoot growth of different plants (Mehta et al. 2016), and in case of *T. foenum graecum*, AgNPs ranging from 8–21 nm were found to be an efficient elicitor of plant growth and diosgenin production at the concentration of 0.2 mg per seedling (Jasim et al. 2017). Another report suggested that application of AgNPs (20 nm in diameter) at low doses (up to 10 μg mL^{-1}) improved germination and growth parameters significantly in the plant (Hojjat and Hojjat 2015). Hojjat and Kamyab (2017) evaluated the effects of silver nanoparticles to ameliorate the negative effects of salinity on germination and growth of fenugreek seeds. According to Sadak (2019), 40 mg L^{-1} concentration of AgNPs significantly enhanced growth, yield, and bioactive content in *T. foenum graecum*. Silicon dioxide nanoparticles are also well known to improve seedling growth and root development (Hutasoit et al. 2013). Application of 50 ppm of nanosized SiO_2 at four levels of salinity stress (0, 50, 100 and 150 mM) significantly improved all growth and germination parameters of *T. foenum-graecum* (Ivani et al. 2018) as listed in Table 8.2. It was hypothesised that germination was accelerated by lowering the mean germination time and stimulation of the respiratory pathway and ATP production by silicon dioxide nanoparticles (Chen and Arora 2013).

8.15 Tissue Culture Techniques for Enhanced Secondary Metabolites Production in *T. foenum-graecum*

Taking into account the immense importance of biochemical constituents present in fenugreek particularly trigonelline and diosgenin several biotechnological approaches have been adapted to scale up the production of secondary metabolites by modulating conditions at laboratory level irrespective of seasonal fluctuations and abiotic stresses which the plant has to face in its natural habitat (Goncalves and Romano 2018). Several strategies have been proved effective in improving biomass and secondary metabolites production in various cultures of fenugreek.

8.16 Callus Culture

The callus is a more or less un-organised de-differentiated amorphous mass of cells from the explant under in vitro cultural conditions. Precursor feeding with nicotinic acid resulted in improved growth and trigonelline content in callus culture (8 weeks old) of fenugreek (Khanna et al. 1975). Another study revealed that 4 weeks old callus culture of fenugreek showed an increase in 3–4 times higher trigonelline content than that of seeds and 12–14 times trigonelline content as compared to root and shoots of the intact plant when cultures fed with nicotinic acid treatment (Radwan and Kokate 1980). Feeding with nicotinic acid decreased callus growth due to enhancement of trigonelline (Lynn et al. 1984). Callus induction with certain phytohormones is also found effective in increasing biomass and secondary metabolite production in the fenugreek plant. Shocking treatment of explant with 10 mg L^{-1} 2,4-D, IAA, IPA, and α-naphthalene acetic acid (NAA) for 1 hour was not only effective in early callus induction but it also enhanced trigonelline production in the generated callus (Radwan and Kokate 1980). The callus raised under these conditions accomplished maximum accumulation of diosgenin that is about 2.2 mg g^{-1} dry weight after 45 days in leaf calli followed by 0.74 mg g^{-1} and 0.60 mg g^{-1} in stem and root calli (Oncina et al. 2000). Culture media treated with 2.0 mg L^{-1} 2, 4-D and 2.0 mg L^{-1} NAA was found effective in callus induction using cotyledons and hypocotyl explants within 5 days (Abd-Elaleem et al. 2014). Further, a combination of 2 mg L^{-1} 2, 4-D and 0.5 mg L^{-1} kinetin was highly effective for callus induction in the case of MS and B5 media when explants were used as stem segments, embryos and hypocotyls (Afshari et al. 2011). Callus induction with 1.5 mg L^{-1} of NAA and 20 mg L^{-1} of BA added to MS medium when subjected to different salinity levels (0, 2, 4, 6 and 8 NaCl g L^{-1}) showed marked fluctuations in fresh and dry weights and potassium ions of callus. Noticeably, salinity at level of 2 g L^{-1} increased all above parameters acting as growth elicitor while at 8 g L^{-1} it showed significant decrease in callus induction and fresh biomass. The negative effects of salinity were overcome efficiently by adding 30 mg L^{-1} SA in culture media and maximum of the above parameters were obtained when cultures were supplemented with 2 gL^{-1} NaCl in combination with 30 mg L^{-1} SA (Al-Madhloom 2018).

8.17 Cell Suspension Culture

A plant cell suspension culture is a sterile (closed) system normally initiated by aseptically placing friable callus fragments into a suitable sterile liquid medium. A significant increase in diosgenin content was observed when subjected to precursor feeding with cholesterol in cell suspension cultures of fenugreek (Khanna et al. 1975; Brain and Williams 1983). Further reports suggest that nicotinic acid applied 50 mg L^{-1} in cell suspension culture showed an increase in trigonelline content (37%) over culture treated with 1 mg L^{-1} of nicotinic acid that improved 31% trigonelline content (Radwan and Kokate, 1980). Among phytohormone treatments, ethephon at 5 ppm brought about an increase of 126% in diosgenin accumulation over untreated cultures through the subsequent increase in isopentenyl diphosphatase activity, however, concentrations of 25 and 50 ppm decreased the production of diosgenin (Gomez et al. 2004). Supplementation of 2, 4-dichlorophenoxy acetic acid (2, 4-D) (1 mg L^{-1}), kinetin (0.1 mg L^{-1}), and sucrose (5%) in cell suspension cultures of fenugreek showed variations in biomass, trigonelline, and 4- hydroxyisoleucine content between days zero and 12. When supplemented with 100 μM of MeJA for 24 h, cell suspension cultures exhibited a marked improvement in the level of trigonelline and 4-hydroxyisoleucine, and administration of such cell extract showed improved hypoglycaemic effects in streptozotoxin induced diabetic rats (Ahmed et al. 2011).

8.18 Hairy Root Cultures

Hairy root cultures are promising for secondary metabolite production of several medicinal plants (Pistelli et al. 2019). The results of Shahabzadeh et al. (2013) indicated that irrespective of fenugreek ecotypes (Karanj and Bushehr) stem explants had higher transformation frequency (81.3%) and greater capacity for induction of hairy roots (8.76) overleaf explants. The maximum values of the transgenic hairy root (8.76), the transformation frequency (79.76%), and the growth rate of transgenic roots (0.77 d^{-1}) were achieved from infection with *A. rhizogenes* strain K599 at OD 600 = 1.2, whereas the lowest belonged to the bacterial concentration of OD 600 = 1.6 (Shahabzadeh et al. 2013). The first report on hairy root culture in *T. foenum graecum* has been established with *A. rhizogenes* strain A4 for diosgenin production using callus culture initiated on McCown's woody plant (WP) medium from leaves, stem, and roots explants obtained from 30 days old seedlings (Merkli et al. 1997). When elicited with chitosan (40 mg L-1), the same culture increased diosgenin content to three folds than non-treated medium. Hypocotyl of seedling used as explant when subjected to infection with *A. rhizogenes* led to induction of hairy root culture. The ethanolic extracts of the culture cells, when subjected to GC-MS analysis, showed enhanced accumulation of sotolone (1.2% of the volatile fraction) and 3-amino-4,5-dimethyl-2(5H)-furanone (17% of the volatile fraction). Hairy root culture serves as a bioreactor for sotolone production, and they serve to be an excellent biological model to study the biosynthetic pathway of sotolone in

fenugreek (Peraza-Luna et al. 2001). Trigonelline compound also successfully produced through hairy root culture initiated using explants from two Iranian masses (Zanjan and Boranzjan). Co-cultivation and injection with different strains of *A. rhizogenes* (A4, 9126 and 15834) resulted in production of transformed hairy roots. Hairy root cultures induced by strains 15834 and 9126 resulted in higher growth index in Zanjan and Borazjan masses, respectively. The highest amount of trigonelline was produced in such root cultures after 28 and 7 days for Borazjan and Zanjan, respectively (Raheleh et al. 2011). Another study revealed that elicitation of hairy root cultures encouraged by three strains of *A. rhizogenes* (ATCC15834, MSU440 and K599) with two elicitors MeJA and chitosan enhanced trigonelline content to several folds. The maximum biomass accumulation resulted in callus induced by ATCC1584 strain. The highest trigonelline contents were reported to be 36.7 and 37.3 mM gm^{-1} dry weight for 100 μM of MeJA and 150 mg L^{-1} of chitosan, respectively (Qaderi et al. 2016).

8.19 Conclusions

The various scientific strategies applied have successfully overcome losses in fenugreek yield resulting from several abiotic stresses and play a vital role in enhancing crop productivity, consequently increasing secondary metabolites production in T. foenum graecum L. non- stress as well as abiotic stress conditions. Among these strategies, tissue culture techniques may appear to be more advantageous as it overcomes damages due to seasonal fluctuations, escapes stress faced by the plant in its natural habitat, and also scales up production of bioactive chemicals from small biomass. However, it requires skilled handling, aseptic conditions and is very costly, which is out of reach and understanding of the common farmers. PGRs also play a vital role in facilitating yield improvement in a model plant. Such PGRs are cost-effective compared to biotechnological approaches and could be handled easily. As fenugreek has to face a wide range of environmental stresses like heat, temperature, flooding, drought, heavy metals, biotic stress, etc., these PGRs effectively alleviate stress conditions in the plants by altering physiological processes activating plant defence system against stress condition. Individual application of PGR may be effective against a wide range of abiotic stress like ethylene that is effective against salinity, flooding, drought, and heavy metals (Gamalero and Glick 2012) and salicylic acid found effective against several abiotic and biotic stresses (Khan and Abourashed 2011). Mineral nutrients in bulk and nanoforms are particularly useful in alleviating the number of abiotic stresses, particularly drought stress, temperature, and heavy metal stress in plants; however, a few reports are available on its application fenugreek. With the advancement in science, several novel PGRs are being explored for their potential in regulating plant growth and development, and some are still obscure and need to be analysed providing immense opportunities to researchers for crop improvement in near future.

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Part II

Chemical Composition, Nutritional Properties and Active Compounds of Fenugreek

9 Extraction, Detection, and Characterization of Various Chemical Components of *Trigonella foenum-graecum* L. (Fenugreek) Known as a Valuable Seed in Agriculture

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Abstract

Trigonella foenum-graecum L. (fenugreek) is cultivated in various countries and consumed for many goals including medicinal applications (anti-diabetic, lowering cholesterol level, antioxidant, anti-microbial, anti-ulcer, lactation aid, and anti-obesity), making food (like stew with rice), roasted grains coffee-substitute, and controlling insects in grain storages. Generally, the seeds and leaves of the crop are commonly applied in food and pharmaceutical industries and contain several active ingredients that is linked to the properties of this plant. The chemical composition of fenugreek crop includes moisture, ash, protein, mucilage, lipids, alkaloids (mainly trigonelline), amino acids (particularly 4-hydroxyisoleucine), saponins (mainly diosgenin), flavonoids (mainly quercetin, rutin, and vitexin), fibers (mainly soluble dietary fiber), polyphenols, coumarin, vitamins (mainly vitamin C), and minerals (mainly calcium, zinc, sulfur, and phosphorus). Fenugreek as a hydrocolloid (known as galactomannan) can be applied as a thickening, emulsifying, stabilizing, gelling, and encapsulating agents and also it has positive effects on the textural and appeal properties of food and pharmaceutical products. In this chapter, a review of the methods for extraction and identification of different substances present in fenugreek is evaluated.

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Keywords

Protein · Mucilage · Lipids · Alkaloids · Amino acids · Flavonoids · Polyphenols · Vitamins · Minerals

9.1 Introduction

Trigonella foenum-graecum L. (fenugreek) is a well-known plant affiliated with the Fabaceae or Leguminosae family. In general, countries in Asia, Europe, and Africa, especially Iran, India, Pakistan, China, and Sudan are known as the main areas for extensively cultivating fenugreek. Meanwhile, India is the main producer of this plant (Snehlata and Payal 2012; Acharya et al. 2006). Different parts of plants can be used as a food component or substance with medicinal properties, but usually the seeds and leaves are of special importance, and fenugreek is no exception to this rule. The seeds and leaves of the crop are commonly applied as an ingredient in food and pharmaceutical industries (Syeda et al. 2008). Usually, each plant has different species that are gradually recognized by research. Regarding the fenugreek plant, it should be noted that there are likely to be 260 species of this plant, of which 18 species have been studied to date, and the results announced for the chemical analysis of fenugreek seeds are also related to these known species. There are many factors that affect the way different species of a plant are determined, which makes it difficult to accurately identify and distinguish between species of a plant. These factors are directly associated with the significant variability in morphology, growth cycle, flower color, leaves, stem, and chemical components between the species (Svecova and Neugebauerova 2010). Each plant, including the fenugreek plant, is known by different names which is related to their planting area. For example, fenugreek is known as hayseed in English-speaking countries, including the UK, as hulba in African and Arabic-speaking countries, including Egypt, and as Shanbalileh in Iran and other Persian-speaking countries (Ahmad et al. 2016). One of the outstanding features of this plant is its resistance to very low temperatures and even frost. In other words, it can be said that this plant grows well in the cold seasons of the year (Acharya et al. 2006). This plant grows very fast and unlike many plants does not need special conditions to grow. It grows well on cultivated or uncultivated lands, hillsides, and plains. This plant needs some amount of sunlight for better growth (Chayut et al. 2014). This plant is an erect, straight branched plant, almost 20–130 cm in length. The stem of this plant is green, but due to the accumulation of anthocyanins during plant growth, it turns pink. Generally, the stems are circular or slightly quadrangular in structure. The diameter of the stem is about 0.5 cm, and the leaves are simple and stipulate. The flowers of this plant show different colors depending on the time and conditions of growth. At the beginning of the growth of this plant, the flowers are yellow, which turn white when the plant reaches the end of growth. They are about 2 cm in size. Generally, the flowering season of the crop is from June to August. The shapes of the seeds are rectangular to oval, and the color of them differs from light brown to golden yellow (Madar and

Fig. 9.1 The seeds of fenugreek

Stark 2003) (Fig. 9.1). In general, the fenugreek plant needs about 4–7 months to fully grow. Also, it needs 5–10 days to germinate and the first leaves of this plant start to grow 5–8 days after germination. The ripening time of seeds is during last summer (August to September) (Amin et al. 2005). Various compounds are known to be in the fenugreek plant which include moisture, ash, protein, mucilage, lipids, alkaloids (like trigonelline), amino acids (like 4-hydroxyisoleucine), saponins, flavonoids, fibers (like dietary fiber), polyphenols, coumarin, vitamins, and minerals (Yadav and Kaushik 2011; Wani and Kumar 2018; Hamden et al. 2011). Leaves of this crop consist of approximately 86.1% moisture, 4.4% protein, 0.9% fat, 1.5% minerals, 1.1% fiber, and 6% carbohydrates. Additionally, various types of saponins including diosgenin have been identified in leaves. The mineral and vitamins exist in leaves include calcium (Ca), zinc (Zn), iron (Fe), phosphorus (P), riboflavin, carotene, thiamine, niacin, and vitamin C (Ravindran et al. 2011). The stem consists of mucilage, bitter fixed oil, protein, and alkaloids. Fenugreek is known for its pleasantly bitter, slightly sweet seeds. Fenugreek seeds can be used either whole or ground in various food formulations including spices, curry powders, and tea. One of the most important characteristics of seeds is their taste, which is neither sweet nor bitter. The use of these seeds has been common among the people of the countries producing this plant since ancient times. Fenugreek seed has a central rough and yellow embryo that is surrounded by a relatively thick layer of white and translucent endosperm (Betty 2008). The seed consists of 45–60% carbohydrates, particularly mucilaginous fiber (galactomannans), 20–30% proteins, 5–7% fixed oils (lipids), pyridine alkaloids, mostly trigonelline, choline, free amino acids (like 4-hydroxyisoleucine), minerals, vitamins, and phytonutrients (Wani and Kumar 2018). Additionally, it is considered to be an acceptable source of vitamin A and

minerals such as calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), and potassium (K). 6.28–9.3% of total weight of fenugreek seed is related to dietary fiber. The oil extracted from fenugreek is an essential oil, and it should be ingested or used on skin after dilution (El Nasri and El Tinay 2007). The presence of volatile compounds in fenugreek seeds causes its unique aroma. There are several methods to increase the amount of chemical compounds in fenugreek seeds that can be used during plant growth or after harvest. These methods include the use of enzymes during the germination, applying tissue and cell culture (static or suspension) and by biological manipulation of yield (Ahmed et al. 2010; Basu et al. 2009; Mehrafarin et al. 2011). Fenugreek, like other plants, is used in different countries and regions of the world for various purposes. In some areas, this plant has medicinal uses and is used as a blood sugar lowering agent, cholesterol lowering agent, and even an anti-microbial agent. While in other areas, it is used as an additive in food formulations. For example, in Iran, the seeds of this plant are used in cooked rice or in Switzerland, they are used to flavor cheese. In some areas, the plant is also used as an insect control in grain storages (Moradi Kor and Bayati Zadeh 2013). In addition to the possibility of using fresh fenugreek leaves, powdered, dried, and frozen leaves can also be used for medicinal and food purposes (El Nasri and El Tinay 2007). In some areas, fresh fenugreek leaves are used in bread and spices. They can also be used in salads and some types of cooked products. Drying the leaves of the plant preserves the aroma and prolongs their shelf life, and thus they can be used as great last-minute additions to curries, sauces, and soup (Hooda and Jood 2005). Conversely, fresh leaves of the plant are applied as common vegetables in the daily diet of people in some countries. The results of previous researches have shown that the leaves performed better than the seeds in meeting the nutritional needs (Wani and Kumar 2018). Today, one of the main goals of food producers is to shift food formulations towards healthy foods, which is due to the increasing demand from consumers to have these types of foods in their daily diet. Healthy foods are foods that, while meeting the nutritional needs of people, also have health-promoting properties and can be consumed as medicine which could prevent diseases in society. The presence of such products in the daily diet of people has become important. These types of foods are called functional foods that include various components like probiotics, prebiotics, vitamins, minerals, and dietary fiber (Nematollahi et al. 2016). It should be noted that fenugreek is a rich source of natural dietary fiber and this has led to the use of this plant in functional foods as an important ingredient. In general, the use of fenugreek seeds and leaves in the diet of people in addition to medicinal uses causes a variety of colors and flavors in food products and improves their texture in preferred way (Wani and Kumar 2018). In addition, fenugreek as a hydrocolloid, which is fenugreek gum (soluble fiber of fenugreek) can be applied as a thickening, emulsifying, stabilizing, gelling, and encapsulating agents and also it has positive effects on the textural and appeal properties. Therefore, the dietary fiber, especially soluble fiber can have a crucial role in improving the nutritional needs of people and can be used in various products including some types of dairy products including yogurts and cereal-based products. The powdered dietary fiber obtained from fenugreek seeds can be incorporated to the formulations of juices and spices.

Additionally, it can be applied to formulate tablets or capsules along with the other vitamins and nutrients certainly needed. Using the seeds of this crop in milk shakes, dressings, bakery and extruded products soups, and candies is being common in recent years which are mostly applied as a stabilizing or emulsifying agent (Wani and Kumar 2018; Im and Maliakel 2008).

9.2 Chemical Constituents of *Trigonella foenum-graecum* (Fenugreek)

9.2.1 Proteins and Amino Acid Composition

One of the most important components in the diet of any person, especially in developing countries, is proteins, which usually, people living in these countries, receive less protein than recommended amounts. Animal protein sources are limited and access to them is declining day by day, so researchers are looking for alternative sources that, while abundant, can meet a person's nutritional needs (El Nasri and El Tinay 2007). Official method of the AOAC (1990) is applied to determine proximate composition of fenugreek seed including protein content. In addition, the calculation of nitrogen is mainly accomplished by Kjeldahl technique where the protein content is measured by multiplying the nitrogen value with conversion factor of 6.25. The crude protein content in fenugreek seed is found to be range between 20% and 30% which was higher than values announced for other legumes (El Nasri and El Tinay 2007). The high protein content of fenugreek seeds allows this inexpensive source to be applied as an alternative to animal proteins which are expensive and hard to produce in most cases (Aljuhaimi et al. 2017). The dominant amino acids in fenugreek seeds are lysine, 4-hydroxyisoleuicine, aspartic acid, arginine, leucine, tryptophan, histidine, and isoleucine which are found to be higher compared with other amino acid values (Aljuhaimi et al. 2017). It has low levels of sulfur-containing amino acids like threonine and high levels of lysine and arginine (Bahmani et al. 2016). The defatting process can be considered as the most important step before using proteins in food formulations. Different solvents with different polarities can be applied for this process and cause significant changes in the physical, chemical, surface, and functional aspects of proteins and derived isolates. A very important point when choosing solvents for the defatting process is their effect on the denaturation of proteins that must be considered (Feyzi et al. 2017). The amino acid composition of protein isolates from various defatting solvents does not differ a lot with each other. There is no significant difference between total amino acid contents of fenugreek protein isolates except for fenugreek protein isolate obtained from chloroform defatted flour that had the lowest total amino acid content. The lowest amino acid content, also its least protein solubility could be associated with the denaturing effect of chloroform on proteins (Rahmati et al. 2017). The results of former researches have demonstrated that many factors can increase the amount of protein in fenugreek seeds including soaking, germination, and roasting. Germination of fenugreek seeds reduces the nitrates and converts them to proteins and

ammonium, which is the main reason for elevation of the amount of protein during this process. Germinated seeds have significant beneficial features over ungerminated fenugreek seeds. They are a great source of essential amino acids particularly leucine, lysine, and tryptophan. Additionally, germination has a positive effect on in vitro protein digestibility. Protein content is high in endosperm of the seed compared to husk (El Nasri and El Tinay 2007). High proportion of protein and amino acids particularly 4-hydroxyisoleucine in fenugreek has high potential for insulin-stimulating activity. The obtained protein fraction is an acceptable source of lysine and can be compared with soybean protein (Khorshidian et al. 2016). Emulsifying and foaming aspects of fenugreek proteins are completely affected by pH levels and salt (NaCl) concentration (El Nasri and El Tinay 2007). Minimum emulsifying capacity is obtained at pH 4.5, where proteins emerge to precipitate and are known as isoelectric point, causing reduction in emulsion formation. Maximum capacity of fenugreek protein concentrate is obtained at pH 12; alkaline pHs enhanced emulsion capacity more than did acidic pHs. Former researches have demonstrated that pH exerts its impact on emulsification aspects mainly by changing the charge on protein molecules. In addition, the emulsifying features of seed proteins are associated with the processing procedure and to the identified amino acids. Emulsion capacity depends on the hydrophobic–lipophilic balance, which is changed by pH (Kiosseoglu et al. 1999). pH values are also the most important parameter in estimating the foaming capacity of protein concentrates including fenugreek protein. The lowest foaming capacity is observed at pH 4.5 (isoelectric point of the protein); at this point, the molecules are in more compact form than at other pH values. In addition, this capacity significantly elevated at pH 10 and 12. The basic elements for a protein to be an acceptable foaming agent are the ability to adsorb immediately at the air–water interface during bubbling and the ability to undergo rapid conformational alterations and re-arrangement at the interface (El Nasri and El Tinay 2007). Suitable and acceptable emulsifying and foaming properties of fenugreek seed protein make it possible to use it in a wide range of food formulations, especially salad dressing, curried meats, ice cream, cakes, and mayonnaise. In addition, the high solubility of fenugreek seed protein makes it possible to apply it in the formulation of various types of products such as beverages. The obtained protein is found to be more soluble at acidic and alkaline pHs than near neutral pH. In some products, such as traditional staples of cereals and tubers, the amount of nitrogen available is low and fenugreek seed protein can be applied as a supplement to enhance the amount of available nitrogen (Abdelaal et al. 1986). Fenugreek protein concentrate has a water-holding capacity (WHC) of 1.68 mL H_2O/g of protein. WHC is a critical aspect of proteins in viscous foods like soups, custards, and baked products because these are expected to absorb water without dissolution of protein, thereby providing higher viscosity and acting as a thickening agent (Seena and Sridhar 2005). Fenugreek protein concentrate has acceptable dispersibility in both acidic and basic solutions. The high dispersibility of fenugreek seed protein is directly related to functional aspects such as emulsifying and foaming features which help to improve these properties during the production of some foods such as cookies (El Nasri and El Tinay 2007). Various researches have illustrated the

promising aspect of plant proteins as surface active agents. They could create and stabilize foam and emulsion systems by migration into water and air/oil interface, decreasing the surface or interfacial tension (Venegas-Caleron et al. 2017). Residual proteins indicate a considerable effect in declining the tension at the oil–water interface (El Nasri and El Tinay 2007).

9.2.2 Fat, Seed Oil, and Fatty Acid Composition

The crude fat could be recognized by extracting the sample in a Soxhlet apparatus applying petroleum ether as a solvent. After evaporation of solvent, the residue must be weighted to determine the fat content. The results obtained from former researches indicated a slight difference in the amount of oil extracted from fenugreek seeds that does not depend on the extraction method, and factors such as the solvent used in extraction are important. For example, ether may be used as a solvent instead of petroleum ether. The fatty oil extracted from fenugreek seeds is of great importance in cosmetic products especially hair styling preparations. Soxhlet extraction technique and n-hexane as a solvent are commonly applied to determine the oil obtained from fenugreek seed. Fenugreek seed contains 5–7% lipids comprising neutral lipids (85%) followed by phospholipids (10%) and glycolipids (5%). Former researches announced that more than 80% of fatty acids in fenugreek seed oil are unsaturated fatty acids particularly linoleic acid (40%), linolenic (25%), and oleic (14%) acids. These acids are dominant in fatty acid profile (Kiralan et al. 2017; Chatterjee et al. 2010; Pandey and Awasthi 2015). GC equipped with FI detector is generally applied to identify the fatty acid composition. Generally, the fatty acids must be esterified as methyl esters before analysis. The oil contents of fenugreek seeds are ranged between 5.8 and 15.2% having a yellowish color. The extracted oil is mainly characterized by the GC-MS analysis which confirmed availability of various compounds such as esters, alkanes, saturated and unsaturated fatty acids, glycerides, phenols, flavonoids, and alkaloids (Akbari et al. 2019). The results obtained from former researches demonstrated that fenugreek oil has an antimicrobial activity against some types of microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium,* and *Aspergillus niger*. According to the former studies, linoleic acid (54.13%), palmitic acid (16.21%), and linoleic acid methyl ester (3.19%) are the main fatty acids in the fenugreek oil structure. Linoleic acid is known as polyunsaturated omega-6 fatty acid, having 18-carbon chain with twin bonds in cis configuration. This fatty acid is known to play an effective role in a person's health due to its anti-cancer activity. It must be mentioned that the effect of using this fatty acid in the treatment of breast cancer has been proven (Akbari et al. 2019). Palmitic acid is known for its biological and antioxidant activity. Additionally, it is one of the most common saturated fatty acids with 16 carbon backbone found in plants, animals, and microorganisms. It has been also applied as a food additive and anti-inflammation; its daily intake may lead to a healthier life (Benzidia et al. 2019). In recent years, *N*-acylethanolamines (NAEs) and their precursors, *N*-acyl phosphatidylethanolamines (NAPEs) have been

established as phospholipid constituents in dried seeds of various plant species. These minor membrane lipid constituents have been implicated in lipid signaling pathway that regulates an array of physiological processes in multicellular eukaryotes like plant defense response and seedling root development (Chapman 2004). Long-chain fatty acid amides are another division of signaling lipids with a physiological role in mammalian nervous system. Oleamide as an important member of this class is a sleep-inducing lipid with diverse action such as antinociceptive (pain reducing) features and also it stimulates increased food uptake. There is an elevated interest in determining natural origins of NAEs, NAPEs, and fatty acid amides for therapeutic uses (Chatterjee et al. 2010). The NAPE and NAE contents of fenugreek have been analyzed by GC/MS which is announced to be about 7.23μg/g and 728 ng/g of the spice, respectively (Srinivasan 2006). The content of oleamide is about 1.8 mg/100 g of fenugreek. The germination is a type of process that consumes plant energy. As a result of this process, the amount of fat in fenugreek seeds is reduced, which is due to the use of fats as an energy source. In other words, germination enhances the fat absorption capacity (Pandey and Awasthi 2015). Decrease in fat content also includes reduction in total unsaturated fatty acids, free fatty acids, triglycerides, phospholipids, monoglycerides, and polar lipids, while those of saturated fatty acids are elevated (El-Aal 1986; Dixit et al. 2005). The fat content of germinated endosperm, seed coat, and sprouts is reported to be about 11.44%, 1.75%, and 6.65%, respectively, whereas ungerminated seed endosperm and seed coat contain about 12.26% and 1.22% fat, respectively. The fatty acid composition results indicated that the ungerminated fenugreek endosperm and seed coat are rich in unsaturated fatty acids such as oleic, linoleic, and linolenic acids, the dominant one being linolenic acid in both ungerminated endosperm and ungerminated seed coat. Among the saturated fatty acids, the prominent one is palmitic acid with smaller amounts of stearic and arachidic acids. Lauric and myristic acids are available in trace amounts. The fatty acid composition of endosperm from germinated seeds demonstrated little variation when compared to ungerminated seed endosperm. The linoleic and linolenic acid contents decreased, while oleic and stearic acids contents elevated (Shakuntala et al. 2011a). Soaking of fenugreek seeds may reduce fat content. Similarly, roasting may also cause reduction in fat content. The impact of roasting upon the fat content of the beans is to decline its actual weight with the shrinkage. In fact, roasting, which accompanies the heating process, removes volatile fatty acids from the seeds. The mechanism of this removal is such that, as a result of roasting, the fat in the seeds breaks and converts to free fatty acids and other compounds. These fatty acids have a relatively high volatility and as a result, they come to the surface of the seed wall and transfer to the outside of the seed. The reduction in the amount of fat during roasting can be justified by the mentioned mechanism (Mathur and Chaudhary 2009).

9.2.3 Fiber (Insoluble and Soluble) Content

Nowadays, availability of fiber-rich foods in the daily diet is highly recommended. The effect of using fiber on improving people's health has been proven. Fiber can reduce calorie intake, elevate chewing time, control overeating, and stabilize body weight. The crude fiber content of fenugreek seeds is in the range of 6.50–11.97% (Khorshidian et al. 2016). Dietary fiber is divided into two main types including soluble and insoluble, and when calculating the total dietary fiber, the sum of soluble and insoluble fibers is considered. Dietary fiber is an indigestible carbohydrate complex that plays a very important role in human health. The soluble type of these fibers has the ability to form gels and the insoluble type does not have this ability. Insoluble dietary fiber is found mostly in fruits and vegetables, but legumes, and especially fenugreek seeds, have soluble dietary fiber. Dietary fiber causes satiety in person and thus reduces the amount of calories received per day in each meal and, ultimately, prevents weight gain and overeating. The mechanism of satiety is through the delay in emptying the gastrointestinal tract. The amount of dietary fiber in fenugreek can be compared to legumes such as guar. About 30% of the weight of fenugreek seeds is soluble dietary fiber, which, like those obtained from guar and psyllium, can form a gel with a suitable viscosity. Conversely, about 20% of the seed weight is composed of insoluble dietary fiber, which is bulk-forming like wheat bran (Srinivasan 2006). The use of dietary fiber in fenugreek seeds reduces the level of LDL in blood, which does this by reducing the reabsorption of bile salts in colon. In addition to dietary fiber, compounds such as hemicellulose, saponins, tannins, and pectin are also involved in this process (Khorshidian et al. 2016). Additionally, the results obtained from previous researches have demonstrated that dietary fiber in fenugreek seeds, in addition to lowering LDL levels, traps toxins entering the body through contaminated food or water, and thus, prevents these toxins from adhering to the mucous membrane of the colon. Also, the availability of dietary fiber in daily diet can indirectly play a role in lowering blood sugar level. As mentioned before, fenugreek seeds can indicate functional properties that is directly associated with the dietary fiber in these seeds, which can play the role of prebiotic and transfer the microorganisms needed by colon to the body and change the microbial flora of the intestine (Mohammadi and Mortazavian 2011). Dietary fiber obtained from fenugreek is very stable, with a long shelf life. It tolerates various types of processing including frying, baking, cooking, and freezing. Slight alteration in the physical conditions of processing can yield dietary fiber with distinct water absorption aspects. Therefore, dietary fiber with a high water retention capacity is made into jelly and spreads and applied as thickener. Dietary fiber can be taken as plain powder mixed in fruit juices or may be added to food items like soups and beverages. Fenugreek dietary fiber has also been incorporated into flour to make chips and breads. By fortifying these products with fiber, one can elevate the total dietary fiber intake (Srinivasan 2006). In recent years, one of the rapid ways to lose weight that has been considered is to use a protein-based diet and eliminate carbohydrates, which can cause health problems. Eliminating carbohydrates such as bread and rice can indirectly cause hair loss. Former studies have shown that by

having enough fenugreek seeds in the daily diet and proper physical activity, you can experience continuous weight loss without side effects of other diets (Khorshidian et al. 2016). One of the main soluble fibers of the fenugreek seeds is galactomannan that reduces the bile salts uptake in the intestine and also declines the digestion and absorption of starch in body (Madar and Shomer 1990). It has been announced that fenugreek seed husk is a noteworthy origin of dietary fiber and phenolic acids which could be an effective origin of natural antioxidants and natural ingredients in functional foods (Khorshidian et al. 2016). The endosperm of fenugreek seed is approximately low in fiber (Naidu et al. 2011). The amount of fiber in raw, germinated, and boiled fenugreek seeds is significantly different, so that raw fenugreek seeds have the highest amount of neutral detergent fibers and acid detergent lignin, followed by germinated and boiled seeds (Sharara 2017). Performing processes such as soaking, sprouting, and roasting is not ineffective in the amount of fiber in fenugreek seeds. Soaking of seeds can reduce the content of all types of dietary fibers. Probably the main reason for the reduction might be associated with the enzymatic degradation of seeds during soaking (Pandey and Awasthi 2015). Like soaking, roasting of the seeds also reduces the amount of fiber. The main reason for this decrease is the retrogradation of starch existed in the seed structure during the roasting process (Mathur and Chaudhary 2009). Unlike soaking and roasting, germination of seeds elevates the amount of fiber. Fiber is a major compound of the cell wall, and germination results in greater synthesis of components such as hemicellulose and cellulose. In this way, the amount of fiber available increases. It should be noted that this elevation is mostly related to insoluble fibers (Pandey and Awasthi 2015; Shakuntala et al. 2011b).

9.2.4 Carbohydrate Content

Total non-polysaccharide carbohydrate content of fenugreek seeds can be calculated applying the standarad method (Brummer et al. 2003). There is a significant amount of mucilage in the endosperm of seeds, which is affiliated with the laxative aspects of fenugreek. Also, it indicates a high water holding capacity and its emulsifying and suspending properties are acceptable. (Bahmani et al. 2016). Fenugreek seed gum is mainly obtained from the endosperm. The endosperm is a relatively thick, white, and semi-transparent layer that is surrounded by a dark brown rough husk. The color of the obtained gum is highly dependent on the color of the husk. The closer the husk color is to dark brown, the darker the gum color will be. There are several methods for extracting gum from plant seeds, but the aqueous extraction method is the most common. The main reason for using this method is its low cost and good extraction yield. In the extraction procedure, precipitation by 95% ethanol must be done to obtain the gum. One of the most important steps during the gum extraction process is its purification. Purification means the removal of non-polysaccharides such as protein, fat, saponins, and other substances to obtain the purified gum. The chemical composition of fenugreek seed gum is mostly related to the extraction procedure applied and variety/cultivar of the seed (Salarbashi et al. 2019). The average

extraction yield of the gum is reported to be about 15.04% (w/w). The difference in reported yield values may depend on factors such as extraction method, plant species, plant growth conditions, plant harvest conditions, and plant growth location (region or country in which the plant has grown) (Salarbashi and Tafaghodi 2018). One of the most difficult steps in purifying the extracted gum is the complete separation of the protein from the polysaccharide. Up to now, no method is known for complete separation of protein from polysaccharide. One common way to remove protein from the gum structure is to use a protease called pronase. The results indicated that by using this enzyme, the amount of protein in the gum structure would be reduced. Commercial gums usually have a small amount of protein in their structure. Proteins are directly related to the emulsifying and foaming properties of gums. Therefore, it can be expected that gum obtained from raw seeds, which has not been purified, will have better emulsifying properties than purified gum. Performing certain processes such as extrusion can cause changes in the chemical configuration of the gum. For example, this process can increase the number of hydrophilic groups that are supposed to react with water during extraction. In addition, performing extrusion may alter the distribution of molecular mass of fenugreek seed gum. In addition, this process improved water solubility and hydration capacity of the gum. But extrusion has no significant impact on water holding capacity of the gum (Chang et al. 2011). As reported in results obtained from former researches, extrusion process had little effect on the emulsifying properties of fenugreek gum. Because the extrusion process done is not sufficient enough, it cannot significantly affect the protein in the structure of the gums, and as a result, the emulsifying properties of the gums do not change much. Unlike gum, the extrusion process improved the emulsifying properties of proteins. This effect is associated with the unfolding of the protein molecules that resulted in exposing their hydrophobic functional groups from the inside, and in consequence, improvement of the emulsifying capacity of proteins (Chang et al. 2011). Gums are considered as hydrocolloids. Hydrocolloids are high molecular weight biopolymers that are applied as thickening, gelling, stabilizing, and emulsifying agents in food formulations. One of the factors involved in the gelling and stabilizing properties of gums is its molecular weight. Fenugreek seed gum indicated a higher molecular weight than other commercial galactomannans, including guar and locust bean gum. Previous researches have shown that high molecular weight in gums can improve their stabilizing activity. The extrusion process also affects the molecular weight of the gum. In other words, the molecular weight of extruded gum is different from that of non-extruded gum. This can be related to alterations occurring in the structure of the gum during extrusion (Liu et al. 2020). Analyzing the type and amount of monosaccharides in gum structure is the first and most important step in determining the potential of using gum in the formulation of food, pharmaceutical, and cosmetic products. Also, the monosaccharides are directly related to the rheological and functional aspects of the gums. The identified monosaccharides in fenugreek seed gum are rhamnose, arabinose, galactose, glucose, and mannose. Mannose and galactose are the major monosaccharides of fenugreek seed gum, indicating a galactomannan structure for this gum. The distinction between the monosaccharide

composition of fenugreek seed gum may be related to the different parameters including extraction method, plant species, plant growth conditions, plant harvest conditions, and plant growth location (region or country in which the plant has grown) (Salarbashi and Tafaghodi 2018; Raghuram et al. 1994). The structure of galactomannan consists of a 1,4 linked β-D-mannosyl backbone with single unit galactose side chains, α-linked at the O-6 oxygen.

The galactose and mannose content of fenugreek gum can be recognized by applying the HPLC-PAD system and comparison of the results with reliable standards (Brummer et al. 2003). Fenugreek galactomannan is considered to be unique due to a 1:1 to 1.2:1 ratio of galactose to mannose molecules (Acharya et al. 2008). This high ratio of galactose substitution helps galactomannan to produce high viscosity at relatively low concentrations and also elevates its water absorption capacity. Eventually, excess glucose uptake decreases during the digestive process. This ability of galactomannans derived from fenugreek seeds can be very effective in people who check their calorie intake daily and are interested in losing weight (Ramesh et al. 2001). Hydrocolloids are insoluble in organic solvents, and galactomannans obtained from fenugreek seeds also follow this rule. They can be precipitated from aqueous solution by adding hydrophobic solvents like ethyl alcohol. The galactomannans are known as good viscosity builders. They indicate non-Newtonian behavior in which the viscosity reduces with the shear rate. The galactomannans are commonly applied when a considerable thickening or stabilizing effect is needed. Having these properties has made it economical to use galactomannans in food formulations (Niknam et al. 2020a). Galactomannans are mostly applied in the formulation of emulsion-based products. The role of galactomannans in such products is not only as a thickening. They have a crucial role in their stability which is known as the main factor in emulsions. Salad dressings are good examples of such systems. The surface activity of hydrocolloids is directly affiliated with the amount of protein in their structure. Thus, crude galactomannans obtained from fenugreek seeds indicated higher surface activity than purified galactomannans (Yousefi et al. 2009). In addition to surface activity, the emulsifying activity of polysaccharides is also associated with the availability of a small amount of protein in their composition which has more tendency to be adsorbed at the oil–water interface and form a stabilizing layer around oil droplets. Compared to commercial galactomannans like guar gum and locust bean gum, galactomannans obtained from fenugreek seeds have greater emulsion stability. Various factors can contribute to this type of behavior, including the ratio of mannose to galactose, molecular weight, and interfacial activity. Galactomannan derived from fenugreek seed can decline the surface tension even to levels lower than guar gum (42 and 55 mN/m) (Khorshidian et al. 2016). The interfacial features of the hydrocolloids (like galactomannans) in emulsions play a very crucial role in the droplet size of emulsions and their long-term stability. Previous studies have demonstrated that the interfacial activity of galactomannans derived from fenugreek seeds is better than other commercial galactomannans like guar gum. The presence of galactomannan in the emulsion structure increases the continuous phase viscosity and, consequently, the droplet motion in the emulsion decreases. The ability to reduce emulsion droplet

size in galactomannan obtained from unpurified fenugreek seed is greater than that of the purified type. This is probably due to the existence of more protein in the unpurified galactomannan structure. In contrast, purified galactomannan has the ability to produce higher viscosities and thus can perform better in the stability of the emulsions. Obviously, with elevating galactomannan concentration, the viscosity increases and the emulsion stability increases (Kasran et al. 2013). The edible film based on fenugreek seed gum indicated acceptable physicochemical and mechanical aspects (Salarbashi and Tafaghodi 2018). One of the most important aspects of hydrocolloids, of which galactomannans are a part, is their bulk density. The bulk density of fenugreek gum obtained from various cultivars differs significantly ranging from 0.615 g/mL to 0.735 g/mL. This parameter mostly affiliated with the solid density of materials and size, geometry and surface aspects of individual particles (Bala Dhull et al. 2020; Rashid et al. 2018; Gadkari et al. 2018). The whipping of gums constructs a stable network resulting in foam formation that can withhold smaller solute particles. Fenugreek gums obtained from various cultivars indicated a significantly high foaming capacity as well as foam stability. The foaming capacity of a gum directly demonstrates its ability to reduce the equilibrium tension at the surface of air/water interface. The foaming stability of fenugreek gum is found to be higher than guar gum, suggesting its application in various products based on foams including meringues and marshmallows (Gadkari et al. 2019; Niknam et al. 2018, 2019a, b, c, 2020b; Kumar Shukla et al. 2017).

9.2.5 Ash Content, Minerals, and Vitamins

The dried fenugreek seeds could be analyzed for the contents of ash using AOAC methods (Acharya et al. 2006). The difference in the ash content could be attributed to the different parameters including extraction method, plant species, plant growth conditions, plant harvest conditions, and plant growth location (region or country in which the plant has grown) (Khorshidian et al. 2016). Total ash content is marginally higher in endosperm, followed by whole fenugreek and husk. The results obtained from previous studies indicated that some processes, including germination, reduced the amount of ash in the seeds which is mostly observed in the endosperm and seed coat (husk) (Shakuntala et al. 2011a). As mentioned before, fenugreek seeds are not a good source of minerals, but some elements are present in acceptable amounts like phosphorus (P) and sulfur (S). It has also been announced that curry made from fenugreek has a high content of calcium (Ca), iron (Fe), and zinc (Zn) (Jani et al. 2009). There are some amounts of magnesium (Mg), manganese (Mn), and selenium (Se) in the fenugreek seeds. In addition to seeds, which are the main origin of minerals, fresh fenugreek leaves also have some mineral elements that can be mentioned, such as magnesium, calcium, phosphorus, iron, sodium (Na), potassium (k), sulfur, manganese, and zinc (Srinivasan 2006). Performing processes such as germination is not ineffective in the amount of minerals in seeds and could make some alterations in their levels. The results derived from former studies announced that the amount of some minerals particularly calcium and phosphorus in the

endosperm and seed coat of the germinated seed elevated during the process which might be associated with the reduction in phytates, tannins, and other anti-nutritional elements that bind the minerals. In contrast, the amounts of some minerals, especially potassium reduced in both seed coat and endosperm during germination compared to its levels in ungerminated seed fractions. It has also been announced that during the germination process, the amount of minerals may decrease or increase only in a part of the seed such as the endosperm. After the process, the levels of magnesium, zinc, manganese, and copper in the endosperm of fenugreek seeds decreased. While the amounts of these elements in the seed coat did not change and demonstrated marginally elevated levels. During the germination process, iron transfer from the endosperm to the sprouts and seed coat was observed. In fact, iron leaked into the soaking solution (Durhan et al. 2002). In addition to the endosperm, various mineral elements are present in the seed coat and particularly, sprout of the seeds, and processes such as germination affect the amount of these elements. The results demonstrated that germination resulted in elevation in the potassium, zinc, and iron levels in germinated sprouts. In contrast, magnesium and calcium levels were reduced compared with ungerminated sprouts (Shakuntala et al. 2011a). The results obtained from former researches demonstrated that boiling fenugreek seeds reduced the amount of minerals, especially phosphorus, calcium, and zinc (Sharara 2017). Soaking of fenugreek seeds has no significant impact on total ash content. Performing this process has resulted in a decrease in elements such as calcium and zinc and an elevation in elements such as phosphorus. The observed reduction in zinc content in soaked and germinated seeds is associated with the leaching of zinc into soaking medium. Comparatively lower contents of mineral when soaked in water might be affiliated with the leaching out of some amount into soaking water (Pandey and Awasthi 2015). Fenugreek is not a good source of vitamins compared to other plants, but it has acceptable amounts of some vitamins, including vitamin C, β-carotene, thiamine, riboflavin, nicotinic acid, and folic acid. These vitamins are mostly found in fresh leaves and fenugreek seeds. Due to the vulnerable nature of vitamins, performing certain processes such as boiling, frying, germination, and irradiation (especially gamma radiation) can reduce their amounts (Srinivasan 2006).

9.2.6 Secondary Metabolites (Bioactive Compounds)

Polyphenols and Flavonoids

Plants are considered to be a rich source of bioactive compounds and phytochemicals and a wide range of these substances including phenols, tannins, alkaloids, saponins, flavonoids, coumarin, and terpenoids are found in plants. In scientific sources, these compounds are known as secondary metabolites (Knott et al. 2017). Many bioactive components have been identified in fenugreek, especially its seeds which are widely shown in Fig. 9.2 (Guardiola et al. 2018).

Bioactive compounds and phytochemicals have a variety of features that encourage food manufacturers to use these compounds in food formulations. The results obtained from previous researches have also indicated that these compounds can

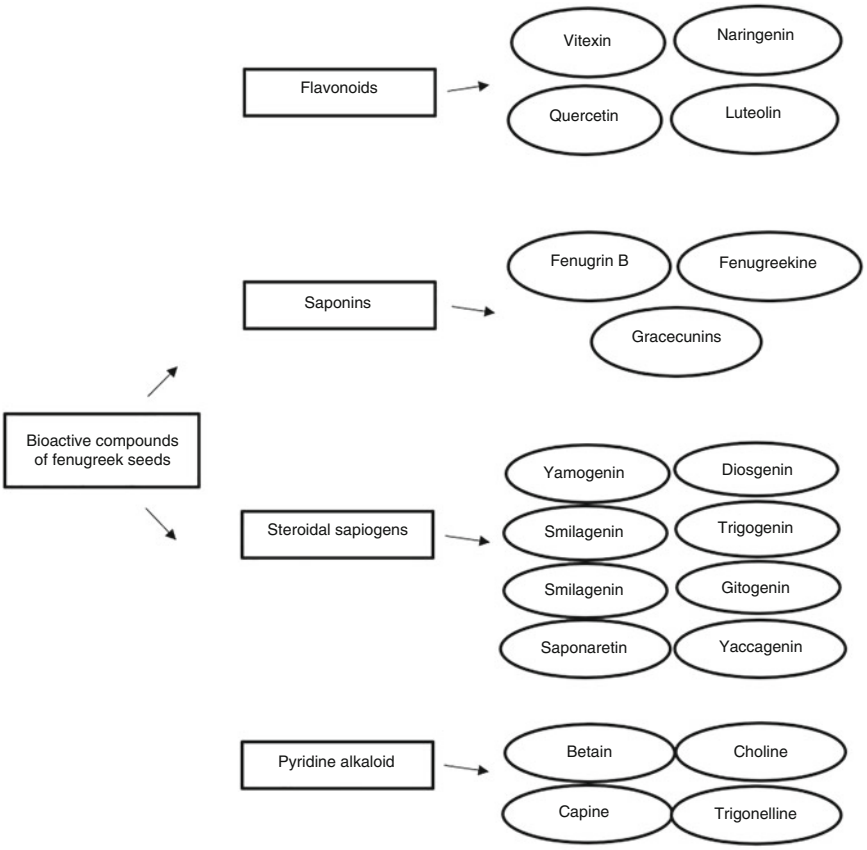

Fig. 9.2 The bioactive compounds of fenugreek seeds

have medicinal aspects and play an effective role in the treatment of some illnesses, especially cardiovascular disease and various cancers. For example, one of these compounds is polyphenols, which can act as antioxidants due to the existence of hydroxyl (OH) group in their configuration. Nowadays, with the advances that have been made in new technologies like nanotechnology, compounds such as polyphenols can be incorporated into the formulation of food products, and the product can act as a medicine in addition to meeting the nutritional needs which is known to be its main task (Riasat et al. 2018). For example, the results of a study demonstrated that regular consumption of foods high in polyphenols in the daily diet can reduce mortality from coronary heart disease. The mechanism of action of polyphenols is that they act as reducing agents and terminate free radicals. They can also act as metal chelators. Additionally, comprehensive research on rats has shown that the use of polyphenols in their diet can lower cholesterol levels and triglyceride concentration in the blood (Belguith-Hadriche et al. 2013). Fenugreek

seeds are a rich origin of polyphenols. The type of process applied to extract the bioactive compounds including polyphenols is effective in their yield. Efforts are based on achieving the highest extraction yield while ensuring that the resulting compounds are of good quality and are not degraded during the process. In addition to the type of extraction process, other factors affect the extraction yield, such as the plant matrix, extraction time, extraction temperature, type of solvent, and the presence or absence of new techniques such as ultrasound or microwave. To calculate the total phenolic content of fenugreek seeds, the extract of the seeds must be prepared and mixed with Folin–Ciocalteu reagent and sodium carbonate. Then, the adsorption of obtained solution must be measured by spectrophotometer at 765 nm. Finally, the amount of phenols available can be determined using the standard curve obtained from gallic acid at various concentrations (Nickel et al. 2016). The results obtained from measuring the adsorption of these compounds by spectrophotometer, which is the main technique for analyzing polyphenols, have shown that alcoholic and particularly, ethyl acetate extracts of the seeds have higher levels of polyphenols (Kenny et al. 2013). To calculate the total flavonoid content of fenugreek seeds, ethanol extract of the seeds must be prepared and mixed with diluted aluminum chloride. Then, the adsorption of obtained solution must be measured by spectrophotometer at 415 nm. Finally, the amount of flavonoids available can be determined using the standard curve obtained from quercetin at various concentrations (Alara et al. 2017). Using HPLC technique as a precise method in determining the amount of bioactive compounds, especially polyphenols and flavonoids, has become common in recent years and is often based upon the retention time of specific constituents, on a specified column, in comparison to a group of reliable standards. HPLC analysis indicated that the extracts obtained from fenugreek seeds were rich in polyphenols, especially apigenin. In addition, this technique has been applied to identify flavonoids in various extracts of fenugreek seeds and the presence of vitexin, tricin, naringenin, quercetin, and luteolin has been proven. Due to the wide range of phenolic and flavonoid compounds, the HPLC technique may indicate some errors and could not determine all components correctly. Thus, other precise techniques including UPLC (ultra-performance liquid chromatography) are applied. These are constructed to apply columns with smaller particle sizes at higher pressures, thus facilitating greater resolution, sensitivity, and speed when analyzing extracts from fenugreek seeds (Kenny et al. 2013). The fenugreek ethyl acetate extract indicated the highest antioxidant activity which is associated with its high phenolic compounds. The amount of polyphenols varies in different parts of the fenugreek seed in which the husk has the highest amount of these compounds. The results obtained from previous researches have indicated that by performing various processes on fenugreek seeds, the amount of phenolic and antioxidant substances increases compared to raw seeds. In contrast, the amount of dietary fiber and phytic acid decreases. One of the common processes is soaking of the fenugreek seeds, which increases phenolic compounds. As the concentration of these compounds increases, the antioxidant properties are also enhanced, which is directly related to the concentration of polyphenols. In contrast, phytic acid levels decrease during soaking which ultimately resulted in significant elevation in in vitro

starch digestibility and in vitro protein digestibility. Like soaking, the germination process of fenugreek seeds also reduces phytic acid levels. Former studies have demonstrated that phytase is inactive in non-germinated seeds. By performing the germination process, this enzyme is activated and decreases the amount of phytic acid in the seeds. Germination causes significant elevation in in vitro protein digestibility and in vitro starch digestibility. This process significantly increases phenolic compounds and, consequently, antioxidant properties (Nickel et al. 2016). It should be noted that the germination process is divided into different stages based on time. The results obtained from previous researches have indicated that the highest amount of phenolic compounds and its antioxidant features are observed in the early stages of germination and gradually, with the passage of time and near the end of germination, the amount of these compounds decreases, but in any case, these components are more than non-germinated seeds (Belguith-Hadriche et al. 2013; Nickel et al. 2016). To describe the germination process, it should be noted that the starting point of this process is when the dried fenugreek seeds begin to absorb water. The decrease observed in phenolic compounds at the end of the germination process compared to the beginning is due to the fact that the seed uses the available compounds, most of which are in the endosperm or seed coat, to complete its seedling growth. The goal of food manufacturers is to be able to produce products that can fully meet the nutritional needs of the individual and without harming the health of him/her. The presence of high polyphenolic compounds in fenugreek seeds, especially in germinated seeds, has made it one of the selected options for use in food products. In general, germination process and heat treatments have a crucial impact on nutraceutical aspects of all leguminous plants (Sharara 2017). Similar to the previous two processes, germination and soaking, roasting of fenugreek seeds also reduces phytic acid levels. As a result of this process, degradation occurs in the structure of phytic acid. Among the three processes discussed, germination results in a more significant reduction in phytic acid content (Ahmad et al. 2016). In contrast, by performing this process, the amounts of phenolic compounds increase and, consequently, the antioxidant aspects enhance. Roasting of fenugreek seeds, in addition to reducing phytic acid levels, destroys other anti-nutritional compounds such as tannins and oxalates. Therefore, elevation in in vitro starch digestibility and in vitro protein digestibility after roasting might be related to destruction of these components. As mentioned earlier, the presence of ascorbic acid in fenugreek seeds has been proven. Unlike polyphenolic compounds, processes such as germination reduce the amount of ascorbic acid in the seeds. In contrast, changes in ascorbic acid levels during the boiling process (heating) are similar to changes in polyphenolic compounds in which by performing this process, the amount of ascorbic acid decreases (Durhan et al. 2002; Knott et al. 2017; Guardiola et al. 2018; Riasat et al. 2018).

Saponins

One of the most important compounds in plants, which is a subset of secondary metabolites, is saponins. These components can protect the plant against pathogens. In addition, the presence of these compounds in plants can reduce the use of

insecticides and prevent the side effects of these chemical-based products on plants. In recent years, the incorporation of saponins in food and pharmaceutical formulations has increased due to its good functional aspects (Augustin et al. 2011). Saponins are classified into two major groups that are triterpenoids and steroidal glycosides and can be distinguished by the sugar chains attached at different positions (Akbari et al. 2019). The saponin extracted from fenugreek seed is classified as steroidal glycosides. The saponins identified by HPLC analysis in fenugreek seeds include graecunins, fenugrin B, fenugreekine, trigofoenosides A-G, yamogenin, diosgenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin, gitogenin, yaccagenin, and saponaretin. Among these compounds, diosgenin and yamogenin are in high levels which are announced to be about 4.8% in seed. These two compounds have estrogenic and androgenic activities and, as a result, demonstrate anti-fertility activity and cause teratogenicity (Al-Yahya 2013). One of the considerable features of saponins in fenugreek seeds is the bitter taste of the seeds. As the consumer is more interested in sweet or neutral flavors, the presence of this flavor has led to widespread restrictions on the use of fenugreek seeds in the formulation of food products. Bitter taste can be omitted by performing various traditional processing techniques such as germination, soaking, and roasting. The results obtained from former researches have demonstrated that soaking and roasting of the seeds can be more effective than germination in removing the bitter taste. As mentioned earlier, performing processes like soaking can act a crucial role in increasing the low methoxy salts of calcium and magnesium. Presence of these salts in fenugreek seeds, in addition to reducing the bitter taste, has some medicinal properties, including lowering blood sugar levels (Jasim et al. 2015).

Diosgenin is widely applied in the manufacture of pharmaceutical products, especially steroidal drugs and some hormones such as progesterone (Jasim et al. 2015; Gyawali and Ibrahim 2014). Diosgenin content of fenugreek varied between 0.43 and 0.52% which is mainly obtained from HPLC analysis with UV detector (Niknam et al. 2019a). As a result of acid hydrolysis of saponin, a compound called sapogenin is produced. Acid hydrolysis is the common method and also the most economical technique for converting saponin. One of the main features of saponin is its inhibition against α-glucosidase. The results obtained from analyzing sapogenin indicated that this compound has more inhibitory aspects against α-glucosidase than saponin (Zhang et al. 2020). It should be noted that during acid hydrolysis, in addition to the production of sapogenin, other bioactive compounds are produced that can elevate the functional aspects of obtained extracts. These compounds include phytosterols and tocopherols, which are known as bioactive compounds and have many medicinal aspects. Also, they can be incorporated to the food formulations to produce functional foods (Herrera et al. 2019). Among the different parts of fenugreek seeds, the seed coat (husk) has the lowest amount of saponin. The amount of saponin in the endosperm is significantly higher than the husk. Therefore, it can be inferred that by dividing the seeds into endosperm and husk, considerable changes may be observed in the amounts of a number of bioactive components (Naidu et al. 2011).

Coumarin

One of the most beneficial bioactive compounds in fenugreek seeds is coumarin. A wide range of constituents including lactone orthodihydroxy cinnamic acid and scopoletin are subsets of coumarin. These components can play a very effective role in improving the performance of anticoagulants like warfarin. In fact, the presence of these compounds reduces the accumulation of platelets in blood vessels in which this aggregation can increase a person's risk of bleeding (Lambert and Cormier 2001).

Alkaloids

One of the most important bioactive compounds in fenugreek seeds is alkaloids that have many functional and medicinal properties. In recent years, the use of these compounds in food formulations has increased significantly. In addition to saponins, alkaloids are also effective in the bitter taste of fenugreek seeds. Like other bioactive compounds, performing various processes can cause considerable changes in the amount of alkaloids. The results obtained from former researches has indicated that boiling, as a common process, increases alkaloids, and germination reduces their amount (Lambert and Cormier 2001). By performing the germination process, the bitter taste of the seeds is reduced which could be affiliated with the reduction in the amount of alkaloids. Up to date, many alkaloid compounds have been identified in fenugreek seeds including trimethylamine, nevirin, choline, gentianine, capaine, betaine, and trigonelline in which, trigonelline, is considered to be more important than others (Herrera et al. 2019; Lambert and Cormier 2001). HPLC equipped with UV detector is mostly applied to identify alkaloids especially trigonelline. Trigonelline content of fenugreek seeds varied between 0.74 and 0.97%. Performing various processes particularly roasting of fenugreek seeds converts trigonelline to nicotinic acid and similar compounds, which are responsible for the specific flavor of the seeds. The presence of this flavor reduces the interest in consuming these seeds by people. As mentioned before, by performing various processes, inappropriate flavor can be eliminated and make it possible to apply the seeds in the formulation of food products (Naidu et al. 2011). In addition, trigonelline has some medicinal features. It can significantly reduce the blood sugar levels. As a result, the presence of this compound in the diet of diabetics can be beneficial. The mechanism of action of this compound to lower blood sugar is that it protects the β-cells in the pancreas and also elevates the insulin sensitivity index (Zhou et al. 2013).

Volatile Compounds

The volatile compounds could be analyzed by GC-ion trap mass spectrometry. There are many types of volatile compounds that have been identified in fenugreek seeds but A-sitosterol and dextroamphetamine are known to be dominant. Performing various processes has a significant effect on the amounts of volatile compounds. So that the highest amount of these compounds is observed in boiled seeds and the lowest amount is indicated in germinated seeds. The reason for the increase in volatile compounds during the heating or boiling process is the destruction of the

structure of high molecular weight compounds and their conversion into smaller molecules. Also, heating may not necessarily damage the structure, but it can cause changes in the structure, which in turn increases the amount of volatile compounds (Laroubi et al. 2007; Sharara 2017).

Sotolone

Recent results obtained from researches done by GC-MS indicated that sotolone (3-hydroxy-4,5-dimethyl-2(5H)-furanone) is the main cause of the specific and unpleasant odor of fenugreek seeds (Mebaza et al. 2009). As mentioned before, one of the key amino acids in fenugreek seeds is 4-hydroxyisoleucine, which is a precursor to sotolone. In addition to sotolone, there are other compounds that may play a role in the specific flavor of fenugreek seeds including butanoic acid, pyridine, and methyl nicotinate (Mazza et al. 2002). Additionally, like sotolone, these compounds have been determined and analyzed by GC-MS. It should be noted that depending on the type of extraction and the solvent applied, there may be changes in the amount of effective constituents in the flavor of fenugreek seeds (Mebaza et al. 2009). The results of former researches have shown that extracts obtained with ethanol as solvent indicated higher amounts of effective compounds in flavor, including volatile compounds. Methanol and dichloromethane as other common solvents had lower levels of these compounds compared with ethanol. It should be noted that each of these solvents can be effective in extracting other specific compounds. For example, ethanol, in addition to its ability to extract volatile compounds that affect the flavor of fenugreek seeds, can also be used to extract compounds with low and medium volatility such as fatty acids and saponins which are not known to be involved in particular aroma of fenugreek seeds. Also, dichloromethane is more effective in extracting compounds that do not play an effective role in the flavor of fenugreek seeds, such as long-chain alkanes. Among the three solvents discussed, methanol plays a more important role in the extraction of sotolone compared with others (Mebaza et al. 2009; Bahmani et al. 2016).

9.3 Fenugreek as a Multipurpose Crop

Recent pharmacological researches authenticated that fenugreek seeds have anti-diabetic, anti-obesity, anti-ulcer, anti-analgesic, anthelmintic, allelopathic, anti-bacterial, anti-fungal antioxidant, and anti-inflammatory activities (Yao et al. 2019). Fenugreek is widely applied in food, pharmaceutical, and cosmetic industries due to its mentioned benefits. In some areas, only the dried or fresh leaves of the plant are used, but in other areas, the seeds are applied as flavorings or mixed with spices (Zou et al. 2012). In addition to industrial uses, fenugreek is also applied as a traditional treatment for burns and swelling. Also, in some countries, especially India, the seed extract of the crop is used to prevent hair loss (Nayak et al. 2013) (Fig. 9.3).

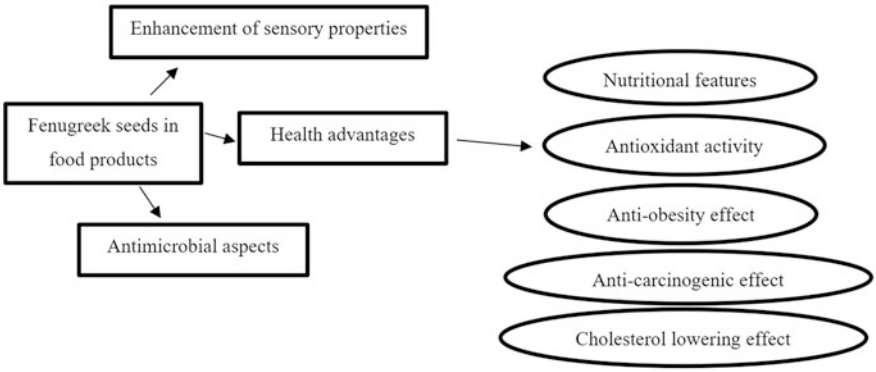

Fig. 9.3 Medicinal and industrial applications of fenugreek herb

9.3.1 Fenugreek as Lactation Aid

Previous researches have shown that the use of fenugreek seeds increases milk flow, but its mechanism of action is unknown. This increase is probably due to the presence of phytoestrogens and diosgenin in the seed chemical composition and structure (Snehlata and Payal 2012).

9.3.2 Fenugreek Having Anti-Diabetic Activity

In animal and human trials, it has been shown that fenugreek seeds, defatted fenugreek seeds and fenugreek gum, known as galactomannan, can effectively decrease the blood sugar level in patients and enhance the insulin response. Therefore, the seeds can be applied as anti-diabetic remedy. Saponins, diosgenin, trigonelline, flavone C-glycosides, 4-hydroxyisoleucine, and dietary fiber are the main bioactive components of fenugreek, which are affiliated with the hypolipidemic and anti-diabetic action (Valette et al. 1984). Today, efforts are being made to use food as a medicine in addition to the task of meeting nutritional needs. As a result, in some countries, people with type 2 diabetes who do not urgently need to use insulin usually use fenugreek seeds to lower their blood sugar instead of insulin. Of course, the effect of this seed is as long as the person makes changes in his/her lifestyle so that the seed can have a noticeable effect (Khorshidian et al. 2016). The soluble dietary fiber in fenugreek seeds, known as galactomannan, by a variety of mechanisms, including altering the intestinal flora, increasing bowel movements, inhibiting the overactivity of digestive enzymes, and satiety by delaying gastric emptying, can lower blood sugar in patients (Zentek et al. 2013). Diosgenin can preserve pancreatic islet β-cells, hepatic glucose kinase, hepatic glucose heteroplasia, and improves the anti-oxidase activity. Trigonelline indicated anti-diabetic activity by enhancement of insulin signaling pathway, attenuation of endoplasmic reticulum stress and oxidative stress in type 2 diabetic rats, affecting the

regeneration of pancreatic islet β-cells, the secretion of insulin, and glucose metabolizing enzymes (Aldakinah et al. 2017). Flavone C-glycosides can inhibit digestive enzymes, activate insulin signaling, and decline the formation of advanced glycation end products. 4-hydroxyisoleucine can stimulate pancreas to secrete insulin (Jin et al. 2014; Sowmya and Rajyalakshmi 1999).

9.3.3 Fenugreek Used for Lowering Cholesterol

Among the various constituents of fenugreek, only the dietary fiber (especially soluble dietary fiber) and saponin demonstrated this activity. To prove this ability, which is related to the soluble dietary fiber found in fenugreek seeds (galactomannan), a comparison was made with other commercial galactomannans, including guar gum. Thus, the rats were given a diet rich in various galactomannans, and some were given only cellulose as control samples. The results showed that rats fed with fenugreek galactomannan had lower cholesterol levels than rats fed with other galactomannans and cellulose. Probably the reason for this ability in fenugreek is due to the unique ratio of mannose to galactose (Evans et al. 1992).

9.3.4 Fenugreek Having Anti-Obesity Activity

The results of some former researches have demonstrated that the use of fenugreek seeds can be somewhat effective in weight loss. As mentioned earlier, there is a large amount of soluble fiber in the fenugreek seed composition that can increase viscosity. This increase in viscosity results in the construction of a gel-like structure which creates a kind of fullness in a person's stomach and reduces the amount of food consumed, and thus can cause weight loss. Conversely, fenugreek seeds have been shown to prevent carbohydrates from entering the blood stream, thus preventing the accumulation of carbohydrates and their conversion into fat and, ultimately, results in weight loss (Evans et al. 1992).

9.3.5 Fenugreek Having Anti-Bacterial Activity

Fenugreek seed oil and aqueous extract have a potent anti-bacterial activity against some types of microorganisms such as *Escherichia coli* and *Staphylococcus aureus* (Verma et al. 2015).

9.3.6 Fenugreek Having Anthelmintic (Anti-Inflammatory) Activity

Some extracts obtained from fenugreek seeds have shown anti-inflammatory activity. Meanwhile, alcoholic extract showed the highest anti-inflammatory activity and

aqueous extract demonstrated this activity to some extent (Buchineni and Kondaveti 2016).

9.3.7 Fenugreek Having Anti-Ulcer Activity

In some sources, it is mentioned that the aqueous extract and gel fraction obtained from fenugreek seeds might have this activity. This ability has been somewhat effective in treating gastritis and ulcers (Srinivasan 2006; Omezzine et al. 2014).

9.3.8 Fenugreek Having Allelopathic Activity

Weeds are one of the main and important factors in reducing the production efficiency of agricultural products. Herbicides have been used to control weeds for decades. The use of herbicides has many disadvantages, including the need for manpower to perform the process and high cost (Omezzine et al. 2014). Nowadays, researchers are looking for safe alternatives to herbicides that can reduce their use on farms to reduce costs while preventing damage to plants, which is one of the main effects of herbicides. Previous researches have shown that some of the secondary metabolites produced by plants exhibit allelopathic activities. Allelopathic compounds produced by plants vary depending on the plant type, genome, physico-chemical properties, planting season, and harvest season. One of the most important parameters in the concentration of secondary metabolites produced is the harvest stage of the plant. It is of special importance to optimize the harvest time of the plant so that it can provide the highest concentration of secondary metabolites. The species of fenugreek is announced to have insecticidal and anti-fungal activity and have considerable allelopathic potential due to the high content of secondary metabolites (Haouala et al. 2008). It is crucial to recognize the developmental stage (vegetative, flowering, and fruiting) with the greatest level of allelochemicals. The identification could be done by HPLC and LC-MS. The plant developmental stage has an impact on the composition of flavonoids in the aerial parts. As reported in previous studies, the extracts of aerial parts obtained from plants, at the vegetative and fruiting stages, had eight types of flavonoids, compared to only six at the flowering stage. Crude extracts of all fenugreek plant organs indicated a strong allelopathic impact on seed germination of test crops and the aerial part extracts indicated the most inhibitory activity. In fact, the maximum inhibition index (1) in germination percentage was demonstrated with material harvested at vegetative stage, followed by flowering and then fruiting stage. This reduction was affiliated with an elevation in stem growth and leaf senescence and abscission or to an elevation in the cell wall accumulation while cell contents regress (Haouala et al. 2008). Flavonoids announced to have allelopathic effects include kaempferol, quercetin, and naringenin.

9.4 Fenugreek Has some Side Effects

As mentioned before, using fenugreek, especially its leaves and seeds, has become particularly important due to its functional and medicinal aspects. Also, this plant, and especially its seeds, can be used in food formulations. However, results obtained from some researches has shown that this plant may have side effects. Using this plant can cause bleeding, facial swelling, and breathing problems. It may also decrease potassium blood levels (He et al. 2015; Sulieman et al. 2000; Ciftci et al. 2011).

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Fenugreek (*Trigonella foenum-graecum*): Nutritional, Health Properties and Food Uses

10

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Abstract

Fenugreek (*Trigonella foenum-graecum*) belongs to legume family and finds its origin from South Eastern Europe and Western Asia, but now grown mainly in India and many parts of the world including Northern Africa and the United States. Over 80% of the total world's production of fenugreek is contributed by India, it occupied an area of nearly 219,000 ha with total production of 247,000 tonnes in 2015–16. Fenugreek seeds are extensively used as spice in Indian cuisine for flavoring, while its leaves, germinated seed, and tender shoots are used as vegetables. The seeds are aromatic, pleasantly bitter, and slightly sweet with antidiabetic and hypocholesterolemic, galactogouge, and carminative properties. They are also reported to have remedial and nutritive properties and trigger digestion process. Fenugreek seeds contain a central hard and yellow embryo surrounded by a corneous and comparatively large, white, and semi-transparent layer of endosperm which is further surrounded by a tenacious and dark brown husk. Fenugreek seeds contain high proteins (27.57%), soluble (30.6%) and insoluble (20.6%) dietary fiber, crude fat (6.71%), and minerals such as calcium, iron, and β-carotene. The seeds are also rich in polysaccharides and galactomannan. In India, boiling, pressure cooking, roasting, or germination of fenugreek seeds are done to make the seeds soft, palatable and to remove its bitterness.

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Keywords

Fenugreek · Bioactive compounds · Antioxidant properties · Health benefits · Food uses

10.1 Introduction

Fenugreek (*Trigonella foenum-graecum)* is a dicotyledonous angiospermic medicinal plant. It belongs to the order Fabales, family Leguminosae, also known as forage crop. It is grown in Canada, The Mediterranean, Northern Africa, Northern India, and Western Asia (Kasran et al. 2013). India is the largest producer of fenugreek in the world (Vidyashankar and Ganaganur 2016) contributing over 80% of the total world's production. It occupied an area of nearly 219,000 ha with total production of 247,000 tonnes in 2015–16 (NHB 2017) with Tamil Nadu, Rajasthan, Uttar Pradesh, Gujarat, and Madhya Pradesh being the major producing areas. Its leaves and seeds are used as spice all over the world to raise the sensory qualities of food. In the early stage, the pods are greenish to slightly purplish but at maturity they turn to brownish. On the surface of long and curved pods there are small hairs (Solorio-Sanchez et al. 2014; Basu et al. 2006).

Fenugreek seeds contain high proteins (27.57%), soluble (30.6%) and insoluble (20.6%) dietary fiber, crude fat (6.71%) (Naidu et al. 2011), and minerals such as calcium, iron, and β-carotene. The seeds are also rich in polysaccharides and galactomannan (Jiang et al. 2007; Dhull and Sandhu 2018; Dhull et al. 2020a). Fenugreek seed oil contains 93% unsaturated fatty acid and 7% saturated fatty acid (Al-Jasass and Al-Jasser 2012). Linolenic and linoleic acids were major contributors of unsaturated fatty acids and were found as 34.85 and 30%, respectively.

Fenugreek leaves consist of about 0.9% fat, 6% carbohydrates, 4.4% protein, 1.1% fiber, 1.5% minerals, and 86.1% moisture (Rao 2003). Several vitamins and minerals such as vitamin C, riboflavin, thiamine, carotene, niacin, phosphorous, calcium, zinc, and iron are also present in leaves. Fresh fenugreek leaves contain β-carotene around 19 mg/100 g and ascorbic acid 220.97 mg/100 g. Fresh fenugreek leaves have better retention of nutrients so it also used as a vegetable in the diet (Yadav and Sehgal 1997).

Fenugreek, a legume crop can enhance soil fertility through biological N_2 fixation and can be used as organic manure also (Hardman 1969; Acharya et al. 2010). Both leaves (Acharya et al. 2011) and seeds (Fedacko et al. 2016) have high level of phytochemicals; so, they are used for culinary as well as medicinal purposes. Fenugreek seed is used to produce synthetic maple syrup (Acharya et al. 2011; Mehra et al. 1996). It intensifies the flavor and color of food material. In many countries, it is utilized as a seasoning and condiment to prepare meals and it have nutritive and restorative properties. It looks like Sweet clover and alfalfa plants having height 1–2 ft. Fenugreek seeds and its green leaves are used in medicinal application and also used in preparation of food materials (Garg 2016). It is used as an emulsifying agent, food stabilizer, and an adhesive because of its high gum, fiber,

and protein content (Dhull et al. 2019a, 2020b). Fenugreek seeds are moderately sweet and slightly bitter. Whole or ground form seeds of fenugreek are used for flavoring in many foods mostly in spice blends, curry powder, and teas. High concentration of dietary fiber in fenugreek seeds helps in improving the texture of food (Meghwal and Goswami 2012). In India, boiling, pressure cooking, roasting, or germination of fenugreek seeds are done to make the seeds soft, palatable, and to remove its bitterness (Mathur and Choudhry 2009).

10.2 Major Bioactive Compounds Present in Fenugreek

Fenugreek consists of several components such as galactomannan fiber, alkaloids like trigonelline, proteins, coumarin, lipids, flavonoids, vitamins, steroidal sapogenins, amino acids, and minerals (Ribes et al. 1986). Various chemical constituents present in the fenugreek are presented in Table 10.1. Several bioactive compounds such as pyridine alkaloids mainly choline (0.5%), trigonelline (0.2–0.38%), carpaine, and gentianine; free amino acids including histidine, 4-hydroxyisoleucine (0.09%), lysine, and arginine; the flavonoids mainly isovitexin, apigenin, vitexin, orientin, quercetin and luteolin and minerals mainly iron and calcium are predominantly present in fenugreek seeds (Mehrafarin et al. 2010). During roasting, huge proportion of trigonelline is degenerated to pyridines and nicotinic acid (Acharya et al. 2006). He et al. (2015) reported the utmost bioactive components that are present in the fenugreek seed include diosgenin, rhaponticin, and isovitexin. Various alkaloids include trimethyl coumarin, trigonelline nicotinic acid, and trigocoumarin found in the fenugreek stem (Khare 2004). Blank et al. (1997) used gas chromatograph instrument to observe the odor and aroma of active

Table 10.1 Bioactive compounds of fenugreek (Source: Yadav et al. 2011; Sowmya and Rajyalakshmi 1999)

Bioactive compounds	Chemical constituents of fenugreek
Alkaloids	Neurin, Carpaine, Trimethylamine, Trigonelline, Gentianine, Betaine, and Choline
Steroidal sapinogens	Saponaretin, Sarsasapogenin, Smilagenin, Neogitogenin, Gitogenin, Diosgenin, Neotigogenin, Yuccagenin, Tigogenin, and Yamogenin
Amino acids	Isoleucine, Arginine, 4-Hydroxyisoleucine, L-tryptophan, Histidine, Lysine and Leucine
Lipids	Monoacylglycerols, Diacylglycerols, Triacylglycerols, Free fatty acids. Phosphatidylinositol, Phosphatidylcholine, Phosphatidylethanolamine (Chatterjee et al. 2010)
Flavonoids	Isovitexin, Quercetin, Vitexin, and Rutin
Saponins	Trigofoenosides A-G, Graecunins, Fenugreekine, and Fenugrin B
Fibers	Gum and Neutral Detergent Fiber (NDF)
Others	Minerals, Coumarin, Vitamins, Lipids, 22% proteins; 28% mucilage; bitter fixed oil, 5% of a stronger-swelling

components present in fenugreek such as eugenol, caproic acid, 3-Isobutyl-2-methoxypyrazine, 3-Amino-4,5-dimethyl-3, acetic acid, olfactometry diacetyl, 3-isopropyl-2-methoxypyrazine, isovaleric acid, 1-Octen-3-one, butanoic acid, sotolon, linalool, 4-dihydro-2 (5H)-Furanone (Z)-1,5-Octadien-3-one, with characteristic aroma like butter, paprika, metallic, pungent, roasty/earthy, sweaty/rancid, spicy, musty, flowery, respectively. It was observed that, sotolon was revealed to be found most predominantly in (5 s)-enantiomeric form (95%) in fenugreek. In another study (Meghwal and Goswami 2012) on the perspiration of human after consumption of fenugreek, the results inferred that the components responsible for the powerful maple syrup odor existing in perspiration after consumption of fenugreek are because of the several compounds such as β-camphor, β-caryophyllene, pinene, terpinen-4-ol, 3-octen-2-one, 2,5-dimethylpyrazine, neryl acetate, and 4-isopropylbenzaldehyde. It was also concluded that 2,5-dimethylpyrazine is a vital compound which was proven to contribute sweat odor.

10.2.1 Lipid

The fenugreek seeds contain about 7.8% golden-yellow colored oil having a bitter taste and disagreeable odor. The oil mainly consisted of neutral lipids (85%) followed by glycolipids (5%) and phospholipids (10%). The unsaturated fatty acids are mainly comprised of oleic (14%), linoleic (40%), and linolenic (25%) acids which can regulate the fatty acid profile in human body (Baccou et al. 1978; Sulieman et al. 2000; Ren and Zhu 2011). The oil found its uses in flavoring of canned foods and syrups and some perfumes (Prasad 2014). The polyunsaturated fatty acid (PUFA) such as linoleic acid has found affecting the growth of muscles, brain, and nervous system (Punia et al. 2019, 2020; Kidwai et al. 2020). PUFA also plays roles in preventing coronary heart diseases, hypertension, rheumatoid arthritis, and ulcerative colitis (Bains et al. 2020). Apart from this, reproductive system, skeletal, dermal, and metabolic system is also benefitted by these PUFAs. Recently, one another PUFA named linolenic acid has found with some important biological functions such as strong in vitro antitumor activity (Dhull et al. 2020c; Dhull and Punia 2020a, 2020b). In evaluating the nutritional quality of oil, fatty acid composition occupies a special place. Intervention studies showed that dietary fatty acids can modify lipoprotein profile of blood plasma and reduce the risk of cardiovascular disease, particularly for PUFA and MUFAs (Sharma and Saini 2020; Punia et al. 2020). Studies on composition of fenugreek oil from different countries showed that linoleic and linolenic acid percentage differed depending on the cultivation places and conditions. The composition of neutral and polar lipid constituents in fenugreek is shown in Table 10.2.

10.2.2 Fenugreek Gum

Fenugreek seed is a rich source of fiber which mainly consists of galactomannans (Madar and Stark 2002; Dhull et al. 2020a). It helps in slow absorption of

Table 10.2 Composition of neutral and polar lipid constituents in fenugreek (Source: Chatterjee et al. 2010)

Lipid species identified of fenugreek	Amount (g/100 g)
Free fatty acids	0.160
Phosphatidylinositol	0.009
Phosphatidylethanolamine	0.036
Phosphatidylcholine	0.110
Monoacylglycerols	0.180
Diacylglycerols	0.280
Triacylglycerols	4.330

carbohydrates resulting in feeling of fullness and helps in reducing stomach disorders. Fenugreek is an excellent source of soluble and insoluble dietary fiber (20–25% and 25–30%, respectively) and its increased dietary fiber consumption may aid in relieving the risk of colon cancer (Grigor et al. 2016; Srinivasan 2019).

The major polysaccharide type found in fenugreek seeds is galactomannans and amounts for more than 50% of the weight of the seed (Raghuraman et al. 1994). Fenugreek seeds contain 26.8% soluble fiber chemically identified as galactomannans (Jiang et al. 2007) with similar properties to guar seeds and psyllium husk soluble fibers (Song et al. 1989). Galactomannans are biopolymers consisted of linear core poly (1,4)-β-D-mannan backbone to which D-galactosyl subunits are linked by 1,6-glycosidic linkages (Im and Maliakel 2008), mannan backbone of fenugreek gum is most fully substituted with galactose side chain (Brummer et al. 2003). The hyper involvement of unsubstituted mannan regions in fenugreek gum becomes nearly impossible because of the presence of disaccharide repeating sequence in its primary structure. Brummer et al. (2003) reported that mannose to galactose ratio in the extracted fenugreek galactomannans differs from 1.02:1.00 to 1.14:1.00. Different galactomannans obtained from various legumes usually differ in its molecular weight, mannans:galactose (M:G) ratio, and degree and place of galactose substitution on core mannan backbone. This M:G ratio affects the physicochemical properties of galactomannans and inversely related to the gum solubility (Garti et al. 1997; Wu et al. 2009). When compared with guar, locust bean, and tara gum; the highest galactose content was found for fenugreek gum resulting in its maximum solubility and hydration (Brummer et al. 2003; Wu et al. 2009).

Brummer et al. (2003) analyzed the effect on the chemical composition of fenugreek gum by adopting different extraction methods. They revealed that the cool water extraction yielded low protein gum having 2.36% protein when compared to the solvent extraction using boiling hexane. By using pronase hydrolysis protein level was reduced to 0.57%. They revealed the presence of stachyose, sucrose, and raffinose percentage in the ethanol-soluble sugar mixture was found to be 2.84%, 0.7%, and 0.5%, respectively. Another author Mansour and El-Adawy (1994) also reported the raffinose and stachyose percentage present in fenugreek gum was 0.49% and 2.01%, respectively. The extracted fenugreek gum contains 0.8% residual protein, therefore forbidding the function of surface activity to the hydrophilic gum alone. By being adsorbed onto oil droplets, galactomannans sterically stabilize the emulsions against flocculation and coalescence. In addition, all galactomannans

also exhibited some emulsifying, interfacial, and surface behaviors (Garti et al. 1997; Gadkari et al. 2018, 2019; Kaur et al. 2018a, b). Due to these properties, it is preferred as a good ingredient in different food applications as compared to natural hydrocolloids (Brummer et al. 2003; Kaur et al. 2018a, b; Punia and Dhull 2019). Various studies have recently spread our awareness of exudates and natural gums and their use in cosmetic, food, pharmaceutical industries (Wu et al. 2009). This has increased natural gum applications, and its prices and demand have encouraged researchers to look new gum sources. Some of the galactomannan hydrocolloids that are naturally derived include fenugreek gum, locust bean gum, guar gum, acacia gum, taro gum, etc., with the variation in mannose and galactose subunit ratios (Brummer et al. 2003).

While other gums are commonly used in foods as stabilizers and thickeners, fenugreek gum with limited use is comparatively primitive. As a hydrocolloid, fenugreek gum can give foods different functional properties, such as texture, gel, emulsion, encapsulating, and stabilizing properties (Gadkari et al. 2018, 2019). In powder form, soluble and insoluble dietary fiber can be utilized in various forms, including dairy, confectionary, bakery products, different nutritional juices, beverages, soups, spices, and seasonings blends, as well as nutraceuticals in capsule or tablet form along with any vitamin or nutrient (Brummer et al. 2003; Im and Maliakel 2008).

Type 2 diabetes can be controlled by hyperglycemic action in animals by galactomannans extracted from fenugreek seeds (Tayyaba et al. 2001; Vats et al. 2003) and in humans (Raghuraman et al. 1994; Sharma et al. 1996). The fenugreek galactomannans have a high water-binding potential and form extremely viscous solutions at comparatively low concentrations; this tends to minimize glucose absorption in the digestive tract (Raghuraman et al. 1994). Major soluble dietary fiber in fenugreek, i.e. galactomannan, plays an important role in reducing sugar level together with other hypoglycemic constituents (Ali et al. 1995).

10.2.3 Proteins

The seeds of fenugreek contain around 25.4 g protein per 100 g (Jani et al. 2009) while 100 g of its endosperm contain 43.8 g protein (Naidu et al. 2011). The fenugreek protein has been found rich in various vital protein and amino acid including globulin, lysine, lecithin, albumin, histidine, and 4-hydroxyisoleucine (Mathur and Choudhry 2009). It has been reported that seeds having high protein (20–30%) and some amino acids such as 4-hydroxyisoleucine and histidine can help in stimulating insulin secretion (Isikli and Karababa 2005). Fenugreek protein can found its application as a nutrient supplement as it is rich in lysine (a limiting amino acid in cereals) and its quality is also found comparable to soybean protein (Meghwal and Goswami 2012). Like most of other legumes, fenugreek seeds also contain essential and non-essential amino acids and its protein is stable to heat and other processing conditions during cooking (Srinivasan 2006); therefore, fenugreek can be considered as a highly nutritive seed.

Plant proteins are used in foods as functional ingredients to improve stability and texture as well as the nutritional quality of the product (Makri et al. 2005). The functional properties of proteins significantly affect the texture as well as cooking and sensory properties of food and decide their ultimate use in different food applications. These properties of proteins are affected by several factors such as size, shape, and conformation, the method and condition of isolation of the protein as well as the method of fat extraction (Luseisano et al. 1984; Finley 1989). Fenugreek protein also contains significant concentration of glutamic acid, aspartic acid, leucine, threonine, and arginine and found with excellent functional properties (Feyzi et al. 2015). Meghwal and Goswami (2012) found that fenugreek protein is more soluble at alkaline pH. The foaming and emulsion properties of fenugreek proteins are much affected by salt concentration and pH levels, both foam and emulsion properties were low at pH 4.5 which is the isoelectric point of the proteins (El Nasri and El Tinay 2007). Besides, they revealed that protein of fenugreek concentrate observed high bulk density (0.66 g/mL), oil absorption capacity (1.56 mL oil per g protein), and water absorption capacity (1.68 mL H_2O per g protein).

10.2.4 Natural Steroidal Sapogenins

Fenugreek is an identified source of natural steroid sapogenin compounds and thus valued in the pharmaceutical industry due to inherent nutraceutical qualities. The diosgenin is the major steroidal compound reported in manufacturing of steroidal drugs and hormones. A disorder, hypocholesterolemia frequently associated with high cholesterol and hyperglycemia, is primarily recognized in human beings nowadays (McAnuff et al. 2002). Yam (*Dioscorea spp*.), cluster bean (*Cyamopsis tetragonoloba L*.), fenugreek, and a few other legumes are the some sources for the preparation of natural diosgenin (Rosser 1985; Mathur and Mathur 2006).

Fenugreek steroidal sapogenin and diosgenin have been reported by Mathur and Mathur (2006), which are in good demand for making the sex hormone, cortisone, used in oral contraceptives. The fenugreek whole seed powder has been used to regulate blood sugar in healthy, obese, and non-insulin-dependent or type-2 diabetic individuals due to the presence of these sapogenins.

Saponins such as neotigogenins, diosgenin, tigogenin, yamogenin, and gitogenin are rich in fenugreek seeds (Taylor et al. 1997). Fenugreek seed contains 0.1–0.9% diosgenin which is extracted commercially and the structure of diosgenin is shown in Fig. 10.1. Fenugreek leaves consist of seven saponins, known as graecunins. These constituents are glycosides of diosgenin. The other bioactive constituents of fenugreek have also been studied, including volatile oils, mucilage, and alkaloids (like fenugreek tissue culture production of choline and trigonelline) (Radwan and Kokate 1980; Brain and Williams 1983). Ghosal et al. (1974) reported the existence of a sapogenin peptide ester, fenugreekine. Yoshikawa et al. (1997) isolated six trigoneosides that were novel saponins based on furostaniol aglycones. Through its natural steroidal properties, the diosgenin content in fenugreek may increase cattle growth (Mir et al. 1997; Acharya et al. 2008). Reduced reliance on synthetic

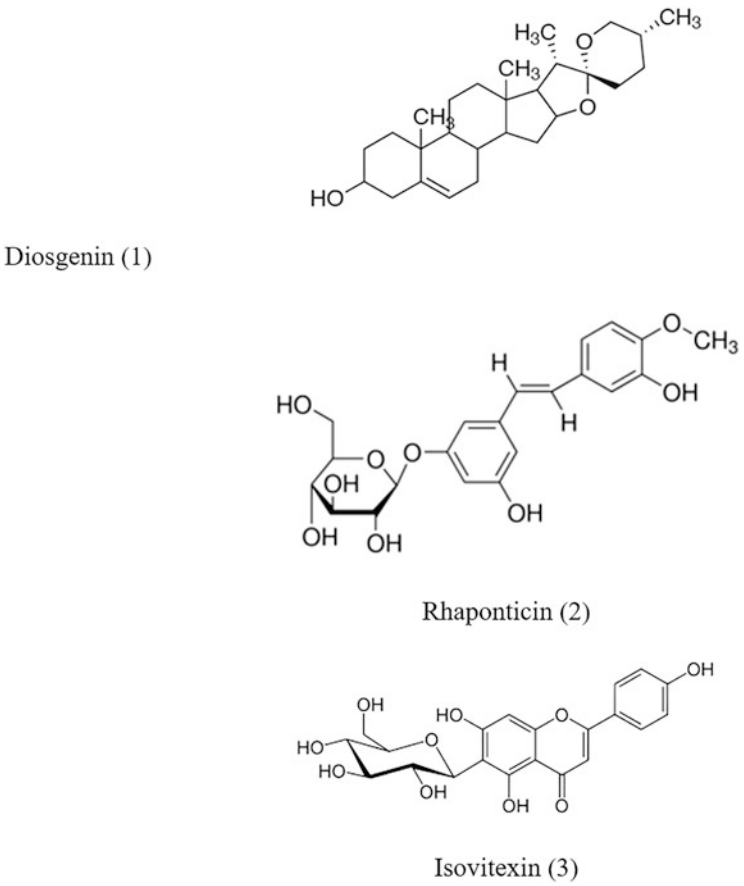

Fig. 10.1 Chemical structure of (1) Diosgenin (2) rhaponticin, and (3) isovitexin

steroids for effective cattle production could reduce the cost of beef producers (Acharya et al. 2006, 2008).

10.2.5 Phenolic Compounds

Phenolic compounds include a number of compounds having a characteristic aromatic ring with one or more hydroxyl groups and a number of substituents. Flavonoids, one of major phenolic compounds, have a basic C6C3-C6 structure and mainly include the flavonols, flavoids, anthocyanin pigments, and isoflavones. Most flavonoids found as glycosides except the flavanols which tend to polymerize to condensed tannins. The tannins could be classified either as hydrolyzable or condensed. Most condensed tannins are polymers of flavan-3-ols (catechins) or flavan-3, 4-diols (leucoanthocyanidins), while most hydrolyzable tannins are

glucose or polyhydric alcohol esterified with gallic acid (gallotannins) or hexahydrodiphenic acid (ellagitannins). Condensed tannins occur widely in cereals and legumes mainly found concentrated in the bran fraction of grains (Dhull et al. 2016; Kaur et al. 2018b; Dhull et al. 2020d). Tannins may be classified as polyphenolic substances (Shimelis and Rakshit 2007). Many phenolics such as gallic acid, catechin, chlorogenic acid, etc., have been reported to inhibit the mutagenic effects of carcinogens as well as reduce the endogenous formation of several carcinogenic compounds (Stich and Rosin 1984). The polyphenolic compounds specially tannins have some anti-nutritional and toxic effects such as decrease in the intake of food/feed, digestive enzymes inhibition and digestive tract malfunctioning, formation of the less digestible tannin-dietary protein complexes, endogenous protein excretion increase, and toxicity of absorbed tannin or its metabolites (Salunkhe et al. 1990).

10.2.6 Phytic Acid

Phytic acid (myoinositol 1,2,3,4,5,6, hexakis-dihydrogen phosphate; PA) is present in grains in concentrations ranging from 0.1 to 6.0%, mainly found in the cotyledon of legumes, oilseeds, and bran of the cereal grains (Reddy et al. 1982). Phytate, i.e. salts of phytic acid, is primarily present as a salt of the mono- and divalent cations K+, Mg 2+, and Ca 2+ and accumulates during the ripening period as primary storage form of both phosphate and inositol in plant seeds and grains (Loewus 2002).

Phytic acid has shown significant negative relationship with the glycemic index (blood glucose response to different starchy foods) (Yoon et al. 1983). Also, plasma cholesterol and triglyceride levels were decreased in the rats with the addition of phytic acid (0.2–9%) (Sharma 1987). Phytic acid can bind to Zn and thus lower the plasma Zn to copper (Cu) ratio; lower ratios tend to predispose humans to cardiovascular disease. Certain enzymes which generate free radicals and result in undesirable oxidative damage are catalyzed by some minerals such as iron and copper. As phytate can bind and chelate these divalent minerals, these have been suggested to have protective effects making them a natural antioxidant. However, due to high negative charge and high reactivity at a wide range of pH, phytates can bind with positively charged ions such as $Fe^{2+/3+}$, Ca^{2+}, Zn^{2+}, Mn^{2+}, etc., and minerals, making less digestible insoluble complexes with low absorption in the small intestine (Vucenik and Shamsuddin 2003), making phytic acid an anti-nutritional factor. Phytic acid also interferes with other negatively charged groups such as proteins mediated by a positively charged mineral. Similarly, it binds with starch by direct hydrogen bonding or indirectly through its associated proteins. Therefore, in the presence of phytic acid, low solubility and digestibility of proteins and starches can be attributed to the formation of these type of complexes (Carnovale et al. 1988).

10.3 Applications of Fenugreek in Various Food Products

Fenugreek is an excellent source of dietary fiber, protein, essential fatty acids, antioxidants as well as minerals and vitamins and can be used as an ingredient in different food preparations. Fenugreek gum mainly soluble fiber is used as emulsifier, thickener, gelling agent, stabilizer in nutritional beverages, dairy products, yogurts, cereal bars, etc. Total dietary fiber and soluble fiber can be used in powder form which may mix with fruit juices, seasonings, and other spice mixes. Fenugreek gum can be directly used in tablets formulation and also in capsules which is enriched with nutrients and vitamins. Fenugreek gum can also be used in various food products such as bagel, soups, milk shakes, flat bread, sweets, candies, and dressings. Bread, baked corn chips, noodles, muffins, fried, cake mix, pizza, tortilla, and bakery flour which is used for the preparation of bakery foods can be enriched with 8–10% soluble dietary fiber. Also, when fenugreek fortified flour is used for the preparation of fried snacks, only 8–15% absorption of oil takes place which is really good for those who wanted less intake of fat (Im and Maliakel 2008). The uses of fenugreek in various food products are presented in Table 10.3.

10.3.1 Fenugreek Used as Gum, Stabilizer, Emulsifier, and Adhesive

The ability of fenugreek protein to stabilize and emulsify the food constituents depends on its interaction with different food constituents. A number of factors such as source of protein, protein extraction method, and interaction of protein with other constituents such as carbohydrate, lipids, and dietary fiber significantly affect the functional properties of proteins (Arogundade et al. 2006). The promising role of fenugreek proteins as surface active agents in creating and stabilizing foams and emulsions by migrating into water and air/oil interface and reducing their surface or interfacial tension has been demonstrated (Feyzi et al. 2017).

Fenugreek dietary fiber which is a galactomannan has excellent emulsifying and stabilizing properties and has huge potential for widespread use in the food industry (Dhull et al. 2020a). Fenugreek gum when added to soy protein isolate (SPI), the emulsifying activity as well as stability increased four times and three times, respectively (Hefnawy and Ramadan 2011). Also, the SPI with fenugreek gum dispersion showed stable emulsifying properties and stability over a wide range of pH, ion strength, and high temperature. Moreover, fenugreek high dietary fiber has been proven to be acting as probiotic in functional food (Lee 2009). In large intestine, soluble fiber of fenugreek acts as excellent substrate for fermentation by the intestinal microorganisms (Sowmya and Rajyalakshmi 1999). Fenugreek gum has been reported with superior emulsifying properties when compared with other galactomannan such as guar or other gums (Garti et al. 1997). Fenugreek dietary fiber of 8–10% has been incorporated in different flours which can be used in the production of baked foods including pizza, bread, cakes, and muffins. Roberts (2011) found the applications of fenugreek to flour, permits to produce functional

Table 10.3 Applications of fenugreek in various food products

Components	Uses	References
Seeds	Bread	Sharma and Chauhan (2000); Raju et al. (2001); Sharma and Chauhan (2002); Isikli and Karababa (2005); Hooda and Jood (2005b); Thomas et al. (2011); Man et al. (2019); Afzal et al. (2016).
Seeds	Food stabilizer, emulsifying agent, and adhesive	Jani et al. (2009); Sowmya and Rajyalakshmi (1999)
Seeds and leaves	Biscuits and cookies	Sharma and Chauhan (2002); Hooda and Jood (2005a); Hegazy and Ibrahium (2009); Hussein et al. (2011)
Seeds	Chutneys, curries, pickles, and condiments	Madar and Stark (2002)
Seeds	Extruded products	Shirani and Ganesharanee (2009); Dhull and Sandhu 2018
Seeds	Galactomannan and dietary fiber	Blank (1996); Dhull et al. (2020c)
Fenugreek gum	Extruded product and breads	Srinivasan (2006); Ravindran et al. (2011); Roberts et al. (2012); Huang et al. (2016)
Seeds	Blend with flour for yellow dye, bread	Srinivasan (2006)
Seeds and leaves	For aroma, color, and flavor	Ramesh et al. (2001)
Seeds	Artificial flavoring and maple syrup	Blank (1996)
Seeds and leaves	Seasoning and spices	Sowmya and Rajyalakshmi (1999); Srinivasan (2005, 2006)
Seeds and leaves	Organoleptic character improver	Srinivasan (2006)

foods that may be generally agreeable to those customers who consumed western pattern diets.

10.3.2 Fenugreek in Bakery Products

Fenugreek as whole seed flour as well as its different constituents such as gum powder, protein isolate, etc., can be used in different bakery products. Fenugreek seed and other legume based products have shown hypoglycemic effects in both diabetic and non-diabetic subjects (Priyali et al. 2000). Rice bran with fenugreek blends were used in preparation of breads and cookies and the results showed that the incorporation of blends increased the baking absorption, decreased loaf volume, overall quality score of bread and increased the spread factor of cookies (Sharma and Chauhan 2002). Biscuits with acceptable quality have been prepared with

incorporation of raw, soaked, and germinated fenugreek flour up to a maximum level 10% which increased total protein, dietary fiber, calcium and iron content of the final products (Hooda and Jood 2005a; Hegazy and Ibrahium 2009). Similarly, bread was prepared by blending raw, soaked, and germinated fenugreek seed powder with wheat flour at different levels. In the supplemented products, increase in protein, dietary fiber, lysine, and minerals contents of bread were observed (Sharma and Chauhan 2000; Hooda and Jood 2005b; Man et al. 2019). Total phenolic content, flavonoid content, and antioxidant activity also found increased in fenugreek flour supplemented bread (Afzal et al. 2016). However, after a certain level of supplementation with fenugreek flour, detrimental effect on loaf volume, crust texture, and sensory properties was observed.

The bitterness of fenugreek flour limits the acceptability in food products prepared with its addition. To encounter this problem, debittered fenugreek flour was produced by soaking fenugreek seed in diluted curd with water (1:1) (Dhull et al. 2019a). This debittered fenugreek flour was used at different levels (5–20%) to produce rusks with improved nutritional, antioxidant properties, and acceptable sensory profile.

The incorporation of fenugreek fiber to the refined flour can help to fortify foods with a balance of insoluble and soluble fibers (Srinivasan 2006). Wheat flour blended with 8–10% fenugreek fiber is used for the preparation of bakery products including muffins, pizza, cakes, and bread with desirable sensory properties. Native as well as extrusion modified fenugreek gum were substituted for wheat flour at different level and the rheological effects and bread making characteristics were determined (Roberts et al. 2012). The volume and texture of gum incorporated breads were found comparable with control bread. Also, increase in dough farinograph water absorption, G' and G'' values as well as increase in peak viscosity, final viscosity, breakdown and setback viscosities were observed. However, fenugreek fiber addition delayed the dough development time but increased the dough stability (Huang et al. 2016). It was also concluded that fenugreek fiber maintained bread quality during storage through its water-holding capacity and the prevention of starch retrogradation.

Parotta (an Indian flat bread) was prepared by replacement of wheat flour with 2.5, 5.0, and 7.5% fenugreek seed powder (Indrani et al. 2010) and the results showed increase in farinograph water absorption, amylograph peak viscosity, spread ratio of parotta while extensograph extensibility and shear force were decreased. The microstructure of parotta dough showed a thick matrix and starch granules appeared to be coated with fenugreek gum.

Srivastava et al. (2012) observed fenugreek seed husk is abundant source of minerals and fibers. Then, husk of fenugreek seed can be mixed in the manufacturing of muffins so it can be rich in fiber content and have a soft texture, medium-fine grain, and good volume with double the part of dietary fiber. Therefore, attributable to high fibre content, a number of studies inferred that fenugreek blend in the baked products up to allowable limit has been found decreasing the insulin resistance and can be used to tackle diabetic patients (Losso et al. 2009).

10.3.3 Fenugreek in Extruded Products

In a study, extruded snacks were prepared by blending different proportions of oat flour, dried green pea flour, fenugreek seed powder and fenugreek leave powder with rice and corn flours and effect on their physical and functional properties were examined (Wani and Kumar 2016). Fenugreek leaf powder shown decrease in lateral expansion, water solubility index, and hardness, while fenugreek seed powder had increased effect on lateral expansion and water absorption index, while decreased effect on water solubility index. Also, fenugreek seed and leaf powder showed negative effect on "a^*" value and positive effect on "b^*" value for color of extrudate. Extrusion was reported to improve the protein, fiber, mineral, and total carbohydrate content of the products with improved bioavailability of minerals attributed to destruction of anti-nutritional factors such as tannin and phytic acids (Wani and Kumar 2015).

The incorporation of debittered fenugreek polysaccharide and fenugreek flour in extruded chickpea–rice based products was carried out and the sensory and physical properties, and glycemic index were analyzed (Shirani and Ganesharanee 2009). More than 2% incorporation of fenugreek flour was not found acceptable in chickpea–rice based extruded products due to recognizable bitter taste of fenugreek. The longitudinal expansion of snacks was increased while radial expansion was slightly decreased after addition of fenugreek polysaccharide. Water solubility index decreased while water absorption index increased in comparison to control.

Dhull and Sandhu (2018) prepared composite flour noodles by blending wheat and fenugreek flour at level of 2%, 5%, 7%, and 10% and reported that water-holding capacity, oil absorption capacity, emulsion capacity, water solubility index, and foaming capacity were increased which was attributed to high fiber content of fenugreek flour. The peak viscosity of flour blends was decreased with increase in the level of fenugreek flour. Composite flour noodles had higher cooked weight, water uptake, and cooking time but gruel solid loss was less compared with control (100% wheat flour) noodles. The preparation of noodles with 7% fenugreek flour was found with satisfactory results for texture, eating, and cooking attributes.

10.3.4 Fenugreek in Traditional Uses

Ayurvedic texts as well as Greek and Latin pharmacopeia has mentioned the medicinal value of fenugreek seeds. In Ayurveda, aphrodisiac properties of fenugreek has been appreciated but modern vaidyas use it for respiratory and digestive problems arising from excess of Kaph (phlegm) and vat (wind). To ease childbirth and enhance milkflow, fenugreek was used in ancient Egypt but it is still used to relieve menstrual cramps and relieving other kind of abdominal pain in modern Egypt and China also. In India, fresh methi ka saag using leaves and stems of fenugreek plant is cooked commonly as winter vegetable. The dried leaves and seeds are used for flavoring of various dishes round the year. The seeds are either eaten raw, soaked and sprouted or used for preparing special ladoo in winters. In the

USA, it is mainly used to make spice blends for soups and stews (Passano 1995). As a popular ingredient in curry powder, pickle, and spice mixture, fenugreek seeds have been used in India, Pakistan, Bangladesh, and other Asian countries for centuries.

10.4 Health and Pharmaceutical Uses

Human health is mainly determined by the food being consumed and many of its constituents such as antioxidants, minerals, vitamins, fiber, lipids, proteins, etc., not only provide nutrition and help in normal functioning of body but also help in preventing metabolic disorder, oxidative damage, onset of chronic diseases and overall aging (Mullaicharam et al. 2013). To cure cancer, atherosclerotic heart diseases, and other disorders, natural plant based antioxidants are gaining interest among the researcher (Rababah et al. 2011). A number of health and pharmacological properties such as anti-carcinogenic, anticholesterolemic, antimicrobial, antioxidant, laxative, carminative, restorative, uterine tonic, emollient, febrifuge, expectoral, galactogogue, anti-inflammatory, hypotensive, antiviral, and demulcent have been reported for fenugreek (Kor and Moradi 2013). Additionally, a number of other activities such as regulating enzyme activity, relieving fever, reducing body fat and body pain, alleviating swelling, boosting appetite, and promoting lactation and sex hormones are also found for fenugreek.

For centuries, besides in the traditional Chinese and Tibetan medicinal practices, the Indian medicinal applications have used fenugreek to treat various diseases in human and animals. Various clinical trials have entrenched appreciable medicinal properties of fenugreek leaves and seeds on study of human and animal subjects as compared with untreated control samples (Acharya et al. 2006, 2010; Basu et al. 2017; Fedacko et al. 2016). Because of the presence of natural, bioactive chemical compounds fenugreek has a broad variety of medicinal, health-promoting, and disease-preventing properties. The fenugreek plant and its products are well known for its various nutraceutical properties. Fenugreek is very useful in reducing high cholesterol (i.e., low-density lipoprotein (LDL)), and high blood sugar (i.e., postprandial blood glucose, triglyceride percentage, and fasting) levels in both laboratory human and animal subjects (Fedacko et al. 2016). Additionally, other health benefits of fenugreek include antineoplastic, antipyretic, antileukemic, antioxidant, and antimicrobial properties (Basu and Prasad 2011; Solorio-Sanchez et al. 2014). Fenugreek is used in the treatment of patients with calcic urolithiasis (Laroubi et al. 2007). Various clinical trials have been established significant results of fenugreek seed extracts such that to decrease glycated hemoglobin (HbA1c) and insulin concentrations, slow down the enzymatic digestion of carbohydrates, to regulate Glucagon-like Peptide-1(GLP-1) signaling. GLP-1 has been positively proved to cure type 2 diabetes (Fedacko et al. 2016). Prophylaxis effect of fenugreek seeds on renal stone formation in rats has also been explored (Laroubi et al. 2007). Fenugreek seeds have medicinal properties including hepatoprotective effect, antidiabetic agent, hypocholesterolemic, galactogogue, gastric stimulant, anticancer,

Table 10.4 Health and pharmaceutical uses of fenugreek

Components used	Beneficial effects	Reference
Seeds	Hypocholesterolemic effect	Srivastava et al. (2012); Zia et al. (2001)
Seeds	Prevents constipation	Sowmya and Rajyalakshmi (1999)
Leaves, seeds	Used as an antioxidant	Naidu et al. (2010); Bhatia et al. (2006); Bukhari et al. (2008)
Seeds	For healthy heart	Blank (1996)
Seeds	Lactation aid	Al-Shaikh et al. (1999); Snehlata and Payal (2012)
Seeds and leaves	Gastro- and hepatoprotective	Blank (1996)
Seeds	Immunomodulatory effect	Meghwal and Goswami (2012)
Seeds	Induces reproduction and growth hormones	Blank (1996)
Seeds	Digestive effect	Platel and Srinivasan (2000)
Seeds, leaves	Anticancer agent	Mathern et al. (2009); Sowmya and Rajyalakshmi (1999)
Seeds and leaves	Wounds and sore muscles treatment	Mathern et al. (2009)
Seeds and leaves	Decreases blood pressure	Sowmya and Rajyalakshmi (1999)
Seeds	Hypoglycemic effect	Roberts (2011)

for anorexia, lactation aid, and antibacterial properties (Srinivasan 2006). These are important medicinal properties of fenugreek, so this crop has equal ability to be used in food industries, pharmaceutical, and nutraceutical industries (Basu et al. 2017).

Fenugreek has a beneficial effect on blood cleansing, and due to the property of diaphoretic, it detoxifies the body with the help of sweating. It smelt in under-arm perspiration and on the skin because of the pungent aroma of fenugreek. It plays an essential role to develop the cells with nutrients and eliminate trapped proteins, toxic waste, and dead cells from the body. If there is blocking in the lymphatic system which means poor fluid retention, diseases, fluid circulation, loss of energy, and pain all around in the person's body. So, it helps in lymph cleaning operation. It also helps to clear congestion in the body, mainly in the lungs, by maintaining the mucus conditions. This also serves as a cleanser for the mouth and a solvent for mucus which also alleviates the urge to cough. Soaked fenugreek seeds are soft which when consumed can accumulate and harden the masses of cellular debris and help in relieving from bronchial symptoms, colds, asthma, pneumonia, catarrh, hay fever, tuberculosis, emphysema, constipation, sore throat, sinusitis, influenza, pleurisy, and laryngitis (Anonymous 2013). Health and pharmaceutical uses of fenugreek are shown in Table 10.4.

10.4.1 Immunological Action

Immuno-modulators are those agents which can increase or decrease the immune responses and this type of effect is known as immunomodulatory effect. The stimulatory immunomodulatory effect of fenugreek aqueous extract at three different levels (50, 100, and 200 mg per kg of body weight) for 10 days was analyzed on the Swiss albino mice immune system and verified from hemagglutination titer, body weight, quantitative hemolysis assay, substantial rise in phagocytic capacity and phagocytic index of macrophages, relative weight of thymus, plaque forming cell assay, phagocytosis, lymphatic proliferation, and latch type of hypersensitivity response (Meghwal and Goswami 2012).

10.4.2 Hypoglycemic Effect

Fenugreek reduces glucose level in body after a collation due to existence of dietary fiber in fenugreek. The procedure has not been completely clarified for this result but the four likely mechanisms for the antihyperglycemic effect of fenugreek are as follows (Garg 2016):

- Inhibition of glucose uptake or absorption from intestine or gastrointestinal tract
- Effect on pancreas, exerting an insulin mimetic or an insulin secretagogue effect
- Enhancement of peripheral blood glucose uptake, normalization of select processes, and increase in insulin receptor density
- Suppression of pro-inflammatory factors, thus reducing insulin resistance.

Fenugreek seed consists of high dietary fiber (soluble and insoluble) and its gum is a galactomannan, i.e. consists of mannose and galactose (Roberts 2011). The reduced glycemic effect of fenugreek gum is mainly associated with the mannose. It was found that uptake of glucose from the jejunum and ileum segments of the intestine in both lean and obese rats was progressively decreased with increasing concentrations of galactomannan (Srichamroen et al. 2009). In particular, the hypoglycemic effect of fenugreek has been recorded in animals and humans with type 1 and type 2 diabetes mellitus. It was suggested that dietary fenugreek probably has its hypoglycemic action by delaying the gastric emptying by direct interference with glucose absorption. Additionally, the release of insulinotropic hormones and gastric inhibitory polypeptides is also reduced by gel-forming dietary fiber. Alloxan-induced subdiabetic and overtly diabetic rabbits were orally administered with active hypoglycemic principle isolated from water extract of seeds to investigate its mechanism of action. The glucose induced insulin response was improved while glucose tolerance curve was attenuated with oral administration of the active compound at a dose of 50 mg per kg body weight over a 15 days span (Puri et al. 2002). It was suggested that stimulation of insulin synthesis and/or its secretion from beta pancreatic cell was the possible reason behind the hypoglycemic effect. The fasting blood glucose of severely diabetic rabbits was lowered significantly after prolonged

administration of the same dose of the active principle for 30 days. But the fasting serum insulin level could elevate to a much lower extent, suggesting an extra-pancreatic mode of action for the active principle. The effect may also be by increasing the sensitivity of tissues to available insulin. The hypoglycemic effect was observed to be slow but sustained, without any risk of developing severe hypoglycemia (Puri et al. 2002).

In one another study, the usefulness of high fiber fenugreek diet in management of diabetes was established in which the effect of fenugreek fortified therapeutic food on blood sugar levels of 24 non-insulin dependent diabetes mellitus patients was investigated. The food was prepared from different legumes such as fenugreek seeds, green gram, Bengal gram, horse gram, and dry peas. The result suggested a decrease in both fasting and postprandial blood sugar levels after supplementation of diet with 30 g of therapeutic food for a period of about 1 month (Kumari and Sinha 2012).

Similarly, when fenugreek extract and Metformin HCl were administered at different dose level to streptozotocin-induced rats (with diabetics) for 6 weeks to investigate its effects on blood glucose, hemorheological parameters, and general properties, the results concluded that fenugreek extract can lower kidney/body weight ratio and blood glucose and also improves hemorheological properties in experimental diabetic rats (Xue et al. 2007).

A study evaluated the hypoglycemic effects of the fenugreek seeds on dogs and the results showed reduced levels of blood glucose, plasma glucagons, and somatostatin and also resulted in reduced carbohydrate-induced hyperglycemia attributed to its galactomannan-rich soluble fiber fraction. Also, in a small study on patients with mild type-2 diabetes mellitus, glycemic control was improved in results of clinical analysis. Insulin sensitivity was increased while glycosylated hemoglobin levels were reduced in fenugreek recipients (Snehlata and Payal 2012).

It is also suggested that saponins present in fenugreek are transformed into sapogenins in the gastrointestinal tract which lower the lipids in the body. The soluble dietary fiber present in fenugreek can retard the rate of postprandial glucose absorption, proving to be a secondary mechanism for its hypoglycemic effect (Basch et al. 2003).

10.4.3 Lactation Aid

Breasts are modified sweat glands and sweat production is stimulated by fenugreek as it contains the hormone precursor which can increase milk formation. Increase in nursing mother milk supply was reported within 24–72 h after first taking of fenugreek herb (Snehlata and Payal 2012). In one study, according to fenugreek level in the feed, i.e. 0%, 25%, and 50%, 21 lactating dairy goats were divided into three groups (A, B, and C, respectively) and effect on milk yield and fat percentage was observed. Keeping all other diets same, milk yield was recorded daily while fat percentage was evaluated weekly. The results showed significant increase in the milk yield and fat percentage of group B than the other two groups, however a low yield of milk and fat percentage was observed for group C in comparison to the

control. Plasma total protein content, albumin, globulin, cholesterol, total lipids, and glucose level showed non-significant differences among the three groups (Al-Shaikh et al. 1999).

10.4.4 Antioxidant Properties

Today there is increasing interest of developing novel food enriched with natural antioxidants extracted from oilseeds, vegetables, fruits, whole grains and their by-products (Dhull et al. 2016, 2019b). Fenugreek is rich in flavonoids including isovitexin, quercetin, apigenin, orientin, luteolin, and vitexin (Sauvare et al. 2000; Shang et al. 1998). Phenolics are used as an antioxidant to hinder the formation of chelating metal ions, free radicals, auto-oxidation chain reactions (Carocho and Ferreira 2013). Higher dry matter content (18.90%), total phenols (0.62%), orthodihydroxy phenols (0.80%), and flavonoids (2.13%) have been reported by Chawla et al. (2004) in fenugreek leaves. An array of polyphenolic compounds with significant health benefits is found in fenugreek. Fenugreek extracts in different solvent such as ethyl acetate (Kaviarasan et al. 2007; Kenny et al. 2013) and alcohol (Naidu et al. 2011; Dhull et al. 2020b) showed high oxygen radical scavenging activity in vitro spectrophotometric analysis. HPLC quantification also showed presence of many compounds including apigenin, number of kaempferol and quercetin glycosides (Chatterjee et al. 2009) and five flavonoids (vitexin, tricin, quercetin, naringenin, and tricin 7-*O*-β-D-glucopyranoside) in fenugreek extracts (Shang et al. 1998). Also, in one another UPLC-MS study of ethyl acetate extract of fenugreek, 18 phenolic compounds were quantified and reported two flavonoids, apigenin-7-*O*-glycoside (1955.55 ng/mg) and luteolin-7-*O*-glycoside (725.50 ng/mg) as most abundant compounds in the extract (Kenny et al. 2013). Fenugreek has a protective effect on enzymatic antioxidants and on lipid peroxidation (Bhatia et al. 2006).

The phenolic and flavonoid compounds present in fenugreek mainly contribute in enhancing its antioxidant capacity (Dixit et al. 2005). The powerful antioxidant attributes of fenugreek have been suggested having beneficial effect on liver and pancreas (Balch 2003). Soaked, germinated, roasted as well as fermented seeds have been found with improved antioxidant properties compared with raw seeds (Pandey and Awasthi 2015; Dhull et al. 2020d, 2020e) because of the fact that processing increases the bioavailability of different constituents of grains. Kaviarasan et al. (2004) reported RBC protective effect of polyphenols in fenugreek from oxidative change inflicted by the peroxide treatment. Fenugreek aqueous extract also showed protective effect against ethanol toxicity and inhibited the lipid peroxidation promoter by production of thiobarbituric acid reactive substances (Thirunavukkarasu et al. 2003). The antioxidative potential of fenugreek was found comparable to other well-known antioxidants, such as glutathione and α-tocopherol (Venkata et al. 2017). The natural antioxidants help to reduce the signs of aging, strengthen the immune system, and improve cellular health (Kaviarasan et al. 2004).

10.4.5 Hypocholesterolemic Effect

Zia et al. (2001) revealed that abnormal blood cholesterol deficiency is known to be a hypocholesterolemic problem, and oral administration of methanolic and aqueous seed extracts at a dose of 1 g/kg of body weight in mice resulted in hypoglycemic results. Roberts (2011) reported fenugreek seeds consist of significant quantities of mannose and galactose which are the main constituents of gum. Out of which mannose is associated to reduce cholesterolemia. "The fenugreek extract has been investigated for its effects on blood lipid, and in experimental rats with diabetics. The streptozotocin-induced diabetic rats were administrated by oral intragastric intubation separately with low dose, middle dose, high dose of fenugreek extract, and Metformin HCl for about one and half month (6 weeks). As compared to diabetic group, rats treated fenugreek extract had lower triglycerides, total cholesterol, higher HDL cholesterol in a dose-dependent manner" (Xue et al. 2007).

10.4.6 Anticancer Effect

One of the world's major causes of death is cancer. The protective effect of fenugreek seeds in experimental cancer models using experimental animals or cell lines has been seen in several documented studies. Fenugreek seed extract significantly inhibited 7,12-dimethylbenz(a)anthracene-induced mammary hyperplasia and reduced its incidence in rats. It was further suggested that the anti-breast cancer protective effects of fenugreek could be due to increased apoptosis (Amin et al. 2005). Further, Verma et al. (2010) studied alcoholic whole plant extracts of fenugreek which showed in vitro cytotoxicity against different human cancer cell lines such as lung (A-549), liver (Hep-2) colon (502713, HT- 29) and neuroblastima (IMR-32). A selective cytotoxic effect of fenugreek extract in vitro to a panel of cancer cell lines has been observed, including T-cell lymphoma (Alsemari et al. 2014). One another study observed effect of ethanol extract of fenugreek on growth of MCF-7 cells which is an estrogen receptor positive breast cancer cell line. The extract decreased the cell viability and induced early apoptotic changes such as flipping of phosphatidylserine and decrease of mitochondrial membrane potential. Also, cellular DNA fragmentation into multiples of approximately 180–200 base pair was reported (Sebastian and Thampan 2007). Cell cycle analysis revealed a sub-G1 apoptotic population along with cell cycle arrest at G2/M phase in fenugreek extract treated cells implicating the role of fenugreek extract-induced apoptosis in its anticancer role. According to an investigation, treatment with fenugreek extract showed growth inhibitory effects on breast, pancreatic, and prostate cancer cell lines but primary prostate or immortalized prostate cells remained unaffected. Inhibition of cancer cell growth by *Trigonella* is attributed to its ability to induce death of cell, despite simultaneous upregulation of growth stimulatory pathways in normal cells (Shabbeer et al. 2009).

10.4.7 Antibacterial and Antifungal Effect

Haouala et al. (2008) studied anti-fungal activity of aqueous extract from different plant parts of fenugreek in different solvents such as ethyl acetate, methanol, and petroleum ether fractions of the aerial parts to find out their activity against fungal strains like *Rhizoctonia solani, Pythium aphanidermatum, Botrytis cinerea, Fusarium graminearum,* and *Alternaria sp*. It was observed that fenugreek had antifungal activity and the immensity of effect fluctuates with species of fungus and plant parts. It could be suggested that fenugreek is an important source of biologically active compounds which can be useful to develop novel and better antifungal drugs. Several studies reported the effectiveness of extracts from fenugreek against *Helicobacter pylori* (Randhir and Shetty 2007; O'Mahony et al. 2005; Randhir et al. 2004). In a study by Mercan et al. (2007), it was observed that honey samples with highest antibacterial activity against *E. coli, S. aureus, and P. aeruginosa* showed maximum pollens from fenugreek than any other plant. Zia et al. (2001) also found that methanol soluble fraction of fenugreek extract exhibits nematicidal activity and gives rise to significant mortality of *Meloidogyne javanica* larvae, and can be used against nematodes. Fenugreek can be used in the treatment of patients with calcic urolithiasis (Laroubi et al. 2007) and also have an anti-inflammatory effect (Chauhan et al. 2010).

10.5 Conclusions

On the basis of scientific information presented in this chapter, fenugreek can be presented as a crop with unique and rare neutraceutical properties with multiple health benefits. High fiber content, protein content, gummy nature, and other bioactive compounds make it a naturally health-promoting herb. It is used in functional food, traditional food, nutraceuticals as well as in physiological utilization such as antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant, and antidiabetic agent. The conventional medical therapy can be supplemented with fenugreek to treat many chronic diseases such as type 2 diabetes, dyslipidemia, joint and gastrointestinal inflammatory conditions, and modulation of fat accumulation in adipocytes, and also can help in preventing or delaying of the onset of neurodegenerative diseases. Lack of safety concerns following acute and chronic dosing is an added beneficial feature of fenugreek. However, some cautions and limitation regarding risks related to pregnancy, fetal development, and allergies need to be followed. Also, a large amount of preclinical data for fenugreek is available but the clinical trials have been of limited quality. The future for fenugreek can be even brighter if additional clinical studies can be performed to increase the weight of evidence with respect to efficacy in humans using the same quality and standards as are used for pharmaceuticals.

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Fenugreek (*Trigonella foenum-graecum* L.): The Magical Healing of Human Health Hazards

11

Suddhasuchi Das and A. B. Sharangi

Abstract

Fenugreek (*Trigonella foenum-graecum*) is an annual herb well-known from ancient times as a spicy food ingredient with exceptional nutritional profile along with substantial medicinal effects. It is native to southern Europe and Asia. The seeds are angular and rich source of gum, fibre, alkaloid, flavonoids, saponin and volatile content. They also contain a considerable amount of fibre, phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid, choline, vitamins A, B1, B2, C, nicotinic acid, niacin and many other functional elements. Fenugreek, as a crop, usually grows well under diverse and a wide range of conditions. It is moderately tolerant to drought and salinity, and can even be grown on marginal lands in profitable way. Owing to these characteristics, fenugreek may well fit in several cropping systems. The high content of fibre makes fenugreek to be used as food stabilizer, adhesive and emulsifying agent to change food texture for some special purposes. Some evidences suggest that fenugreek may also be regarded as anti-diabetic, anti-carcinogenic, remedy for hypocholesterolaemia and hypoglycaemia, anti-oxidant, antibacterial agent, gastric stimulant and anti-anorexia agent. The present article aims to review the potential applications of fenugreek and its healing effects to various human health hazards.

Keywords

Chemical composition · Bioactive Compounds · Fenugreek · Health benefits

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11.1 Introduction

Herbs have been used in all parts of the world not only as food but also as potent drugs for thousands of years. They do not work like chemical drugs and they are not substitute of them (Horne 1996; Vuorelaa et al. 2004). Medicinal plants are used by 80% of the world population especially in developing countries to alleviate disorders and improve the general health, predominantly due to the universal conviction that plant-derived drugs are without any side effects along with being economical and locally accessible (Gupta and Raina 1998). Increasing demand of consumers for healthy foods has urged the food industry to develop food products that promote health. Foods that provide significant nutrition, exert health advantages, inhibit disease and/or assist health have become more readily accepted by the industry and can be used as successful marketing tools. This has caused the emergence of functional foods that comprise a wide range of components such as probiotics, prebiotics, vitamins, minerals and dietary fibre (Nematollahi et al. 2016). In this respect, some herbs have been considered for their application as an anti-oxidant, anti-microbial, health promotion and food development.

Fenugreek (*Trigonella foenum-graecum*) is an annual plant belonging to the family Leguminosae. It is one of the prominent spices used in preparing culinary day to day human food and regarded as healthy as well foods in today's world. The seeds and green leaves of fenugreek (Fig. 11.1 a and b) are also used in food over and above the medicinal applications since the beginning of human history. It not only enhances the aroma, taste and colour of the food but also modifies the texture of food materials (Table 11.1). Seeds of fenugreek spice have medicinal properties such as hypocholesterolaemic, lactation aid, anti-bacterial, gastric stimulant, for anorexia, anti-diabetic agent, galactogogue, hepatoprotective effect and anti-cancer. These beneficial physiological effects of fenugreek are mainly attributable to the intrinsic

Fig. 11.1 Fenugreek (**a**) young plant, (**b**) mature, dried and cleaned seeds

Table 11.1 Morphological characteristics of fenugreek (*Trigonella foenum-graecum*)

Morphological characteristics	Description, colour and texture	References
Plant habit	Erect or prostrate, straight or profusely branched	Flammang et al. (2004), Srinivasan (2006b)
Stem	Circular to slightly quadrangular, greenish, often characterized by pinkish colour due to anthocyanin accumulation under field conditions. Mucilage is a dominant ingredient of the seeds	Moradi and Moradi (2013), Blank (1996)
Leaf	Fenugreek leaves are trifoliate, triangular stipules, 10–30 mm long, 5–15 mm wide, truncate at apex, narrowed towards the base, margins shallowly serrate to dentate glabrous	Brar et al. (2013)
Flower	Yellow when young but white on maturity	Mehrafarin et al. (2011)
Seed	Fenugreek seed has a central hard and yellow embryo which is surrounded by a corneous and comparatively large layer of white and semi-transparent endosperm	Srinivasan (2006a), Betty (2008)

dietary fibre constituents which have promising nutraceutical values as well (Srinivasan 2006a).

11.2 Historical Uses of Fenugreek

Fenugreek has a long history as both a culinary and medicinal herb in the primeval world. Applications of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies (Morcos et al. 1981). The Greeks and Romans used it for cattle fodder (hence the Latin *foenum graecum* meaning Greek hay). In ancient Rome, fenugreek was supposedly used to relieve labour and delivery. In traditional Chinese medicine, fenugreek seeds are used as a tonic, as well as a treatment for weakness and oedema of the legs (Yoshikawa et al. 1997). In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant (Patil et al. 1997).

11.3 Composition and Active Constituents

Fenugreek has dominant anti-oxidant properties concurrent to its health benefits. Fascinatingly, germinating seeds are more advantageous than un-germinated dry seeds in this regard. Alternatively, the aqueous fraction of fenugreek shows great anti-oxidant activity than flavonoids and phenolics (Meghwal and Goswami 2012a; Khole et al. 2014). Fenugreek contains a fairly high amount of flavonoids, alkaloids, saponins and other anti-oxidants (Fig. 11.2). It contains a major class of phenolics

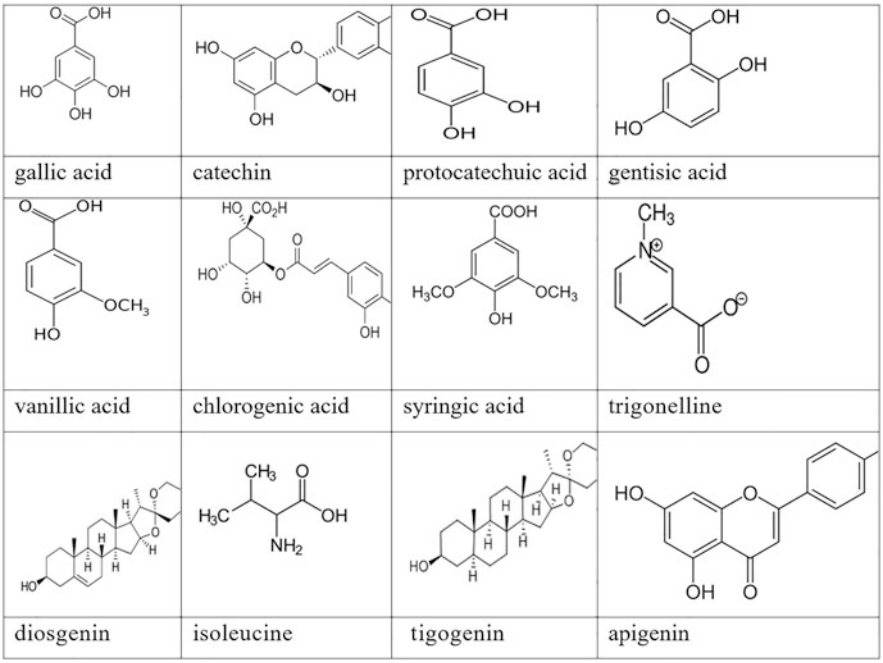

Fig. 11.2 Biologically active compounds of fenugreek

like gallic acid (1.7), protocatechuic acid (4.0), catechin (0.4), gentisic acid (35.8), chlorogenic acid (0.7), vanillic acid (58.5) and syringic acid (0.3) as mg per 100 g of the seed extract (Rababah et al. 2011). Fenugreek endosperm contains 35% alkaloids, primarily trigonelline (Jani et al. 2009). Flavonoid constitutes more than 100 mg/g of fenugreek seed (Naidu et al. 2011). All these compounds are classified as biologically active as these have pharmacological effects on the human body when administered. That is why they are promoted in daily diet to manage hypercholesterolaemia, cancer and diabetes mellitus as they possess hypoglycaemic, anti-lipidaemic, anti-carcinogenic and cholagogic properties (Meghwal and Goswami 2012b). However, volatile oils and alkaloids, the two foremost constituents, are to be removed before use as they cause awful aroma and bitter taste.

Fenugreek seed contains 45–60% carbohydrates, mainly mucilaginous fibre (galactomannans); 20–30% proteins high in lysine and tryptophan; 5–10% fixed oils (lipids); pyridine-type alkaloids, mainly trigonelline (0.2–0.36%), choline (0.5%), gentianine, and carpaine; the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin; free amino acids, such as 4-hydroxyisoleucine (0.09%); arginine, histidine and lysine; calcium and iron; saponins (0.6–1.7%); glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin); cholesterol and sitosterol; vitamins A, B1,C, and nicotinic acid; and 0.015% volatile oils (n-alkanes and sesquiterpenes), which are thought to account for

many of its presumed therapeutic effects (Granick et al. 1996; Snehlata and Payal 2012; Yoshikawa et al. 1997).

The main steroidal sapogenins obtained from fenugreek seeds are diosgenin and yamogenin which are used as steroid intermediates in the pharmaceutical industry. The occurrence of diosgenin [(25R)-spirost-5-en-3β-ol] in the seeds of fenugreek has been well expected for over 50 years (Marker et al. 1947). Other saponins and steroidal saponins present in fenugreek include fenugrin B, fenugreekine, trigofoenosides A-G, tigogenin, neotigogenin, gitogenin, neogitogenin, yuccagenin and saponaretin (Yadav et al. 2011). The plant alkaloid Trigonelline (0.3–0.4%) was first isolated from the seeds of fenugreek. It is a pyridine alkaloid, known for its hypoglycaemic and hypocholesterolaemic activity. Trigonelline (N-methylnicotinic acid) is derived from nicotinic acid and the reaction is catalysed by S-adenosyl-L-methionine (SAM)-dependent nicotinate enzyme N-methyltransferase. Nicotinamide and nicotinic acid, the products of pyridine nucleotide cycle (PNC) give rise to trigonelline.

As fenugreek is rich in several phytochemicals, alkaloids, carbohydrates, steroidal saponins, amino acids and minerals are present in fenugreek, it can be used for nutritional, nutraceutical, medicinal and therapeutic purposes (Aasim et al. 2018). Fenugreek has been extensively used as a flavour enhancer in several traditional cuisines. Additionally, the medicinal properties of fenugreek such as anti-carcinogenic, anti-diabetic, anti-oxidant, hypocholesterolaemic, anti-lithogenic anti-microbial and immunological properties make it an important compound to be used in food and pharmaceutical industries (Reddy et al. 2019). Fenugreek is also used as an emulsifier and stabilizer in different types of food products. Moreover, use of fenugreek extracts or powders has also been reported for developing bakery and extruded products (Wani and Kumar 2018).

11.4 Nutritional Values of Fenugreek

Fenugreek is having with a huge assortment of nutrients and bioactive compounds essential for recuperating the health and functionality of biological systems. The fenugreek seeds have 58% carbohydrates, 23–26% proteins, 0.9% fats and 25% fibre. Similarly, fenugreek leaves have 6%, 4.4%, 1.1% carbohydrates, proteins and fibre, respectively (Wani and Kumar 2018). Furthermore, fenugreek also contains different types of minerals such as potassium (603 mg/100 g), magnesium (42 mg/100 g), calcium (75 mg/100 g), zinc (2.4 mg/100 g), manganese and copper (0.9 mg/100 g) and iron (25.8 mg/100 g). Vitamin C (220 mg/100 g) and β carotene (19 mg/100 g) are also considered as the important components of fenugreek. (Wani and Kumar 2018; Al-Jasass and Al-Jasser 2012).

11.4.1 Proteins

Fenugreek endosperm is highly rich in protein such as globulin, albumin, histidine and lecithin. Seed of fenugreek has a high proportion of protein ranging from 20% to 30% as well as amino acid, 4-hydroxyisoleucine, which contains high potential for insulin-stimulating activity (Isikli and Karababa 2005). Fenugreek proteins are stable enough, and are not affected during booking. Moreover, debitterized fenugreek seeds are rich in protein and lysine contents (Srinivasan 2006b).

11.4.2 Vitamins and Minerals

Mineral content is relatively low in fenugreek except phosphorus and sulphur, which are present in fairly good concentrations (Nasri and Tinay 2007) It has also been reported that curry made from fenugreek has a high amount of calcium, iron and zinc (Jani et al. 2009). Another research confirmed that germinating seeds have pyridoxine, cyanocobalamine, calcium pantothenate, biotin and vitamin C (Parthasarathy et al. 2008).

11.4.3 Volatile Content

In 1997, researchers found a few volatile compounds based on the fenugreek aroma detection by the help of gas chromatography, namely acetic acid, linalool, isovaleric acid, butanoic acid, 3-isopropyl-2-methoxypyrazine, olfactometry diacetyl, eugenol, caproic acid, 3-Amino-4, 5-dimethyl-3, 3-isobutyl-2-methoxypyrazine (Blank et al. 1997).

11.4.4 Fenugreek Gum

The fenugreek gum can be utilized for thickening, stabilizing and emulsifying food agents (Brummer et al. 2003). Fenugreek gum is less exploited in the food industry as compared to other gums. Fenugreek gum is originated from the endosperm of the seeds and it consists of mannose and galactose (Youssef et al. 2009). Whenever making bread with wheat flour with combination of fenugreek, the prepared dough showed more water absorption in spite to the dough made without fenugreek gum.

11.4.5 Fenugreek Fibre

The fibre of fenugreek seed extract can regulate metabolism of glucose in the digestive tract. It acts as a source of natural anti-oxidants (Raju et al. 2001). It has been estimated that 100 g of seeds give more than 65% of dietary fibres which can exert short term beneficial effects by sinking energy intake and escalating satiety as

studied with obese people. Fenugreek fibre can also bind to cancer causing toxins of the intestine and remove them. Furthermore it lowers the rate of glucose absorption and helps in controlling blood sugar level (Meghwal and Goswami 2012c).

11.4.6 Alkaloid, Flavonoids and Saponin

Fenugreek contains different alkaloids, flavonoids and saponins (Kumar et al. 2012; Uemura et al. 2011) that the latter one is in the highest concentration (Singh and Garg 2006a). Alkaloid and volatile constituents of fenugreek seeds are the two major components which cause bitter taste and bad odour (Fæste et al. 2009). The level of flavonoid in fenugreek is more than 100 mg per g of seed (Madhava Naidu et al. 2011). The main alkaloids, flavonoids and saponins are present.

As a traditional medicine Fenugreek is highly rich in phytochemicals such as flavonoids, steroids and alkaloids, which have been identified and isolated by the pharmaceutical companies or industries for the manufacture of hormonal and therapeutic drugs (Fotopoulos 2002). The use of fenugreek seeds in eczema or other inflammatory situations is one of the more historical medicinal uses and the practice is still in use today in many countries. The extracted oil from fenugreek is almost about 6–8% of the weight of seed. It was also used as a tonic and treatment for weakness and oedema of the legs (Basch et al. 2003).

11.5 Role of Fenugreek in Healing of Human Health Hazards

11.5.1 Anti-Bacterial and Anti-Fungal Effect

Reports on anti-bacterial properties of fenugreek seed against the important human pathogenic bacteria so far are very much scarce (Farrukh and Aharnad 2003). The anti-fungal activity of fenugreek was reported (Table 11.2). Based on the studies carried out in fenugreek, worldwide report shows that the seeds of this plant possess

Table 11.2 Beneficial effects of fenugreek

Component used	Beneficial effects	References
Seeds	Hypocholesterolaemic effect	Srivastava et al. (2012)
	Lactation aid	Snehlata and Payal (2012)
	Immunomodulatory effect	Meghwal and Goswami (2012c)
	Prevents constipation	Sowmya and Rajyalakshmi (1999)
	Hypoglycaemic effect	Roberts (2011)
Leaves	Digestive and appetizer	Sowmya and Rajyalakshmi (1999)
	Gastro- and hepatoprotective	Blank (1996)
	Anti-cancer agent	Mathern et al. (2009)
	Wounds and sore muscles treatment	Mathern et al. (2009)
	Decreases blood pressure	Sowmya and Rajyalakshmi (1999)

strong anti-bacterial activity (Palombo and Semple 2001). The anti-bacterial activity of Fenugreek's leaves, seeds and stem in aqueous, methanol and acetone extract against *E. coli* and *Staphylococcus* isolated from a spoiled cabbage. A supplementary study found methanol extract to display the maximum zone of inhibition as compared to the aqueous extract. In comparison of all the extracts the leaves extracts were found to be maximum as compare to the extracts of seeds and stems. The results obtained during this experiment were highly effective and comparable with the commercial antibiotic at 100 µl concentration of Fenugreek extract (Sharma et al. 2017).

11.5.2 Anti-Diabetic Activities

Hannan et al. (2007) have reported that soluble fibre of fenugreek delays digestion and absorption of carbohydrate thereby improving homeostasis of glucose. Researchers have attributed this performance to extensive gel formation and low viscosity of the resulting gels inside the intestine, delaying the gastric emptying and decreasing the intestinal transit time of the food mass. Hammerness et al. (2003a, b) have reported that adding 100 g fenugreek powder containing 50% dietary fibre for a period of 10 days decreased 25% blood glucose level among the type II diabetes patients.

11.5.3 Anti-Oxidant Activities

Flavonoids of Fenugreek extract have been investigated to possess anti-oxidant activity (Moskaug et al. 2005). A study on fenugreek seed extract has been reported to prevent lipid peroxidation and haemolysis in RBC. Fenugreek seeds have also been proved to raise the anti-oxidant levels and reduce the liver per oxidation in liver of diabetic rats (Anuradha 2001).

The seed extract exhibited scavenging of hydroxyl radicals and inhibition of hydrogen peroxide-induced LPO in mitochondria of rat liver cells. The OH scavenging activity of the extract was demonstrated by pulse radiolysis and the deoxyribose system. Cellular structures are protected from oxidative damage due to the presence of anti-oxidants in the fenugreek seeds extract. An aqueous methanolic extract of fenugreek was investigated for its anti-radical and in vitro anti-oxidant activity in various model systems. The results obtained from different methods provide some important factors responsible for the anti-oxidant activity of fenugreek seeds (Kaviarasan et al. 2007).

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11.5.4 Anti-Cataract Activities

Cataract is the opacification in the eye lens and it remains the leading cause of visual abnormality, also contributes 50% of blindness worldwide (WHO 2005). The anti-cataract potential of *Trigonella foenum-graecum* was evaluated in selenite induced in vitro medium. The medium was supplemented with selenite and aqueous extract of *T. foenum-graecum* to the test group. An increasing level of malondialdehyde and diminishing level of GSH were seen in control as compared to standard lenses. Glutathione is amazingly restored and malondialdehyde levels are decreased by *T. foenum-graecum*. It also plays a significant role in re-establishment in the anti-oxidant enzymes, viz., superoxide dismutase, glutathione, peroxidase, catalase and glutathione-s-transferase. Fenugreek protects against the experimental cataract due to its anti-oxidant properties and also significantly restored the GSH level in a dose-dependent manner (Gupta et al. 2009).

11.5.5 Enzymatic Activities

Fenugreek has excellent capability to restore the performance of key enzymes in human and animal models. *Trigonella* when administered in rats can restore the changed enzyme activities and decrease hyperglycaemia. The combined dose of insulin, vanadate and fenugreek can treat the transformed levels of superoxide dismutase, glutathione peroxidase and anti-oxidant enzymes catalase, respectively, in liver and kidney of experimental diabetic rats. Activities of glucose 6-phosphatase and fructose-1, 6-biphosphatase in the liver and kidneys of diabetic rats are diminished by applying fenugreek seed extract (Kakani et al. 2009).

Anti-Fertility Activities

The antifertility effect was evaluated due to the addition of 30% fenugreek seeds to feeding diet of male and female rabbits and reported the following results: (a) the circulation of plasma progesterone concentrations at 10 and 20 days of gestation considerably augmented with no any side effect on the pre-breeding oestrogen concentrations, (b) significant decrease in developing foetuses in the female rabbits, (c) the plasma concentration of the androgen hormone and sperm concentration were halved, (d) appearance of toxicity in male rabbit, (e) anti-fertility effect of fenugreek seed in female rabbit, (f) testis weight in male diminished with damage to the seminiferous tubules and interstitial tissues (Raghuram et al. 1992).

11.5.6 Cholesterol Lowering Effect

Khorshidian et al. (2016) have attributed cholesterol lowering ability of soluble fibre to bind bile acids, which are therefore excreted rather than recycled to the blood stream thus reducing blood cholesterol. Basch et al. (2003) reported that fenugreek

seeds have ability to lower serum cholesterol, triglyceride and low-density lipoprotein in hypercholesterolaemia suffering patients and experimental models.

11.5.7 Anti-Cancer Perspectives

Cancer is undoubtedly one of the primary causes of death nowadays around the world. Severe side effects take place with commonly used therapeutic medicines with the ability to increase the life span of patient from few months or some years only. Plant-based active components have shown their potential (Mohammadinejad et al. 2019). In this regard, active ingredients of vegetables and fruits are being utilized to prevent the chances of cancer (Tohidi et al. 2017). Efforts are on-going to use the other approaches and ideas which can be effective in the prevention of cancer. Studies are available in which animals and cell lines were used as the investigational models of cancer proving the positive consequences of fenugreek seeds against this deadly disease (Yadav and Baquer 2014).

11.5.8 Hypoglycaemic Activities

Hypoglycaemia is a condition of human body in which there is an abnormal decrease in the sugar level of the blood. Singh and Garg (2006b) reported that fenugreek seeds have hypoglycaemic and hypocholesterolaemic effect as supported by findings during the experiments on animals. It has been reported to improve peripheral glucose utilization, contributing to improvement in glucose tolerance and exerts its hypoglycaemic effect by acting at the insulin receptor level as well as at the gastrointestinal level (Meghwal and Goswami 2012a).

11.5.9 Against Gall-Stone and Gastric Ulcer

Trigonella foenum-graecum seeds showed significant anti-ulcer effect. The outcome of fenugreek seeds is equivalent to omeprazole that is used as proton pump blocker in managing of gastrointestinal issues such as gastritis, gastroesophageal reflux disease, gastric ulceration and duodenum ulceration. In a rat model wherein gastric ulcer was induced by ethanol, the gel portion and aqueous fenugreek seed extract have consequences on mucosal glycoproteins and anti-secretory action plays a defensive role against ulcer. Ethanol-induced mucosal injury and lipid peroxidation can be avoided by improving the prospective of the mucosa of gastric against oxidation by the fenugreek seed consumption. The soluble gel fraction of fenugreek effectively prevents the formation of gastric lesion and its results are superior than omeprazole. Gastro protective and anti-secretory activities of fenugreek seeds are due to the presence of polysaccharides and flavonoids presence in the gel portion of fenugreek (Pandian et al. 2002).

11.5.10 Against Obesity

Hydroxyl isoleucine ameliorates insulin resistance caused by obesity. Precisely, the hydroxyl isoleucine downregulated a tumour necrotic factor-transforming catalyst which actually causes the change of mTNF to sTNF. The studies also provide the information about the pathway of signal transduction and upgraded the insulin confrontation, which is induced by obesity in adipocytes (3 T3-L1) (Gao et al. 2015). Similar results have been described in obese Zucker rats administering the fenugreek seeds resulting with subsequent decrease in tumour necrosis factor intensities, important rise in receptors of membrane and TNF receptor 2. In additional research, it was demonstrated that fibre present in fenugreek ominously suppresses the hunger and amplified in obese experimental units. Dietary supplementation of fenugreek is proved to have significant effect on loss of weight for short period of time (Raju and Bird 2006). Upon administering fenugreek powder to obese rats for 14 weeks, a considerable change in nutritional values, body measurements and decrease in the body weight was detected. Galactomannan that is present in the seeds of fenugreek captures and excretes the sugars from body before it moves in the blood, this causes the loss of weight (Mathern et al. 2009).

11.5.11 Analgesic Activities

Fenugreek seeds showed potential analgesic activity in comparison to an established analgesic drug, diclofenac potassium. For displaying the activity, aqueous and methanolic extracts of fenugreek seeds are used by following tail flick method (Yadav and Kaushik 2011). Vyas et al. (2008) opined that active herbal principles of fenugreek possess potential analgesic activities.

11.6 Potential Dangers

Fenugreek has some side effects too. For instance,

- it may increase the risk of bleeding,
- it may reduce potassium levels in the blood,
- it may cause loose stools in some women,
- it may induce facial swelling,
- it may create breathing difficulty.

Also, it can produce uterine contractions and hypoglycaemia in some mothers, etc. (Yadav et al. 2011).

11.7 Conclusion

Fenugreek, loaded with fibre, protein and valuable bioactive components, is having promising therapeutic applications. The medicinal and functional properties of fenugreek seeds have been studied over the last few years. Anti-diabetic, anti-oxidant, anti-carcinogenic, hypoglycaemic activity, hypocholesterolaemic activity are the major medicinal properties of fenugreek demonstrated in various studies. Based on these several healthful benefits, fenugreek can be recommended to be a part of our daily diet and incorporated into foods in order to produce functional foods. Fenugreek is a unique spice whose properties are being discovered with the renewed interest in traditional medicine. As rich sources of protein, lipids, fatty acids and minerals, fenugreek seeds and leaves cater to the body's needs for essential nutrients and deliver numerous health benefits. This eco-friendly plant has a high number of potential applications in the production of food and feed, medicine, cosmetics and pharmaceutical industries due to its nutrient and nutraceutical content. Fenugreek is traditionally supposed to be and ostensibly consumed as a medicinal plant since prehistoric time and is undoubtedly considered safe to human health. Its nutritional value and biologically active compound profile are unquestionably appreciated by medical science. Moreover, drought, saline and heavy metal tolerability, wide adaptability to various climatic regions and marginal lands are the potentialities of this crop to hold a righteous place in agricultural systems. Potential research directed at exploring whether and how fenugreek might constructively manage various human disorders has the promise to disclose new insights into how this traditional natural product could make us happier.

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Part III

Physiological, Biotechnological and Molecular Responses of Fenugreek

Nodulation Process, Nitrogen Fixation, and Diversity of Fenugreek Rhizobia

12

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Abstract

Trigonella foenum-graecum is a medicinal and culinary plant with varied health benefits. The improvement of its growth in weak soils depends on symbiotic soil bacteria called rhizobia which contribute to the autonomy of legumes towards nitrogen. Biological nitrogen fixation is one of the most beneficial processes for legumes. This process allows the plant to become autotrophic to nitrogen, one of the most limiting components for growth. Thus, the Fabaceae have acquired the possibility to invade and settle in areas and regions which are difficult for the cultivation and growth of other plants. It was found a great phenotypic diversity of fenugreek rhizobia and they were more tolerant to environmental stresses than the reference strains. Also a number of molecular methods have been discussed to reveal the genetic diversity of different strains, species, genera or families of rhizobia.

Keywords

Rhizobia · Fenugreek · Diversity · Symbiosis · Host range · NBF · Nodulation

12.1 Introduction

Fenugreek (*Trigonella* L.) is an annual medicinal, herbaceous plant member of the Fabaceae (Legumes) that originates from Asia and North Africa. Similar to clover, this genus encompasses approximately 80 species of annuals (Burnie et al. 2006). It is widely cultivated worldwide as a spicy crop in major continents (depending on soil and climatic conditions) around the world, including North Africa,

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Mediterranean, Europe, Middle East, China, India, Pakistan, Iran, Afghanistan, some regions of the Far East and Southeast Asia, Australia, the USA, Canada, and Argentina (Petropoulos 2002; Acharya et al. 2006; Burnie et al. 2006; Maletic and Jevdjovic 2007; Acharya et al. 2007). The seeds and leaves of this annual aromatic herb plant are used for various medicinal purposes since ancient times. The vernacular name of the fenugreek in Arabic is "Hulba," in England, it is called "fenugreek or fenigrec," in Pakistanis and Indians, it is called "Methi," in Italian called "Fieno Greco," and in French called "Senegre" (Petropoulos 2002; Mehrafarin et al. 2011).

The legume family or Fabaceae is ranked third among the Angiosperms in number of species (after Orchidaceae and Asteraceae) with more than 20,000 species worldwide, classified into around 727 genera (Cronk et al. 2006). It is a huge family which has been divided into six subfamilies: *Caesalpinioideae* DC., *Cercidoideae* LPWG, *Detarioideae* Burmeist., *Dialioideae* LPWG, *Duparquetioideae* LPWG, and *Papilionoideae* DC (LPWG 2017). Species range from small arctic and mountain grasses to huge tropical forest trees (Judd et al. 2001). Tree forms are predominant in hot countries, while herbaceous forms are mostly found in temperate regions (Guignard and Dupont 2005). However, among the species described so far, only a small proportion belonging to the *Papilionoideae* and some of the *Caesalpinioideae* from the *Mimosoideae-Caesalpinieae-Cassieae* clade have been studied for their nodulation and nitrogen-fixing symbiosis with rhizobia (Sprent et al. 2017).

Some fodder legume species can be grown in low nitrogen soils and serve as excellent green manure. This is the case of acacias (*Acacia* spp., *Vachellia* spp., *Senegalia* spp.), Clovers (*Trifolium*), alfalfa (*Medicago sativa*), sparse (*Onobrychis viciifolia*), lupines (*Lupinus* spp.). Others are cultivated for their high nutritional value thanks to their high energy and protein content such as broad beans (*Vicia faba*), peas (*Pisum sativum*), chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*), and beans (*Phaseolus* spp.). Some are considered as oilseeds, soybeans (*Glycine max*), and peanuts (*Arachis hypogaea*). Among woody plants, one can cite black locust (*Robinia pseudoacacia*), which is useful for reforestation and restoration of degraded soils, as well as ornamental plants such as *Laburnum anagyroides* and *Wisteria sinensis*. There are also species of industrial interest, namely the indigo tree (*Indigofera tinctoria*), *Haematoxylum brasiletto,* and *Haematoxylum campechianum*, which produce pigments, and *Crotalaria juncea* which produces textile fibers. There are many legume species with medicinal applications such as *Ononis spinosa*, licorice (*Glycyrrhiza glabra*), fenugreek (*Trigonella foenum-graecum*), clover (*Trifolium arvense*), tamarind (*Tamarindus indica*), sweet clover (*Melilotus officinalis*), the trefoil (*Lotus corniculatus*), the galega (*Galega officinalis*), the carob tree (*Ceratonia siliqua*), the anthyllide (*Anthyllis vulneraria*), and the bean (*Phaseolus vulgaris*) Some shrub legumes such as members of the genera *Cytisus* and *Chamaecytisus* play an important role in sylvo-pastoral systems by presenting an undeniable fodder interest (Chahboune et al. 2011).

Legumes are pioneer plant species that can grow in poor soils and allow their enrichment in nitrogen and other nutrients; thereafter, these soils can be colonized by other plant species. They constitute an important source of vegetable protein for

animal and human food. Several species in this family are able to fix atmospheric nitrogen in symbiosis with soil bacteria called rhizobia. However, only about 20% of legumes have been studied for nodulation ability and nitrogen fixation (Mahmood and Athar 2017).

Nitrogen (N_2) is a limited nutrient in the soil to support plant growth. This chemically inert gas constitutes about 78% of the atmosphere and its reduction is an energy-intensive process. Some legumes have the ability to fix nitrogen directly from the atmosphere thanks to the symbiosis they establish with certain soil bacteria called rhizobia. The term rhizobia is derived from the genus name *Rhizobium* (Frank 1889) which refers to the nitrogen-fixing symbiotic bacteria associated with the nodules of legumes. The rhizobium–legume symbiosis is an essential process not only for the plant to acquire nitrogen in reduced form, but also for the rhizobia to obtain the nutrients necessary for their development. Biological fixation of nitrogen is the transformation of atmospheric nitrogen gas by soil microorganisms into combined nitrogen easily exploited by the legume plant. This process may constitute an alternative to the use of chemical nitrogen fertilization or at least reduces its use in agricultural systems.

The major experimentations carried out on nodulation, inoculation, and nitrogen fixation were fundamentally realized for the development of the application of biofertilizers. However, afterward the importance of these bacteria in agriculture has been demonstrated, and the research and studies of the plant–microorganism interactions have been diversified.

These studies were carried out with the aim to increase legumes production without deterioration of the environment, within the framework of sustainable agriculture. Indeed, unfavorable climatic factors, intensive land use, and inadequate agricultural practices may lead to the degradation of natural ecosystems and the increase of marginal lands. Thus, rhizobium–legume symbioses are considered as a potential application for the restoration of degraded lands by regenerating and maintaining their fertility. The exploitation of the natural diversity of legumes and the creation of collections of native rhizobial strains could contribute to the revalorization of the cultivation of legumes and to the subsequent re-installation of plant cover on degraded soils.

12.2 General Notions on the Biological Fixation of Atmospheric Nitrogen in Legumes

Nitrogen is considered as a major element in the nutrition of humans, animals, and plants. It is an essential nutrient source for agricultural production since the content of the various forms of assimilated nitrogen (ammonium, nitrates, or organic compounds) is low in the soil. As a result, atmospheric nitrogen (N2), the major constituent of the atmosphere but chemically inert, is restricted to some N2-fixing microorganisms living generally in a free state or in symbiosis with plants. Indeed, the biological process of atmospheric nitrogen fixation (ABNF) varies from one species to another and it is generally processed by prokaryotes, namely *Klebsiella,*

Azotobacter, Frankia, or *Rhizobium*. This process could be compared to the Haber–Bosch chemical reaction in an industrial context, which has been developed 100 years ago for the synthesis of chemical fertilizers. Furthermore, ABNF is of great importance as it contributes to the development of modern agriculture. However, the excessive use of chemical fertilizers in agriculture may generate different environmental impacts, such as the release of CO_2 and nitrogen monoxide N_2O, the main sources of greenhouse gas emissions as well as soil eutrophication (Gresshoff et al. 2015).

It has been reported that ABNF by soil native bacteria is significant, even using non-leguminous plants such as sugar cane. Many legume species are used in both modern agriculture and forestry, due to their contribution to the ABNF. Indeed, grain legumes such as soybeans (*Glycine max* L.Merr.), beans (*Phaseolus vulgaris* L.), faba bean (*Vicia faba*), and chickpea (*Cicer arietinum* L.) are major sources for food nutrition. Similarly, fodder species such as *Trifolium*, and medics (*Medicago*) contribute to a high ABNF intended for protein synthesis mainly in animals. More than 200 million tons of N_2 are added annually to the biosphere thanks the ABNF by legumes.

12.2.1 The Legume–Rhizobia Symbiosis

The establishment of a symbiosis between rhizobia and legumes is under the control of a sequence of events starting with bacterial infection and ending with the formation of a differentiated nitrogen-fixing nodule. The symbiosis has long been studied in legumes, first at the morphological level, then at the biochemical and genetic level thereafter. The organogenesis of nodules depends on a complex molecular dialogue between the two partners of the symbiosis. The plant is immobile and must wait for the compatible bacteria to enter in contact with its roots in the rhizosphere. However, the mobility of bacteria and chemotaxis play only a limited role in recognition (Ferguson et al. 2010).

In the rhizosphere there is an intense development of microbial populations around the root thanks to the release of many carbon molecules (sugars, organic acids, hormones, vitamins, and phenolic substances) by exudation, secretion, or autolysis of old cells. These organic substances are of low molecular weight and easily decomposed by microorganisms, which attracts symbiotic and pathogenic bacteria to the roots (Hoffmann 2003). However, plants have the ability to differentiate between pathogenic or symbiotic signals thanks to many cell-surface immune and symbiotic receptor complexes (Zipfel and Oldroyd 2017).

Among the roots exudates, flavonoids are considered as strong inducers of nodulation genes, called *nod* genes (Brencic and Winans 2005; Poinsot et al. 2016). Flavonoids play a very important role in the initiation of symbiosis and interaction with the protein NodD. They control the synthesis of NodD factor, a LysR protein belonging to the family of transcription regulators (Gage 2004; Brencic and Winans 2005). The NodD factor forms a complex with a particular flavonoid, which binds to conserved sequences upstream of the *nod* operons, called (*nod* Box),

and activate the transcription of several nod genes promoters. The presence of suitable flavonoids induces changes in the DNA topology at the nodD gene locus in the promoter region, allowing the gene transcription initiation by RNA polymerase (Chen et al. 2005).

The nod genes control the biosynthesis of the lipo-chito-oligosaccharides Nod factors (Spaink 2000; D'Haeze and Holsters 2000; Poinsot et al. 2016). The Nod factors secreted by bacteria provoke multiple responses which are essential for host plants nodulation. They induce many early nodulation events in the epidermis of the plant, the cortex, and the pericycle. At submicromolar concentrations these molecules are responsible for the depolarization of the membrane potential, the formation of infection threads, the deformation of root hairs, the division of cells of the root cortex, the formation of primordia of nodules, and the induction of gene expression of early nodulins (Siczek et al. 2013).

Rhizobia have a large group of genes involved in the early stages of nodulation. Nod factor (NF) production genes are common in all these bacteria (*nodA, nodB, nodC, nodD*). The latter (*nodD*) is responsible for the synthesis of the structure of (NF); the other genes are specific for particular species (Sergeevich et al. 2015). After the recognition phenomenon, the bacteria adhere to the root hairs and subsequently form an infectious cord which will penetrate the root. The absorbent root hairs will deform under the action of bacteria and cause the development of the nodule (Pandya et al. 2013).

The bacteria will divide into the incurved root hair and form an infectious thread that will spread inside the cortex and infect adjacent root cells (Yoro et al. 2014). The cells of the internal cortex become different and their mitotic activity is reactivated before and during the formation of the infection thread and its progress to the internal root tissues. The consequence of this mitotic activity is the formation of a structure called the nodular primordium. When the infection thread reaches the primordium, the cells of the middle cortex that have not been crossed by bacteria differentiate into meristematic cells. The infection thread branches out further and penetrates part of the cells located below the meristem leading to the development of a mature nitrogen-fixing nodule, which has four specific zones, the meristem, the infection zone, the fixation zone, and the senescence zone (Timmers et al. 1999).

Rhizobia are attracted to the root hairs by a wide range of substances, mainly by the phenylpropanoids exuded by the root (Verma et al. 2020). Greater production of these compounds is observed under nitrogen deficiency conditions (Hoffmann 2003). The flavonoids present in root exudates induce the expression of the bacterial nod genes that govern the production of Nod factors.

The establishment of symbiosis requires coordination between the plant and the expression of bacterial genes, which is regulated by the mutual exchange of molecular signals (Perret et al. 2000; Spaink 2000; Jones et al. 2007). The bacterial genes responsible for nodulation and nitrogen fixation are located in megaplasmids (pSym) in the genera *Rhizobium* and *Sinorhizobium*, or on the chromosome in the symbiotic islets in *Mesorhizobium*, *Bradyrhizobium*, and *Azorhizobium* (MacLean et al. 2007; Jones et al. 2007).

1. the common nodulation genes *nodABC*, essential for nodulation and in which the productions of mutations lead to a Nod (−) phenotype (Perret et al. 2000);
2. Host-specific genes (*nodFE, nodH, nodG, nodPQ,* and others) which determine the range of host plants and influence the rate and frequency of nodule formation (Doyle and Luckow 2003);
3. A family of *nodD* regulatory genes (Spaink 2000).

12.2.2 Synthesis of Nod Factors and Regulation of Nodulation Genes

Rhizobia activate the synthesis and secretion of lipo-chitooligosaccharides (LCOs), called Nod factors as response to the exudation of compatible inducers by the host plant legume. The first step which consists of gathering the Nod factors elements is achieved by the N-acetyl-glucosaminyltransferase coded by the *nodC* gene (Geremia et al. 1994). The chain elongation is carried out by NodC at the unreduced terminal region (Kamst et al. 1997, 1999). Then the NodB deacetylase removes half of the N-acetyl from the terminal region of N-acetylglucosamine (oligosaccharides) (John et al. 1993; Spaink et al. 1987, 2000). Finally, an acetyltransferase coded by *nodA* binds the acyl chain to the free C2 carbon of the final non-reduced region of the oligosaccharide (Debelle et al. 1986; Röhrig et al. 1994). Likewise, NodI and Nod J are involved in the export of other factors Nod (Cardenas et al. 1996; Fernandez-Lopez et al. 1996; Spaink et al. 1995). However, recent studies have suggested that nodA and nodC are also very important elements, particularly in the specificity of the host plant for nodulation (hsn) (Roche et al. 1996). The nodA varies in its specificity towards the host plant thus contributing to the definition of the host spectrum. For example, the replacement of the nod A of *E. meliloti* by the nod A of *R. tropici* contributes to the production of other nod factors acetylated with C16: 2 (51), while the nod A of the strain ANU289 of Bradyrhizobium sp. is unable to achieve the transfer of nodEFs from R. leguminosarum-bv.viciae (multi-unsaturated fatty acids) to the final acceptor (Ritsema et al. 1996). Furthermore, nodC is a determinant of the length of the skeleton of the nod factor and therefore a determinant of the specificity of the host (Kamst et al. 1995; 1999).

The *nodABC* genes are important in the synthesis of dimers and pentamers of N-acetyl-D-glucosamine possessing symbiotic activity in certain plants (Banfalvi and Kondorosi 1989; Plazinski et al. 1994). Other Nod factors play an important role in nodulation as well as in the interaction of rhizobia with their host plants. These secondary Nod factors contribute to the protection of the primordial Nod factors from degradation. These constituents play a support and protective role for the basic structures. The loci that control these additions are specific to certain rhizobia and are known by the genes (hsn).

12.3 Nodulation Process

During the rhizobium–legume interaction, the bacterial infection is totally beneficial to the plant, through the nitrogen supply provided by the bacteria. But it is also beneficial for the bacteroïd, because it receives from the plant the saccharide units necessary to produce the energy essential for the fixing of nitrogen and consequently for its development. In addition, the nodule forms a unique biological niche providing the bacteria with protection against oxygen and avoiding any competition, encountered in the natural environment, with other bacterial species.

After attachment of the bacteria on the surface of the host roots, mutual recognition between the two organisms is established, leading to a sequence of morphological and biochemical events. The first visible indication is the deformation of absorbent root hairs induced by the Nod factors.

Rhizobia invade the roots through tubular structures called infection threads, which penetrate the root cortex, triggering the formation of the nodular primordium (Jones et al. 2007). Cell division of the nodular primordium is preceded by the inhibition of auxin transport, caused by endogenous root flavonoids, which shows their crucial role as development signals in the initiation of nodular primordium (Wasson et al. 2006; Subramanian et al. 2006).

The bacteria then penetrate into the absorbent root hairs by invagination of the plasma membrane of the hair cell. The invagination is extended by a tubular structure: the infection thread which branches out and progresses from cell to cell towards the cortex (Smit et al. 2007). Infection of a cell by the infection thread is preceded by a rearrangement of the cytoskeleton, a displacement of the nucleus towards the center of the cell, and the formation of a structure similar to a phragmoplast (Brewin 2004). Subsequently, in cells that are invaded by the cords of infection, cell division is reactivated (Brewin 2004). The bacteria are then released inside the cortical cells. Therefore, rhizobia infect cells of the nodular primordium by an endocytosis-like process (González-Sama et al. 2004).

Once inside the nodular host cell, the rhizobia are surrounded by a membrane derived from the plasma membrane of the host cell called the peribacteroidal membrane or symbiosome membrane, forming hence a new cell compartment called the symbiosome (Whitehead and Day 1997; Oke and Long 1999). Then the bacteria forms of rhizobia will differentiate into bacteroid forms which are the real nitrogen fixers. During the infection process, the plants defense responses are suppressed or attenuated (Brewin 1991; Jones et al. 2007; Luo and Lu 2014).

12.4 Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is a process in which bacteria interact with the plants. One of the major BNF occurs in the interaction between rhizobia and legumes. “Symbiosis is the acquisition of an organism(s) by another unlike organism(s), and through subsequent long-term integration, new structures and metabolism(s) emerge” Zook (2015).

Nitrogen-fixing symbiosis between rhizobia and legumes is categorized as a mutualistic symbiosis, in which both sides benefit: rhizobia are fed and hosted by the plant, and legumes receive combined nitrogen provided by rhizobia. BNF is a natural process by which atmospheric dinitrogen gas (N2) is reduced to ammonia:

$N_2 + 16\ ATP + 8\ e^- + 8\ H^+ \rightarrow 2\ NH_3 + 16\ ADP + 16\ Pi + H_2$ (Lindström and Mousavi 2019).

The atmospheric nitrogen fixation and its reduction to ammonium (NH_4^+) are ensured by nitrogenase. However, bacteria are unable to fix nitrogen because the oxygen inhibits this enzyme and blocks its gene transcription. The atmospheric nitrogen fixation and its reduction to ammonium (NH_4^+) are ensured by nitrogenase. However, bacteria are unable to fix nitrogen because the oxygen inhibits this enzyme and blocks its gene transcription. The Nitrogenase is protected by leghemoglobin, a symbiotic protein, which plays a role in oxygen transport by maintaining low but sufficient O2 levels in the cytoplasm of plant root cells. This low oxygen level is necessary for oxidative phosphorylation and provides low oxygen levels to the bacteroids. (Verma and Long 1983). The atmosphere in the nodular environment then being favorable, the nitrogenase becomes active and catalyzes the reduction of N_2 to NH_4^+.

On the bacterial side, symbiosis is mostly controlled by accessory genes, which are often in rhizobia located in transmissible elements such as plasmids, symbiosis islands, and chromids. Unlike the "essential" core genes that are carried by chromosome, the accessory genes are dispensable for the bacteria. Accessory genes encode various characters of bacteria; for instance, pathogenicity, antibiotic resistance and toxins, and in rhizobia symbiotic elements. Since protein-coding accessory genes are specialized in ecological adaptation, they might have been acquired independently and might have evolved separately from core genes. The accessory genes are carried on transmissible elements, and they are more likely to undergo horizontal gene transfer (HGT) (Martens et al. 2008; Vinuesa 2010).

The horizontal transfer of nodulation genes between rhizobia is common, geographically widespread, not specific to any genus. The transfer of symbiosis genes between rhizobia or rhizobia to other bacteria adapted to local soil conditions can allow them to become rhizobial symbionts of previously incompatible legumes growing in these soils.

Nitrogen fixation can occur also in other plant families than Leguminosae. For instance, some rhizobia can fix nitrogen in symbiosis with the plant *Parasponia*. Actinorhizal plants can be nodulated by Frankia (Dawson 2008; Normand and Fernandez 2009; Op den Camp et al. 2011). Angiosperms that form nitrogen-fixing root nodules are clustered in the Eurosid I clade (Soltis et al. 2000).

12.5 Diversity and Phylogeny of Rhizobia

Different alpha and beta-proteobacterial bacterial species can establish a nitrogen-fixing symbiosis with plants of the *Fabaceae* family (Sprent et al. 2017). These bacteria are collectively called rhizobia and have the unique ability to induce the formation of nodules on the host plant roots. The individual rhizobia species have specific host ranges that allow them to nodulate either a limited or an extensive set of legumes (Perret et al. 2000).

The first classifications of rhizobia were essentially based on nodulation and cross-nodulation tests with the host plants. Generation time was also considered among the most suitable criteria for distinguishing between rhizobia. Indeed, they have been classified according to two groups: the slow growing rhizobia and those with rapid growth (Fred et al. 1932; Vincent 1970; Young and Haukka, 1996). In the initial classifications of Bergey et al. (1923), rhizobia were described as aerobic, non-sporulating Gram negative bacteria. In addition, the major criterion of this classification was their ability to induce nodular structures on the host plants.

However, many conflicting observations have been elucidated with regard to the ancient criteria adapted for the classification of rhizobia. Consequently, new comparative methods were introduced to develop a better classification, namely serological methods, RNA/DNA or DNA/DNA hybridization and plasmid analysis (Zakhia et al., 2004).

Jordan (1984) used digital taxonomy, GC content in DNA, serological response, extracellular composition, carbohydrates utilization, type of metabolism, sensitivity to antibiotics and bacteriophages, protein composition and type of bacteroids, to suggest the new genus *Bradyrhizobium* and subsequently reclassify the previously defined species. Thus, the three species *R. leguminosarum*, *R. trifoli,* and *R. phaseoli* were combined in a single species of *R. leguminosarum*. Two other slow growing species *R. meliloti* and *R. loti*, belonging to the genus *Rhizobium*, were then included in the genus *Bradyrhizobium* (Jordan 1984).

Young et al. (2001) proposed new combinations between the different genera of rhizobia based on the comparison of the sequences of 16S rRNA. They used phylogenetic similarities between *Agrobacterium, Rhizobium,* and *Allorhizobium* to include *Agrobacterium* species within the genus *Rhizobium* and defined the new species: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, R. undicola, and *R. vitis* (Young et al. 2001; Berrada and Fikri-Benbrahim 2014).

Ribosomal DNA sequencing has been the most suitable approach for comparing rhizobia sequences and the symbionts associated with various legumes were classified into α and β -Proteobacteria (Berrada and Fikri-Benbrahim 2014). Moulin et al. (2001) found that some South African legume species were nodulated by the members of Burkholderia from the β-proteobacteria. The nodulation genes *nodABC*, *nod,* and *nifH* in the symbiotic β-proteobacteria are similar to those of rhizobia of the α-proteobacteria subclass which assume that β-Rhizobia acquire these symbiotic genes by horizontal transfer (Rivas et al. 2009).

A number of molecular methods have been used to reveal the genetic diversity of different strains, species, genera, or families of rhizobia. However, with the advent of new, more practical methods, such as multilocus sequence analysis (MLSA) which has been widely used for estimation of genetic diversity and definition of new rhizobia species and has even been proposed to replace DNA-DNA hybridization (Martens et al. 2007, 2008). While others lost their value, such as multilocus enzymatic electrophoresis (MLEE), PAGE of total bacterial proteins (SDS-PAGE), two-dimensional electrophoresis of total bacterial proteins, amplified fragment length polymorphism (AFLP), restriction analysis 16S rDNA amplified

(ARDRA or PCR-RFLP of the 16S rRNA gene) and RFLP analysis of the amplified 16S–23S intergenic space (IGS).

The taxonomy of Rhizobia is actually based on the molecular and genomic analyses. Hence, the isolation and characterization of new isolates from different host plants led to the discovery of new groups and species. Actually, more than 136 species have been grouped into 18 genera belonging to the α and β-proteobacteria subclasses, (Mousavi et al. 2015; Wdowiak-Wróbel et al. 2017; Bournaud et al. 2017).

Currently, there are 180 described species of symbiotic nitrogen-fixing bacteria within the Alphaproteobacteria (α-rhizobia) and Beta-proteobacteria (β-rhizobia) (Wang et al. 2019). They are regrouped in different families, so far, Rhizobiaceae (*Rhizobium, Ensifer* (syn. *Sinorhizobium*), *Allorhizobium, Pararhizobium, Neorhizobium,* and *Shinella*), Phyllobacteriaceae (*Mesorhizobium, Aminobacter,* and *Phyllobacterium*), Brucellaceae (*Ochrobactrum*), Methylobacteriaceae (*Methylobacterium* and *Microvirga*), Bradyrhizobiaceae (*Bradyrhizobium*), Xanthobacteraceae (Azorhizobium), and Hyphomicrobiaceae (*Devosia*). Other three symbiotic N2-fixing genera, *Paraburkholderia*, *Cupriavidus,* and *Trinickia* belong to the family Burkholderiaceae of the beta-Proteobacteria subclass (De Lajudie et al. 2019). However, only a limited number of genera in these families have the capacity to fix nitrogen in symbiosis with *Fabaceae.*

12.5.1 Symbiovars

It has been reported that symbiotic genes (*nodA, nodC*, and sometimes nifH) may have very great similarity in the genomes of different rhizobial species, conferring them the ability to nodulate the same species of legume. Inversely, different strains of the same rhizobial species may have divergent genes that give them the ability to nodulate different legume species and therefore a distinct host range. Thus, species and even strains have been differentiated into symbiovars, that is, a set of strains with a similar nodulating host range (Rogel et al. 2011).

The analysis of symbiotic genes and mainly the nodC gene, and sometimes the gene nodA also, allowed the definition of different newly described biovars, which are presently called symbiovars (Rogel et al. 2011). The old biovars were defined on the basis of nodulation tests in different legumes (Jordan 1984). The number of symbiovars in the classical rhizobia is continually increasing within the different genera and species of rhizobia (Mnasri et al. 2012; Guerrouj et al. 2013; Bejarano et al. 2014; Cobo-Díaz et al. 2014; Rogel et al. 2014; Ramírez-Bahena et al. 2016; Delamuta et al. 2017; Mohamad et al. 2017; Tampakaki et al. 2017; Bouhnik et al. 2019; Missbah El Idrissi et al. 2020; Lamin et al. 2020). New symbiovars have even been described in non-rhizobial genera such as *Microvirga* and *Phyllobacterium* with the mediterranense symbiovar of the species *M. tunisiensis* (Msaddak et al. 2018; Missbah El Idrissi et al. 2019). Other symbiovars will most likely appear in the near future.

12.6 Fenugreek Rhizobia

The nitrogen-fixing bacteria associated with plants of the three genera *Trigonella, Melilotus,* and *Medicago* are specific and known as *Ensifer* (*Sinorhizobium*) *meliloti* or *Ensifer medicae* (Reeve 2010). The *Medicago* microsymbionts are able to nodulate *Trigonella* and *Melilotus*. However, Hou et al. (2009) reported that the species *Trigonella archiducis-nicolai* is nodulated in the Tibet high altitudes by Ensifer meliloti and a new species of *Rhizobium* they named *Rhizobium tibeticum* which is able to nodulate also *Medicago lupulina*, *Medicago sativa*, *Melilotus officinalis*, *Phaseolus vulgaris,* and *Trigonella foenum-graecum.* In China, You et al. (2008) and He et al. (2011) reported that *Trigonella arcuata*, an ephemeral legume plant, is nodulated exclusively by *Ensifer meliloti* strains, although their nodC and nifH symbiotic genes phylogenies diverged from the reference strains of *E. meliloti*. They were member of the symbiovar meliloti, as they nodulated *M. sativa* and *M. edgeworthii*, but not *Glycine max* or *P. vulgaris* (He et al. 2011).

There are very few data about the bacteria isolated from *Trigonella foenum-graecum* root nodules.

Singh et al. (2008) found that the bacteria isolated from the root nodules of *T. foenum-graecum* have optimal temperature of 29.4 °C and the optimal growth was obtained at pH 7. These bacteria utilize glucose, sucrose, and starch as sole carbon source. They produce amylase and cellulose and they were sensitive to chloramphenicol, kanamycin, and streptomycin.

Gaur et al. (2018) characterized different strains isolated from the root nodules of fenugreek collected from different districts of Western Rajasthan. Following the nodA and 16S rRNA sequences phylogeny; they concluded that the strains are closely related to *E. meliloti* and *E. medicae* although they clustered in a different clade.

In addition to photosynthesis, BNF is one of the most beneficial processes for legumes. This process allows the plant to become autotrophic to nitrogen, one of the most limiting components for growth. Thus, thanks to BNF, the Fabaceae have acquired the possibility to invade and settle in areas and regions which are difficult for the cultivation and growth of other plants.

The fenugreek organic cultivation profits from BNF, thanks to the choice of efficient inocula containing efficient rhizobial strains with high nitrogen-fixing capability. These rhizobia should especially survive in different soils while keeping their infectious power and their competitiveness against native strains which are usually less effective.

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Diversity of *Trigonella foenum graecum* Microsymbionts in Morocco

13

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Abstract

Trigonella foenum graecum is a medicinal and culinary plant with varied health benefits. The improvement of its growth in weak soils depends on symbiotic soil bacteria called rhizobia which contribute to the autonomy of legumes towards nitrogen. In this work, we characterized 75 bacteria isolated from root nodules of Fenugreek grown in different soils of the Eastern Morocco with the aim to select some performant strains to use as inoculum for the improvement of the plant growth and yield in weak soils of Moroccan semi-arid areas. We found a great phenotypic diversity of fenugreek rhizobia and they were more tolerant to environmental stresses than the reference strains. The strain S3D has similar behavior as *Ensifer meliloti* strain USDA1002, whereas the other strains analyzed were different from the studied reference strains. There was no relationship between rep-PCR fingerprinting and phenotypic properties. The nearly 16S rRNA gene sequence of some representative strains showed they are closely related to the genus *Ensifer* with the highest similitude values (100%) with *E. meliloti* USDA1021. Sequencing of the symbiotic *nodC* gene from some representative strains revealed they were close to *nodC* sequences of different *Ensifer meliloti* strains. Host range tests revealed that all the fenugreek rhizobia nodulated several legumes from different Fabaceae tribes. In conclusion, the strains analyzed are very infective and may be used as inoculum in the arid areas of Morocco.

Keywords

Rhizobia · Fenugreek · Diversity · Symbiosis · Host range · 16S rDNA gene sequencing

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13.1 Introduction

Leguminosae or Fabaceae is one of the most abundant and diverse plant families. Six subfamilies are recognized in Leguminosae: Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae, and Papilionoideae (Azani et al. 2017). This family contains over 19,000 species, in 750 genera, distributed worldwide. However, among the species described so far, only a small proportion has been studied for their nodulation ability, with nearly 2000 being identified as potential nitrogen fixers (Allen and Allen 1981; Dommergues et al. 1999). Legumes have the ability to fix nitrogen in association with rhizobia within the nodules elicited on their roots or stems. During the plant–bacterium interaction, an interchange of chemical signals called molecular dialogue between soil bacteria (rhizobia) and legumes involving flavonoids from plants and Nod factors of rhizobia will lead to infection of root hairs and the formation of nodules on legumes roots (Cooper 2007). Within the nodule, rhizobia reduce atmospheric nitrogen (N_2) to ammonia (NH_4^+) by the nitrogenase enzyme complex. This process has major ecological and agricultural importance as it permits the cultivation, installation, and survival of legumes in soils where demanding crops are more difficult to cultivate.

Trigonella is one of the first genera in which nodules were first recorded in 1542 (Allen and Allen 1981). Fenugreek (*T. foenum graecum*) is quite nutritious, being high in proteins, ascorbic acid, niacin, and potassium. This old cultivated medicinal plant is widely grown in the Mediterranean countries, India, North Africa, and the USA as a food, condiment, medicinal, dye, and fodder (Duke 1981; Foury 1954; Mir et al. 1998; Mishkinsky et al. 1977; Ajabnoor and Tilmisany 1989; Ernst 1990; Gabay 2002).

Symbiotic nitrogen fixation in legumes is a major contributor to soil fertility and depends on the compatible interaction between the plant and rhizobia (Van de Sande and Bisseling 1997). Rhizobia constitute a polyphyletic group and genera distributed in two subclasses of Proteobacteria, most of them in the alfa-Proteobacteria, including traditionally described rhizobia, namely *Rhizobium, Ensifer*, *Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium* as well as *Neorhizobium* and *Pararhizobium*. Recently, some bacteria isolated from legume nodules were phylogenetically placed out of the conventional groups of rhizobia, including the genera, *Aminobacter, Devosia, Methylobacterium, Microvirga, Ochrobactrum, Phyllobacterium*, and *Shinella*. Some symbiotic nitrogen-fixing bacteria belonging to the subclass of beta-Proteobacteria include three other genera, namely *Paraburkholderia, Cupriavidus,* and *Herbaspirillum* (Mousavi et al. 2014; Castro et al. 2016; Wdowiak-Wróbel et al. 2017; Bournaud et al. 2017).

Rhizobia associated with this plant are culturally and bio-chemically similar to alfalfa rhizobia and have been identified as *Ensifer* sp. (ex *Sinorhizobium*) species (Allen and Allen 1981). Members of the genus *Ensifer* are best known for their ability to form nitrogen-fixing symbioses with forage legumes of the three genera, *Medicago* L., *Melilotus* Mill., and *Trigonella* L. (Eardly et al. 2017).

Zurdo-Piñeiro et al. (2009) reported that the rhizobia isolated from *Trigonella monspelliaca* in the Canary Islands, identified as *E. meliloti*, nodulated also

Phaseolus vulgaris, whereas Eardly et al. (2017) found that the strains nodulating *T. suavissima* L., the only member of the tribe Trifolieae that is endemic to Australia formed a distinct clade within *E. meliloti*. Kumar et al. (2018) reported the nodulation of *T. foenum graecum* in India by *E. meliloti*, *Burkholderia* sp., and *Rhizobium leguminosarum*. However, little work has been undertaken to evaluate the diversity of fenugreek nodule bacteria in the southern Mediterranean region, excepting the report by El Batanony et al. (2015) who found that *T. foenum graecum* is nodulated by *E. meliloti* in Egypt. To our knowledge, this is the first work carried out on the taxonomy of fenugreek rhizobia through the Mediterranean area.

In this work, we aimed to analyze the symbiotic, phenotypic, and genetic diversity of rhizobia nodulating *Trigonella foenum graecum*, isolated from different soils in the Eastern region of Morocco, known by its semi-arid to arid climate and to evaluate their host range, aiming their use to inoculate other introduced legumes.

13.2 Material and Methods

Soils were collected from seven sampling sites in semi-arid regions of Morocco (Table 13.1). They were kept air dry in paper bags until used in the laboratory which was done within 2 weeks of collection. The soil from each site was placed in 3 pots 1,5 liters size and then in each of the three pots, three fenugreek seedlings were planted. The seedlings were grown in a greenhouse and were irrigated regularly with distilled water. The bacteria were isolated from root nodules as described by Vincent (1970). Pure isolates were maintained at −80 °C in 50% glycerol (in LSTM, Montpellier, France) and at +4 °C in Yeast Extract-Mannitol (YEM) which was routinely used as complete medium for rhizobium culture. The soil characteristics and the designation of the isolates used in this study are presented in Table 13.1.

13.2.1 Phenotypic Tests

Tests were performed in broth tubes or agar plates inoculated with an exponentially growing liquid culture. The tolerance of the rhizobial isolates to high temperatures was tested on TY broth medium (Beringer 1974) at 25, 30, 35, 40, 43, and 45 °C. The ability of isolates to grow in acidic or basic media was determined on YEM agar Petri dishes whose pH had been adjusted and buffered to 5, 5,5, 6, 7, 8, 9, 9,5, or 10, as described by Zerhari et al. (2000). The response of isolates to drought stress was tested on tubes containing YEM medium with different concentrations of PEG 6000 (0%, 5%, 15%, 20%, or 25%). The salt tolerance of the isolates was tested at 0, 1%, 2%, 3%, 4%, 5%, or 6% of NaCl (w/v). Utilization of 17 carbohydrates at 1% (w/v) as the sole carbon source was investigated on a modified YMB medium in which yeast extract was replaced by NH_4Cl (0,1%, w/v) and mannitol by one of the carbohydrates tested as reported by Mohammed et al. (2000). The following carbohydrates were tested: l(+)-rhamnose, d(+)-galactase, myo-inositol, d(+)-mannose, d(+)-fructose, citrate de calcium, l(+)-arabinose, d(+)-maltose, starch, lactose,

Table 13.1 Origins of the soils used to trap fenugreek rhizobia. -): no nodule found on the roots after 12 weeks of culture in growth chamber. +): at least one nodule was obtained per plant

Soil sampling site	Strains isolated	Type of substrate	Bioclimatic status
Lalla Mimouna 34°01′15.0"N 2°53′54.0"W	S1A, S9A to S9I	Calcareous marls	Arid with cool winter $0\ °C < m < 3\ °C$
Debdou 33°59′19.3"N 3°02′06.2"W	S3C, S3D, S3E and S3G	Schist and granite	Semi-arid with cold winter, $m < 0\ °C$
Aïn Almou 34°51′42.1"N 2°25′40.0"W	S8A, S8C	Schist and granite	Sub-humid with cool winter, $m < 0\ °C$
Ahfir 34°56′34.6"N 2°06′22.4"W	S10A to S10K and STR1 to STR40	Marls and erosion elements	Semi-arid with temperate winter $3\ °C < m < 7\ °C$
Irsane 34°29′43.1"N 2°28′30.5"W	S12A, S12D	Limestone and marls	Arid with cool winter $0\ °C < m < 3\ °C$
Ain Noucha Chrifia 34°48′18.8"N 2°25′11.4"W	S17A to S17D	Limestone and marls	Semi-arid with cold winter and hot summer $3\ °C < m < 7\ °C$
Tafoughalt Forest 34°48′53.7"N 2°24′30.9"W	S18B	Limestone and marls	Sub-humid with cool winter $0\ °C < m < 3\ °C$

sucrose, d(+)-raffinose, dextrins, trehalose, d(+)-glucose, ribose, and salicin. The heavy metal resistance of the isolates was determined on TY agar plates containing the following heavy metals (μg ml^{-1}): $CuSO_4.5H_2O$ (500); $AlCl_3$ (100); $AlCl_3$ (450); $HgCl_2$ (5); $CdSO_4$ (50); $ZnSO_4$ (250); Lead-acetate (1000); $MnCl_2.4H_2O$ (500); $MnSO_4$ (500); $MgCl_2$ (1000); $MgSO_4$ (1000); $BaCl_2.2H_2O$ (1000); and $CoSO_4$ (150). The intrinsic antibiotic resistance was tested on TY agar plates containing the following filter sterilized antibiotics (μg mL^{-1}): ampicillin (20), carbenicillin (20), chloramphenicol (30), gentamycin (10), geneticin (20), neomycin (20), rifampicin (20), spectinomycin (20), nalidixic acid (20), and tetracycline (5). Hydrolysis of urea and reduction of nitrate by the rhizobial isolates were investigated as described by Lindström and Lehtomäki (1988). The gelatinase activity was investigated as described by Missbah El Idrissi et al. (1996). The catalase activity was determined by the method of Graham and Parker (1964). The evaluation of oxidase activity was determined as described by Ourarhi et al. (2010). The aptitude of the strains to produce melanin was determined by the method of Cubo et al. (1988).

13.2.2 Numerical Analysis

A computer cluster analysis of 75 phenotypic traits was carried out for the 18 bacterial isolates. The resemblance between pairs of isolates was calculated using the Pearson correlation coefficient and presented as a dendrogram with the unweighted pair-group method with arithmetic average (Sneath and Sokal 1973).

13.2.3 Plant Nodulation Tests

The fenugreek seeds were surface sterilized for 5 min in 3% sodium hypochlorite, thoroughly rinsed with sterile distilled water and transferred to sterile water agar (0,6% w/v) plates and then incubated in darkness at 25 °C to promote germination. All isolates were tested for their ability to re-nodulate fenugreek seedlings. Inoculation and seed treatment were performed by using the method of Vincent (1970). The plants were cultured in a growth chamber under a constant temperature of 23 °C and a light/dark photoperiod of 16/8 h and watered with nitrogen-free nutrient solution. Nodulation was checked after a period of 4–6 weeks. Indirect effectiveness of the nodules for nitrogen fixation was estimated by visual assay of red leghemoglobin presence in cross sections and by the dark green intensity of the leaves compared to uninoculated control plants.

13.2.4 Screening for Host Range of Nodulation

Seeds of some legumes were scarified with 97% sulfuric acid and washed thoroughly with sterile distilled water and then transferred to sterile water agar (0,6% w/v) plates to germinate in a temperature of 25 °C. Seedlings were transferred to jars containing Jensen's N-free solution and inoculated with 1 ml of rhizobial strains. In each case two control treatments were included: uninoculated treatment without N and uninoculated treatment plus N (100 mg KNO_3 $liter^{-1}$) in the nutrient solution. Jars were transferred in a culture chamber as previously described. The appearance of nodules was assessed between the fourth and the twelfth week of growth. All the tests were done in duplicate.

13.2.5 Extraction of Bacterial DNA, PCR Assays, and DNA Sequencing

Bacterial total DNA was extracted as described by Brenner et al. (1982). For 16S rDNA amplification, template DNA was denatured for 5 min at 94 °C; then the PCR was carried out for 30 cycles (1 min at 94 °C,1 min at 55 °C, 3 min at 72 °C for each cycle) as described by Guerrouj et al. (2013).

For rep-PCR, the genomic DNA from each strain was amplified, using ERIC (I and II), (Versalovic et al. 1994) as described by Benata et al. (2008). Cluster

analysis of the electropherograms was performed with the Statistica 6 software. The resemblance between pairs was calculated using the Pearson correlation coefficient and presented as a dendrogram by the unweighted pair group method with arithmetic average (UPGMA). For the specific amplification of the *nodC* gene, the two oligonucleotides nodCFn and nodCi (Laguerre et al. 1997) were used following the protocol described by Guerrouj et al. (2013).

The PCR products were sequenced using Big Dye1 Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA) on a 3130xl model sequencer (Applied Biosystems). Analysis of the electropherogram was carried out with the sequencing analysis software version 5.3.1 (Applied Biosystems). The 16S rDNA and *nodC* genes were sequenced using the same primers as for PCR amplification, i.e. fd1-rd1 primers for 16S rDNA and nodCr- nodCF primers for *nodC* gene at the sequencing facilities of the UATRS (CNRST, Rabat).

The sequences obtained were compared with those from GenBank using the BLASTN program (Altschul et al. 1990). They were aligned using ClustalX software (Thompson et al. 1997). Distances calculated according to Kimura's two-parameter model (Kimura 1980) were used to infer phylogenetic trees with the neighbor-joining analysis (Saitou and Nei 1987) with MEGA 6 software (Tamura et al. 2013). Bootstrap analysis was based on 1000 re-samplings.

13.3 Results

13.3.1 Phenotypic Characteristics

75 bacterial isolates were isolated from fenugreek root nodules grown in different soils collected from many sub-humid, semi-arid, and arid regions of the northeastern Morocco (Table 13.1). They were all fast-growing rhizobia with mean generation times ranging from 3 to 5 h.

The phenotypic and genotypic characterization was carried out to describe the isolates. Some rhizobial strains kindly given by Pr Diegane Diouf (LCM, IRD-ISRA-UCAD, Belair, Dakar, Senegal) were used as references to compare their phenotypic properties with our isolates.

Excepting strains S3D, S10F, STR9, STR10, STR37, and STR43 that acidified the medium, all the other strains neither produced acid nor alkali. Interestingly, seven strains grew very well on PGA medium Graham and Parker (1964). All the strains produced catalase and oxidase and none produced indole; 83% produce urease and more than 50% produced melanin, whereas 13% grew normally in 8% of KNO_3.

The fenugreek rhizobia grew in media with pH values ranging from 4.5 to 8.5. However, the growth of all the strains was improved at pH values between 7 and 8.5, while 38% grew in pH 4.5. All the strains grew at 35 °C and a large number also at 45 °C. They were also tolerant to salinity. All the isolates grew in presence of 340 mM of NaCl, more than 50% grew in 520 mM, approximately 25% tolerated

850 mM and 18% tolerated 1,7 M of NaCl. We have already shown that fenugreek rhizobia were very salt tolerant (Abdelmoumen et al. 1999).

Growth responses to antibiotics were very variable. All the strains isolated from fenugreek nodules grow vigorously in the following tested antibiotics concentrations: ampicillin, spectinomycin, streptomycin, neomycin, gentamycin, nalidixic acid, chloramphenicol, rifampicin, and carbenicillin. The majority of the references were sensitive to these antibiotics. Tetracycline was the most potent antibiotic; it inhibited most of the strains used as references as well as fenugreek rhizobia. Most of strains were tolerant to heavy metals. Fenugreek rhizobia were tolerant to lead; while 25% of reference strains were sensitive. Mercury and aluminum inhibited the growth of all the reference strains.

13.3.2 Numerical Analysis

A dendrogram constructed from the similarity matrix by UPGMA revealed a phenotypic diversity of the strains. The strains were clustered in two major groups with several subgroups and clusters (Fig. 13.2) that differed by many characteristics and the diversity or similarity of properties in a group of strains was not correlated with their original soil conditions.

13.3.3 Host Range of the Isolates

The host range tests confirmed the high diversity of rhizobia nodulating fenugreek in the northeast region of Morocco. The ability of fenugreek rhizobial strains and *Sinorhizobium meliloti* (USDA1002), to nodulate different legumes was also assessed. None of strains nodulated *Cicer arietinum, Lens culinaris, Phaseolus vulgaris, A. cyanophylla, A. senegal, Retama raetam, Retama monosperma, Cytisus albus,* and *Cercis siliquastrum.* Strain *S. meliloti* USDA1002 and the fenugreek rhizobia tested nodulated *Trigonella foenum graecum, Medicago sativa,* and *Medicago arborea.* However, there was a great diversity in their aptitude to nodulate other legume species (Table 13.2). The reference strain USDA1002 and the strain S3D produced nodules only with *Trigonella foenum graecum, Medicago sativa,* and *Medicago arborea*, while all the other fenugreek rhizobia tested formed nodules with *Leucaena leucocephala*. *Colutea arborescens* was nodulated by seven of the eleven strains tested. *Erythrina caffra* was nodulated by seven strains. Strain S3G showed a very wide host range nodulating *Cytisus alpinus*, *Cytisus laburnum*, *Erythrina caffra*, *leucaena leucocephala*, *Medicago arborea*, *M. sativa*, *Parkinsonia andersonii*, *and Acacia nilotica*. It is the only strain that also nodulated *P. andersonii. A. seyal*, and *A. horrida* were exclusively nodulated by strains S10k and S17C, respectively. *Vigna unguiculata* was nodulated by strains S9D and S9I. *Cytisus laburnum* was nodulated by strains S9D, S10D, and S10J. Strain S10F differed from the others by its ability to nodulate *Pisum sativum*.

Table 13.2 Host range of fenugreek rhizobia and *Sinorhizobium meliloti* 1002

Bacterial strains / Legumes tested	*Ensifer meliloti* USDA1002	S3D	S3E	S3G	S9D	S9I	S10D	S10F	S10J	S10K	S12A	S17C
Acacia horrida	−	−	−	−	−	−	−	−	−	−	−	+
Acacia nilotica	−	−	−	+	−	−	−	−	−	−	−	−
Acacia seyal	−	−	−	−	−	−	−	−	−	+	−	−
Colutea arborescens	−	−	+	+	+	−	+	+	+	−	−	+
Cytisus alpinus	−	−	+	+	−	−	−	−	+	−	−	−
Cytisus laburnum	−	−	−	+	+	−	+	−	+	−	−	−
Erythrina caffra	−	−	−	+	+	+	+	−	+	−	+	+
Leucaena leucocephala	−	−	+	+	+	+	+	+	+	+	+	+
Medicago arborea	+	+	+	+	+	+	+	+	+	+	+	+
Medicago sativa	+	+	+	+	+	+	+	+	+	+	+	+
Parkinsonia andersonii	−	−	−	+	−	−	−	−	−	−	−	−
Pisum sativum	−	−	−	−	−	−	−	+	−	−	−	−
Trigonella foenum graecum	+	+	+	+	+	+	+	+	+	+	+	+
Vigna unguiculata	−	−	−	−	+	+	−	−	−	−	−	−

13.3.4 Genetic Analysis

The overall diversity of isolates was first determined by ERIC–PCR DNA fingerprinting. Examples of the patterns are shown in Fig. 13.1. Cluster analysis using STATISTICA 7 program grouped strains by genomic similarity and resulted in the dendrogram shown in Fig. 13.2. Eight main clusters were formed and some of the isolates occupied isolated positions, although the final level of similarity was not more than 75%, which supported the possibility of lineage differences within the isolates from *Trigonella foenum graecum.*

13.3.5 16S rDNA and *nodC* Sequencing

We selected some strains representative of different rep-PCR clusters to investigate the systematical divergence of the strains based on their 1 6S rDNA sequences. The 1 6S rDNA sequences of seven strains showed they were members of the genus *Sinorhizobium.*

A phylogenetic tree inferred from 1 6S rRNA gene sequences (Fig. 13.3) indicates that all the representative strains clustered with the published strains of *Sinorhizobium meliloti.* The sequences identities are reported in the figure. Pairwise alignments between globally aligned sequences of strains with various *Sinorhizobium* type species indicated they have identity values that vary between 99.93% and 100% with *Sinorhizobium meliloti* 1021 and *Sinorhizobium meliloti* LMG 6133.

The amplification of the *nodC* gene using the primer pair *nodCF—nodCl* resulted in an amplicon of approximately 930 bp. We sequenced the *nodC* gene of some strains used in the cross inoculation tests which showed different host patterns. The results showed some differences in the *nodC* classification which was different from 1 6S rRNA gene sequencing results. A phylogenetic tree (Fig. 13.4) showing the relationship between the *nodC* genes of representative strains and other species in various rhizobial genera revealed that nodC genes of these strains are related to different taxa. The sequences identities are reported in the Figure. The sequences were very close to different strains of *S. meliloti*.

13.4 Discussion

All the bacteria isolated from fenugreek root nodules were fast-growing rhizobia. The phenotypic and genotypic characterization was carried out to describe the isolates. Some rhizobial strains kindly given by Pr Diegane Diouf (LCM, IRD-ISRA-UCAD, Belair, Dakar, Senegal) were used as references to compare their behavior versus our isolates. The results showed a high level of diversity between the isolates and difference with the strains used as references.

Physiological and biochemical studies are the basis for detailed polyphasic taxonomy but cannot be used alone in taxonomic analysis. However, phenotypic

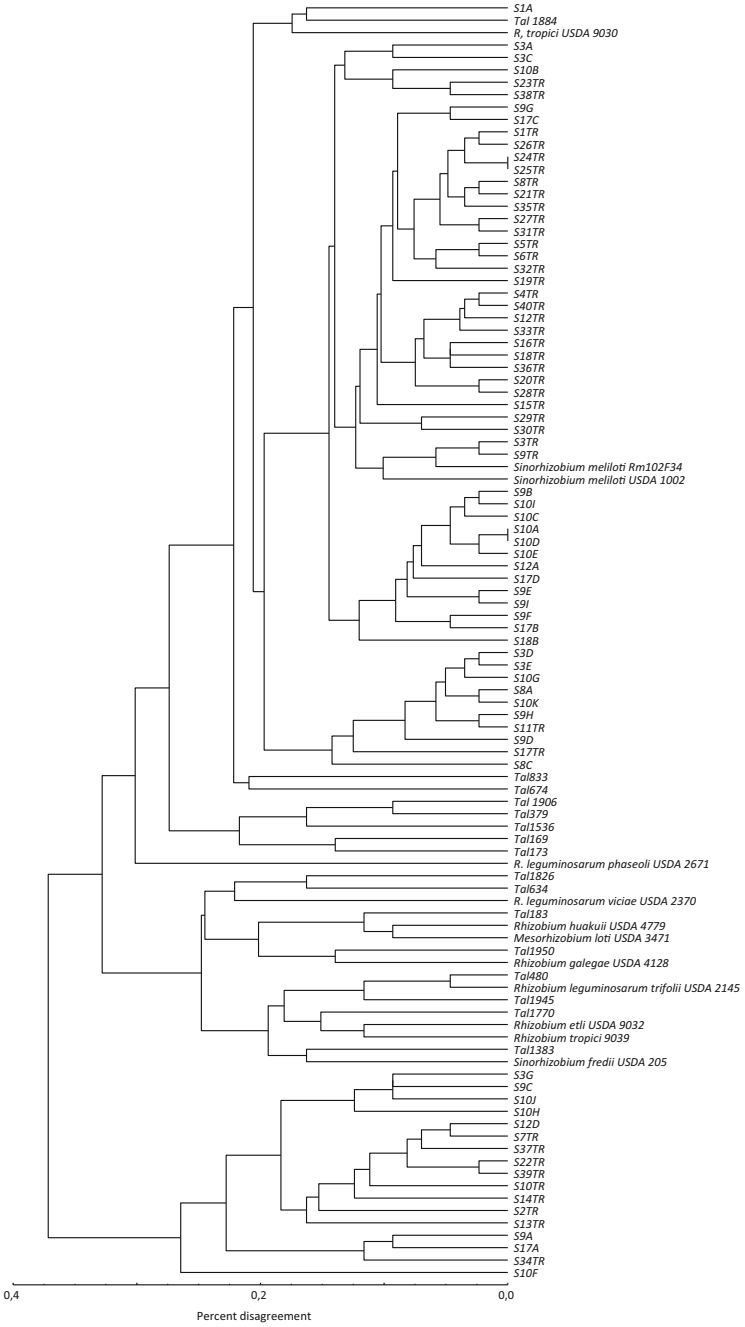

Fig. 13.1 Dendrogram of similarities between fenugreek rhizobia and reference strains according to their phenotypic properties. The dendrogram was constructed using UPGMA method and the STATISTICA5 program

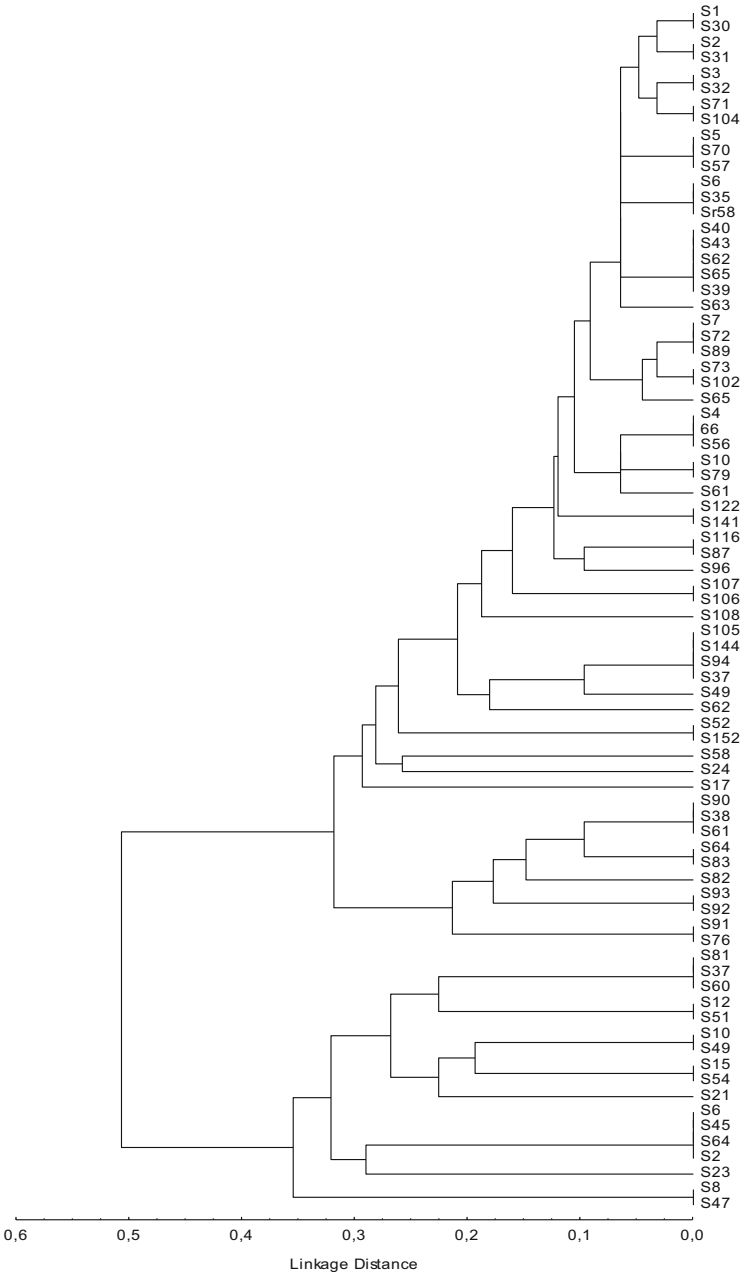

Fig. 13.2 Dendrogram generated by UPGMA clustering from ERIC-PCR fingerprinting of 75 fenugreek rhizobial strains isolated from soils in eastern Morocco

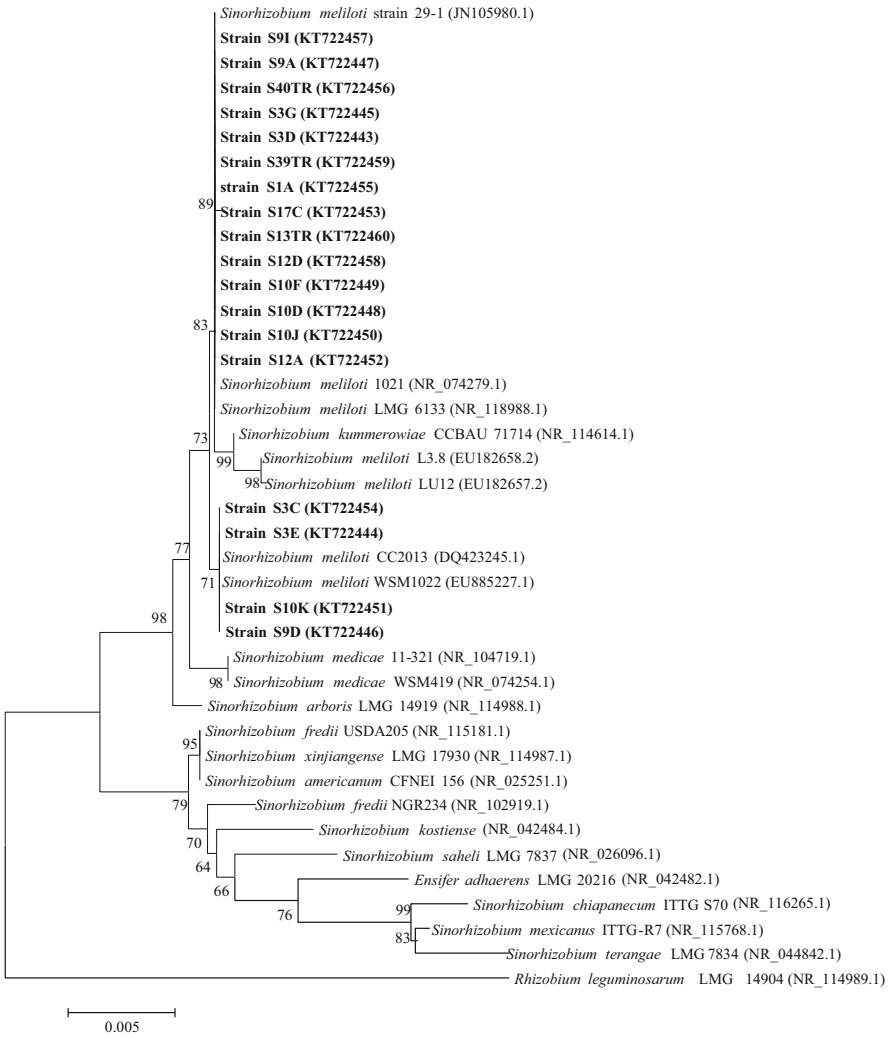

Fig. 13.3 Neighbor-joining phylogenetic tree based on partial 1 6S rRNA sequences of strains isolated from nodules of *Trigonella foenum graecum* and phylogenetically related species within the genus *Sinorhizobium.* Bootstrap values are indicated as percentages derived from 1000 replications. Bar 1 nucleotide substitution per 100 nucleotides. The tree is rooted with *Rhizobium leguminosarum* LMG 14904

characteristics are necessary for the characterization and selection of rhizobial isolates adapted to marginal soils and to provide information about their diversity. Interestingly, some strains grew on PGA medium. This property is uncommon in rhizobia (Vincent 1970). The majority of the isolates were neutral, producing neither acid nor alkali and a large number tolerated high salt concentrations and some grew at 45 °C. All the strains were isolated from continental Mediterranean area, with hot

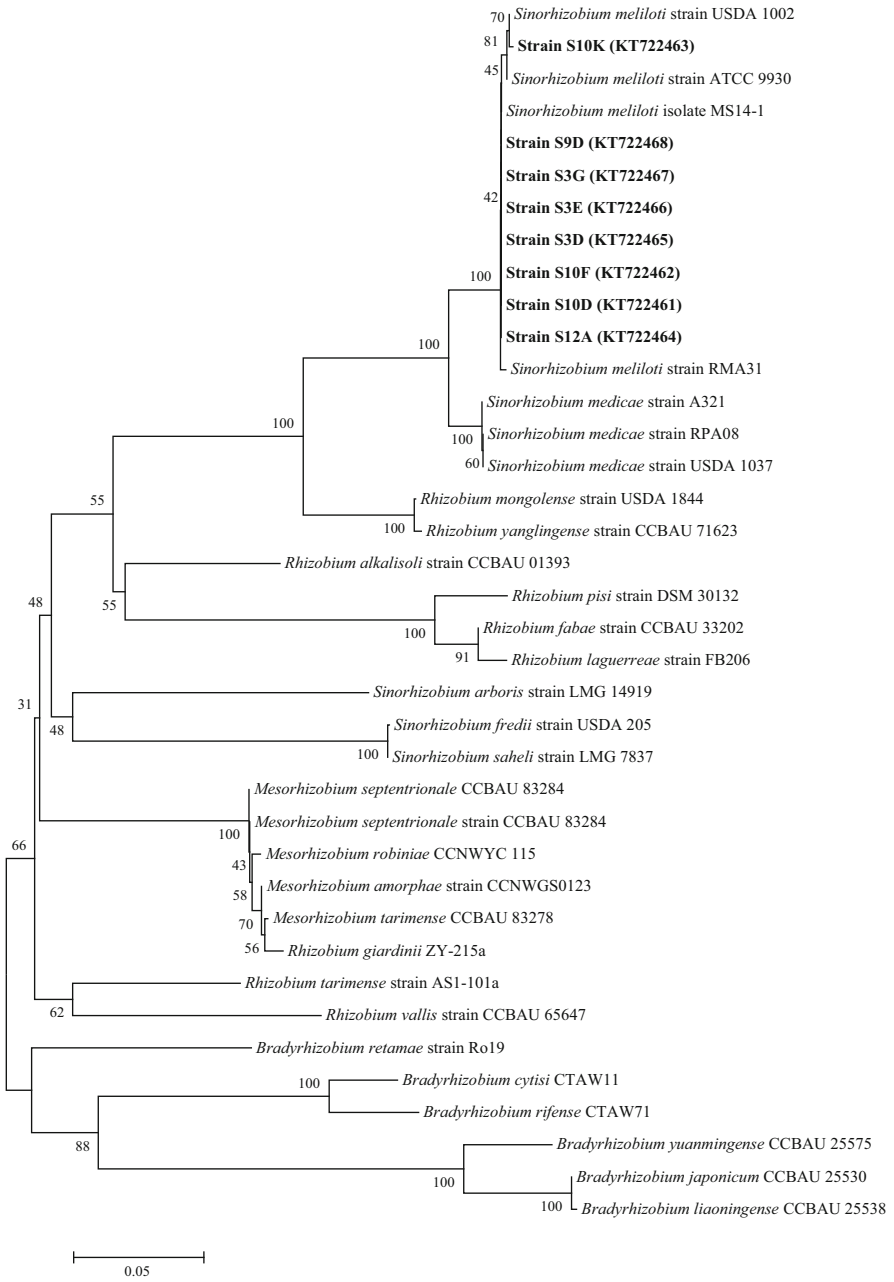

Fig. 13.4 Neighbor-joining phylogenetic tree based on *nodC* protein sequences of strains from nodules of *Trigonella foenum graecum* and some described species rhizobia, using MEGA 6 program. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site

summer and cool winter seasons. The ability of the strains to tolerate extreme conditions could be considered as an advantage allowing the survival of the rhizobia in soils during the summer hot season and their aptitude to nodulate the plant once the conditions are favorable. However, Munnevar and Wollum (1981) and Robert et al. (1982) observed that rhizobia isolated from hot regions presented no advantage when compared to strains isolated from temperate regions. The isolates growth responses to antibiotics were very variable and most of strains were also tolerant to heavy metals. The phenotypic diversity was revealed by a numerical analysis and construction of a dendrogram from the similarity matrix by UPGMA method.

The genetic diversity of isolates was first determined by ERIC–PCR DNA fingerprinting. This method is highly discriminating, enabling identification of genetic diversity at the intraspecies level (Versalovic et al. 1994). However, the method is more suited to indicate overall genomic structural similarities than phylogenies. Although the technique is suitable for strain discrimination and is not used primarily for taxonomical placement, it can, nevertheless, reveal a certain degree of chromosomal structural conservation (Laguerre et al. 1997).

The large host range tests confirmed the high diversity of rhizobia nodulating fenugreek in the northeast region of Morocco. The ability to nodulate different leguminous species was variable. All the strains tested were able to nodulate *Medicago sativa*, *M. arborea*, and *Leucaena leucocephala.* They also were able to nodulate differently other legume species. This wide host range may be mainly due to the diversity in Nod factors produced by the isolates. Boivin and Giraud (1999) reported that the analysis of nod genes or Nod Factors could be used to develop a polyphasic approach for the symbiotic characterization of rhizobia. However, the occurrence of strains with the same host specificity but belonging to different taxonomic groups suggests the possibility of lateral gene transfer.

The sequencing and phylogeny of 16S rDNA showed that the strains have 100% similarities and clustered with the published strains of *Sinorhizobium meliloti* species. This was expected as it is known that species of the genera *Medicago, Melilotus,* and *Trigonella* are nodulated by the same group of rhizobia. We confirmed our results by the sequencing of the *nodC* symbiotic gene. Bacterial nodulation (nod) genes are involved in the production of Nod factors, which act as specific signals triggering nodule formation. The *nodABC* genes, basically common to all root nodulating rhizobia and absent from all other biota, are known to be essential for the synthesis of the lipo-oligosaccharide backbone (Sachiko et al. 2001).

The sequencing and phylogeny of the *nodC* genes showed that all the strains analyzed were related to different strains of *S. meliloti* (Fig. 13.4). However they are not related to any described symbiovar of *S. meliloti.* Although *S. meliloti* is well known for its capacity to nodulate alfalfa, and *Melilotus*, some biovars were recognized in this species, such as bv. *acaciae*, bv. *medicaginis*, bv. *lancerottense,* bv. *mediterranense* and bv. *meliloti* Sachiko et al. (2001). They produce Nod factors similar to those described by other rhizobia or mesorhizobia nodulating different legumes.

In conclusion, the 75 rhizobia isolated from root nodules of fenugreek in eastern Morocco soils showed a high physiological, symbiotic, and genetic diversity.

Despite edapho-climatic and geographical differences in location of the sampling sites, all the isolates belong to *Ensifer meliloti* species. However, they belong to different symbiovars, which explains the large host range and specificity between the plant and the microsymbiont partner in the soils of Oriental Morocco. Their host range was broader than that of described sinorhizobia and this was confirmed by sequencing of the *nodC* gene. The ability of fenugreek to be nodulated by diverse rhizobial strains owing different properties and able to grow in different stress conditions is an advantage to the plant as it will always find a bacterial partner who will feed it continuously with nitrogen in different environments.

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14 Impact of Various Environmental Stress Factors on Productivity, Quality, and Secondary Metabolites of Fenugreek (*Trigonella foenum-graecum* L.)

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Abstract

Plants are subjected to various environmental stress factors as biotic (pathogens, pesticides, herbicides, insecticides, etc.) and abiotic stress (freezing, radiation, salinity, floods, drought, high temperature, heavy metals). These stress factors decrease the crop production and chemical constituents by affecting the plants at the different growth stages. Fenugreek (*Trigonella foenum-graecum* L.) occupies an important position among these plants, which belongs to the Fabaceae family and is widely considered as an antidiabetic and anticholesterol herb. These stress factors affect the morphological properties, yield attributes, and secondary metabolites of fenugreek. Field experiments revealed that several abiotic factors such as water stress, drought, low and high soil pH values, salinity, and nutrient deficiencies reduce plant growth by affecting various physiological and biochemical processes in the plant. Furthermore, population of root nodulating soil bacteria face same adverse environmental conditions as their legume partner. In addition, the pesticides induce oxidative stress and cytotoxic effects in fenugreek seedlings with appreciable reduction in mitotic index, induction of chromosomal abnormalities in root meristematic cell, and decreased level of accumulation of some key cell cycle regulators. Therefore, strategic measures should be taken in management of these environmental stresses. For example, conventional breeding or biotechnological approaches should be maintained to put forth new fenugreek species resistant to stress factors and these techniques also help the fenugreek producers to obtain higher yielding species under environmental stress conditions. Also, rhizobia strains should be developed to abiotic and biotic stress tolerance. In this review, the effect of various environmental stress factors on the

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growth and development of fenugreek has been summarized. Also, it is outlined the recent advancements and future perspective about such topic on fenugreek.

Keywords

Abiotic and biotic stress · Fenugreek · Secondary metabolites · Yield

14.1 Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a valuable medicinal plant and an annual crop belonging to the Legume (Fabaceae) family. Its seeds contain various bioactive compounds like flavonoids (quercetin, rutin, vitexin), saponins (graecunins, fenugrin B, fenugreekine), amino acids (isoleucine, 4-hydroxyisoleucine, histidine, leucine, lysine), alkaloides (trimethylamine, neurin, trigonelline, choline, gentianine, carpaine, and betain). These bioactive compounds are used against allergies, appetite, bronchial, cholesterol, diabetic retinopathy, gas, gastric disorders, lung infections, mucus excessive, throat/sore, abscesses, anemia, asthma, boils, body odor, bronchitis, cancer, swollen eyes, fevers, gallbladder problems, heartburn, inflammation, sinus problems, ulcers, uterine problems, etc. (Chatterjee 2015).

Plants are subjected to various environmental stress factors as biotic (pathogens, pesticides, herbicides, insecticides, etc.) and abiotic stress (freezing, radiation, salinity, floods, drought, high temperature, heavy metals). These stress factors affect the morphological properties (plant height, number of branch and pot, etc.), yield values (seed and biological yield), and secondary metabolites (diosgenin, trigonelline) of fenugreek. In addition, these stress factors decrease the crop production and chemical constituents by affecting the plants in the different growth stages and the performance of the photosynthetic mechanism (Elleuch et al. 2013; Karmakar 2014; Hazrati et al. 2016). These stress factors have been employed in plant species in response to changing conditions of in vitro or in vivo growth by many researchers (Elleuch et al. 2013; Karmakar 2014; Kapoor and Pande 2015; Hazrati et al. 2016). They reported that abiotic stress factors influence on growth and secondary metabolite production of the plants (Isah 2019). These influences depend on both genetic characteristics and the changing ecosystem. For example, influence of climate change on bees, butterflies, soil microflora, etc. also affects plant ontogeny, adaptation, and productivities. Such climate change drastically influences water availability, salinity, and several adverse soil conditions which will have direct bearing on original yields. As a result, all stresses lead to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm to vacuoles (Isah 2019).

14.2 Influence of Abiotic Factors on Fenugreek Growth and Secondary Metabolites Production

14.2.1 Drought Stress

Drought is one the most important limiting factors for crop production and it is becoming a severe problem in many regions of the world. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by evapotranspiration (Allen and Ort 2001). Drought impacts include growth, yield, membrane integrity, pigment content, osmotic adjustment, water relations, and photosynthetic activity (Benjamin and Nielsen 2006; Praba et al. 2009; Farooq et al. 2009). The increase of drought stress decreases the primary absorption rate and thus the germination percentage decreases (Iqbal et al. 2015). The findings showed that when exposure of plants to drought stress substantially decreased the leaf water potential, the yield decreases as the physiological activities of the plant are negatively affected. Also, water stress decreases in biological yield as compared to non-stress condition. Biological yield is an important criterion for improvement in yield, which is strongly influenced by the environment conditions. Harvest index, an important criterion for improvement in the yield, is strongly influenced by environment conditions (Siddique et al. 2001; Chauhan et al. 2017).

According to Johnson and Franz (2002) the applied water stress decreased the root biomass, flower development, and height of above ground parts of two fenugreek genotypes. When assessing mature plants at the end of the pot experiment, both cultivars grown under drought stress showed a significantly higher number of nodes (average 25) than those grown under the optimal irrigation regime (average 22).

Unlike results were obtained on different fenugreek genotypes, there is reduction in pod formation as compared to flowering in water stress. However, under drought stress, these genotypes were less affected by water stress at both the stages. Also, when chicken manure was applied, significant increases were obtained in plant height, leaf number, and plant fresh weight under drought stress (El-Missery 2003). Similar results of changing heritabilities of agronomic traits with environment conditions in fenugreek and other crops were reported by other researchers (Ali et al. 2008; Marzougui et al. 2007; Statti et al. 2004). Sadeghzadeh-Ahari et al. (2010) indicated that phenotypic coefficient of variability values is higher than genotypic coefficient of variability values almost for all the traits under both under rainfed (RF) and irrigated (IR) conditions, which reflect the influence of environment on the expression of traits. These results are in agreement with the findings of many researchers in different fenugreek genotypes (Abdel-Rahman 1999; Sultana et al. 2001; Tabatabaei and Fakhrzad 2008; Chaum et al. 2010) who found a wide variation for grain yield potential among the genotypes (Figs. 14.1 and 14.2).

Moreover, drought conditions increased the activity of other antioxidant enzymes along with proline, trigonelline, and melatonin. As a result, concentrations of hydrogen peroxide (H_2O_2) and malondialdehyde also increased under stress

Fig. 14.1 Fenugreek grown in water stress conditions in our experimental area

Fig. 14.2 Fenugreek grown in irrigation conditions in our experimental area

conditions. With the treatment of melatonin (MEL), the reactive oxygen species (ROS) scavenging enzymes increased, but chlorophyll degradation significantly decreased. These results revealed that in fenugreek increased endogenous melatonin and trigonelline by increasing overall physiological responses, thereby tolerating water deficiency. The finding demonstrated that trigonelline increased a fourfold in the plants exposed to a water deficit compared to control treatment (Zamani et al. 2019). Furthermore, trigonelline is considered an active compound that acts as an osmoprotectant and osmoregulator under abiotic stress conditions, inducing leaf movements in plants. In addition, it is assumed that trigonelline acts as a cell cycle regulator and a compatible solute in fenugreek under abiotic conditions (Evans and Tramontano 1984; Cho et al. 1999). On the other hand, researchers indicated that arid and semiarid regions are required immense possibilities of organic production of fenugreek. Although the use of effective rhizobium cultures as inoculums for organic production of fenugreek has not gained much momentum due to lack of scientific information, the use of rhizobium has proved beneficial in improving the soil nitrogen and getting higher yield (Farook et al. 2012).

Zamani et al. (2019) reported that using effective rhizobium strains for adverse environmental conditions prevailing in Rajasthan, it may require a great attention on rhizobia research. According to the results of the data, shoot length significantly decreased, while representing an increase in water deficit. In addition, the effects of drought stress on shoot weight were more drastic than for root weight, thus

performance of root is better than shoots under drought stress in fenugreek. Moreover, it was also found that Exogenous melatonin (MEL) significantly increased in fresh shoot weight in water deficit. It may be attributed to drought stress influenced the changes in nitrogen as well as in other macronutrients uptake, blocked the amino acids biosynthesis, suppressed the vegetative growth and simultaneously, caused a shift in diosgenin metabolism in young fenugreek plants, reflected in lower diosgenin content in mature seeds. Moreover, plants exposed to stress growth conditions accelerate the nitrate accumulation in plant tissue and slow down the protein synthesis (Larcher 1995; Matičič 1997; Saker et al. 1997).

As a result, fenugreek is a drought stress resistant plant, but is slightly affected by vegetative and generative growth. On the other hand, drought stress increased its biochemical content. All these features make fenugreek an attractive option for farmers, seed companies, the dairy and beef industries and pharmaceutical industries in World. That is why, in order to support the farmer, researchers should continue the traits such as growth vigor, flowering time, days to maturity, and might be implemented as selection criteria to improve pod yield in fenugreek breeding programs under dryland condition.

14.2.2 Salinity Stress

Soil salinity is one of the most important global problems affecting different aspects of plant metabolism and structure. Salinity creates a dilemma for plants, disrupting plant growth and development through cytotoxicity and nutritional imbalance due to excessive intake of ions such as sodium (Na^+) and chloride (Cl^-). In addition, salinity is typically accompanied by oxidative stress due to the formation of ROS (Gao et al. 2007; Negrão et al. 2011; Maathuis et al. 2014). ROS causes lipid peroxidation, protein breakdown, and enzyme inactivation. Salinity stress also causes ion deficiency or imbalance due to the competition of nutrients such as K^+, Ca^{+2}, and Mg^{+2} with toxic ions Na^+ and Cl^-. Under saline conditions, K^+, Ca^{+2}, Mg^{+2} concentrations decreased in the air parts and roots, while Na^{+2}, Cl^-, and SO_4^{-2} concentrations increased (Yaldiz et al. 2018). Salinity is a major restriction for crop production worldwide (Sadeghzade-Ahari et al. 2010). Several studies have justified that salinity stress was found to cause a significant decrease in protein content and membrane stability index compared to control in fenugreek. Membrane lipid peroxidation, catalase activity, malondialdehyde, and other aldehyde content are stated to increase in salinity stress. It has also been noted that increased catalase in the plant shows the tolerance capacity to protect the plant from oxidative damage caused by NaCl (Rahnama Ebrahimzadeh 2005; Koca et al. 2007).

In another similar study, the effect of salt stress on membrane lipid peroxidation, catalase activity, and protein content was investigated in five different salinity levels (0, 50, 100, 150, and 200 mM) in fenugreek. It has been stated that lipid peroxidation content has increased due to malondialdehyde (MDA) and other aldehydes production. Although MDA value increased with all NaCl concentrations, it was found to be higher in high salt concentrations. In particular, it was stated that the peroxidation

rates have increased compared to control plants at 100 (113%) and 200 mM (373%) NaCl, respectively (Pasandipour et al. 2014).

In line with above researchers (Lima et al. 2002; Lee et al. 2001; Sudhakar et al. 2001; Sairam and Srivastava 2002; Bandeoglu et al. 2004), they reported that an increase in H_2O_2 and MDA concentration upon salt stress has been reported in different plant species and it is shown that this is related to stress levels and well correlated with lipid membrane damage.

In earlier studies Madhloom (2018) reported that five concentration of sodium chloride salts (0, 2, 4, 6, and 8 g/l) and salicylic acid (0, 10, 20, 30, and 40 mg/L) were added to the culture medium, and found that salicylic acid levels and salinity level of (2 g/l of NaCl and 30 mg/L of SA) increased fenugreek callus induction, fresh weight, dry weight, and potassium ions of callus.

The effects of 100 ppm of salicylic acid, citric acid, and proline on some of morphological traits, chlorophyll content, antioxidant enzymes activities and isozyme pattern were investigated in fenugreek seedling under salinity stress using different concentrations of NaCl (0, 1000, 2000, and 3000 ppm) during germination stage by Behairy et al. (2017). They showed a significant decreasing of germination percentage, shoot length, seedling dry weight, seedling vigor, and chlorophyll content with the increasing NaCl concentration.

Ghorbanpour et al. (2011) also observed that there was a decrease of germination velocity, and percentage was greater compared to other characteristics, and they also said fenugreek seed has higher tolerance to drought stress compared to salinity stress. Similarly, increasing salt concentration reduced the fenugreek growth, result of osmotic potential in root medium, ion specific toxicity, and nutrient deficient (Ashraf and Orooj 2006; Munns et al. 2006).

In another study, Mickky et al. (2019) reported that fenugreek seeds were germinated in presence of 0, 50, 100, 150 and 200 mM NaCl for 2 and 5 days; and the seedlings were evaluated for their morpho-physiological features. They found that fenugreek seedling dry mass and seedling bulk vigor were only affected a small ratio by salinity at both ages, seedling fresh mass was adversely affected only at the first age, while germination percent, germination index, seedling length, and seedling length vigor index were all restrained by salinity at both ages. And they also found that up to 200 mM NaCl, early germination phase may have slight impact on its morpho-physiological performance. Likewise, effects of salinity on fenugreek were recorded by Soughir et al. (2013) as well as Gupta (2016) that salinity induced activity of some antioxidant enzymes like catalase, peroxidase, ascorbic peroxidase, and polyphenol oxidase. Similar results were also reported by Nair et al. (2017) that fenugreek seedlings accumulated total soluble sugars and proline in response to salinity. In addition, Dadresan et al. (2015) concluded that salt-stressed fenugreek plants accumulated sodium as compared to control (non-salt) condition.

These results are in line with those obtained by Khodary (2004) and Pasandipour et al. (2012) and salinity stress induced the reduction in germination and seedling growth of fenugreek. Seeds primed with different concentrations of salicylic acid proved to be effective in salinity tolerance at the germination stage of fenugreek. As a result, although fenugreek has tolerance to some degree of abiotic stress factors

such as salinity, drought, temperature and heavy metals, strategic measures should be taken in the management of these environmental stresses. In addition to conventional breeding, biotechnological methods should be studied to develop new fenugreek species resistant to salt stress. At the same time, more productive fenugreek species will help fenugreek producers under salt stress conditions developed by these methods. In addition, rhizobium strains should be developed for salt stress tolerance.

14.2.3 Heavy Metal Stress

Heavy metals can bind to functionally important areas of biomolecules and thus inactivate them. They may be inhibition of the enzymatic reaction and disruption of metabolism (Van Assche and Clijsters 1990). However, it has been noted that heavy metals induce the formation of free radicals (FR) and ROS either by direct electron transfer containing metal cations or as a result of metal-mediated inhibition of metabolic reactions (Halliwell and Gutteridge 2004). So, the rate of FR and ROS formation, and the cellular yield and detoxification and repair mechanism depend on the degree of cell damage under heavy metal stress (Halliwell and Gutteridge 2004; Sinha and Saxena 2006; Sinha et al. 1997).

According to Oncel et al. (2000) and Sinha and Saxena (2006), the accumulation of proline is considered as an indicator of environmental stress, including exposure to heavy metals, and is also noted to have significant protective roles. These researchers also indicated that the physico-chemical properties of the soil were negatively affected, and also contaminated with high levels of metals, especially toxic metal Cr. Several other studies showed that heavy metals as a toxic compounds significantly increased the isoflavonoid production (Mithofer et al. 2004; Skorzynska-Polit et al. 2004; Michalak 2006).

In previous studies, the presence of metal accumulation in many plants was emphasized that growth was inhibited exposed to high concentrations of heavy metals. After prolonged metal exposures, sensitive plants have been found to develop visible toxicity symptoms such as chlorosis and necrotic lesions (Singh et al. 2004; Sinha et al. 2005).

There is not much information about the tolerance of the plant grown in soil with heavy metal pollution (Onder et al. 2007). Fenugreek plants were grown with tannery mud (TS) in different proportions to evaluate the participation of carotenoids and the content of ascorbic acid, cysteine, thiol, free proline. They found that these bioactive substances induced the cope with stress after accumulation of metals in the plant (Singh et al. 2004).

Metalloid arsenic (As) is a common environmental toxic substance. As is known to inhibit plant growth through the induction of oxidative stress (Khalid et al. 2017; Shahid et al. 2015; Rosas-Castor et al. 2014; Neidhardt et al. 2015). It poses great health risks in edible parts of crops like cereals, fenugreek, and legumes grown in fields contaminated with As (Mombo et al. 2016). In previous studies, the plant of exposure to As has been reported to lead to ROS formation by converting arsenate to highly toxic arsenite (Shahid et al. 2015).

Although As-contaminated areas are used for fenugreek cultivation, the toxic effect of the plant has not been evaluated until now. There are also records of great reduction in the growth, nutrients, and yield of different legumes due to exposure to As (Imran et al. 2013; Onishi 1969). In line with these findings, in the pot study using different doses of As (10, 20, and 30 mg As/kg soil) in fenugreek, the development and antioxidant metabolism of plants were investigated by Talukdar (2013). Photosynthetic properties and yield components were found to be significantly increased in As 20 compared to the control (0 mg As/kg), but significantly decreased in As30. Three important antioxidant enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) were analyzed to understand the connect between plant growth and As doses. The enhanced expression and formation of new isoforms in the three enzymes under As treatment strongly indicated a key role of H_2O_2 metabolism in the tolerance of fenugreek plants to As stress. So, different morphological, physiological, and biochemical parameters may pave the way for the improvement of fenugreek genotypes under metal (loid) stress.

In recent years, the heavy metal nickel (Ni) in sustainable agroecosystem is a major problem in plants. The toxicity effect on the photosynthetic properties of plants depends on their species, developmental stages, environmental conditions, Ni level, and exposure time in soil (Krupa et al. 1993; Marschner 1995; Kabata-Pendias and Pendias 2001; Assuncao et al. 2003; Yusuf et al. 2011; Chen et al. 2009). Similarly, Gajewska et al. (2006) indicated that Ni affects the uptake of other necessary metal ions, necessary to sustain the plant's vital activities and causes oxidative stress. This negativity in metabolic and physiological activities causes a decrease in yield and quality.

Recently, Gupta et al. (2013) observed that metals such as Hg, Pb, and As negatively affect vegetative growth of plants. High heavy metal levels in the environment such as Ni, Cd, Co, Cu, and Pb can pose a potential hazard due to their toxic effects and accumulation along the food chain (Kovacik et al. 2009; Lou et al. 2013; Abd-Alla et al. 2014; Cai et al. 2014). Ahmad et al. (1997) stated that rhizobium was very effective in decontamination and recycling of organic compounds, therefore it could be used in environmental cleaning, and also an incentive effect was observed on the production of flavonoids compared with a non-inoculated control sample of Ni-treated.

In addition, researchers have determined that as the Ni concentration increases, it has a detrimental effect on node gene expression. In studies with different plant types, phenylalanine ammonia lyase (PAL) activity was found to increase with excessive heavy metal stress (Kovacik and Backor 2007; Kovacik et al. 2008; Yan et al. 2008; Wang et al. 2010). As a result of these findings, the researchers suggested that increased PAL activity may be associated with vaccination of fenugreek plants with *R. tibeticum* together with flavonoids, such as hesperetin and apigenin under excessive Ni stress.

In a similar study, the application of Ni at different concentrations (0, 50, 100, 150, and 200 mg/kg) has been reported to have a significant negative effect on the dry mass accumulation of the root and shoots of the plant length, number of nodules, leaf area (Vijayarengan 2004). These studies are supported by many studies

with different legume plants (Gyorgypal et al. 1988; Schlaman et al. 1998; Suominen et al. 2003; Begum et al. 2001).

Yusuf et al. (2012) indicated that Ni can increase the nitrogen fixation, nodulation, and fenugreek growth in contaminated soils. It has been strongly emphasized that an agriculturally acceptable inoculant carrier and flavonoid components can be combined throughout the vaccination formulations. It is indicated that it is necessary to supply the physiological necessities of rhizobial inoculants in adapting the formulation technologies to increase the growing of legumes in sewage sludge and heavy metals.

It is emphasized that the harmful effects of Ni present in the soil may be reduced by activation of *R. tibeticum* with hesperetin and apigenin. Therefore, it can be recommended to legume crops cultivation in soils contaminated with highly toxic heavy metals and sewage sludge. Increasing sludge rate induced in metal accumulation (Fe, Cr, Zn, Cu, Pb), except Mn, in all plant parts. While the roots had higher Fe (3682.93 mg/g dw) and Cr (105.95 mg/g dw) than the shoots, high Mn, Zn, Cu, and Pb accumulation was determined in the shoots (Sinha et al. 2007).

Furthermore, many factors affect the metal accumulation, such as physiological-chemical characteristics of the metal, amount of humic matter and other metals, soil mineralogy, pH, and amorphous Fe and Al content. Also, different metal accumulation in different plant parts may result from different cellular mechanisms of plants and translocation of plants (Shenker et al. 2004). Recently, Abd-Alla et al. (2014) also strongly indicated that the supplement with flavonoids to the growth medium of *Rhizobium* increases the growth and nodulation of plants grown in cobalt-contaminated soils. The effects of copper on germination and growth of fenugreek were investigated under different concentrations of $CuSO_4$. While the germination percentage was largely unaffected by concentrations of copper below 10 mM, the root growth increased with increasing copper concentration up to 1 mM Cu^{2+}. Fenugreek roots and shoots showed a gradual decrease in growth, which was more obvious at the roots. Excess copper caused an increase in H_2O_2 production and lipid peroxidation in all plant parts and showed oxidative stress (Elleuch et al. 2013). Similarly, Cook (1978) concluded that copper accumulated firstly in roots of fenugreek when subjected to copper stress.

As seen from previous studies, there are few studies on secondary metabolites of fenugreek grown in heavy metal soils. Fenugreek seeds also contained large amounts of the toxic metals Cd and Pb, and therefore studies should be focused on this subject (Fig. 14.3).

Fig. 14.3 Powdery mildew disease in our experimental area

14.3 Influence of Biotic Factors on Fenugreek Growth and Secondary Metabolite Production

14.3.1 Cercospora Leaf Spot

Cercospora leaf spot (CLS) is an important constraint to use of fenugreek as a crop (Fig. 14.4). *C. traversiana* is the only species of the genus Cercospora infecting fenugreek (Cook 1978; Ryley 1989; Voros and Nagy 1972), which falls under the division Ascomycota (Agrios 1997). Cercospora leaf spot is a destructive and widespread disease of fenugreek (Acharya et al. 2010; Malhotra 2010). Prasad et al. (2014) reported that the fenugreek genotypes tested varied greatly in their reaction to *C. traversiana* for the agronomic traits tested, and also suggest that further work (including field work) was required to confirm the resistance response of fenugreek. Besides reducing yield, CLS has also adverse effect on seed quality (Ryley 1989). Cercospora has 17.6–28.8μm long and 1.78–3.01μm wide, unbranched, slightly separate, uniform, slightly bent conidiophores (Prasad et al. 2014; Ryley 1989). The spread of these conidia, which are found in different living plant parts and rotting debris, can occur by splashing rain and wind (Acharya et al. 2010).

There are several studies to determine appropriate design for plant analysis of crops towards varied pathogens. Felsenstein et al. (1998) have determined the resistance of fenugreek leaves, stems, roots, and pods to the disease in a controlled environment. Notably, they noted that a detailed leaf test (DLA) was quickly screened without the use of a large area and a large amount of vaccines. Alike, DLA has been used to discriminate the resistance level in various plants, from herbaceous plants to large tree species with high success rates (Browne and Cooke 2004; Hansen et al. 2005; Huang et al. 2016; Jackson et al. 2008).

Fig. 14.4 Cercospora leaf spot in our experimental area

Temperature is very effective in survival, spread, disease appearance, and severity of pathogens (Agrios 2005; Jacobsen et al. 2004; Terefe et al., 2015). The effect of temperature on this pathogen has been studied by many researchers in many plants (Alderman and Beute 1987; Carisse et al. 2000; Cooperman and Jenkins, 1986; Khan et al. 2009; Paul and Munkvold 2005). It was determined that the optimum temperature for maximum disease severity of CLS was around 25 °C, conidia germination and micelle growth were very well and maximum symptoms were seen. In addition, when there is physical damage or injury to plants by human or natural factors, this disease can develop and lead to direct economic damage (Cooperman and Jenkins 1986; Paul and Munkvold 2005).

Savatin et al. (2014) concluded that physical damage easies the entry of pathogen into plants and eventually increases the severe disease. Fenugreek, which grown in field conditions, could be harmed by natural reasons like hail stones, wind, insects, frost and snowfall, and together with mechanical damage during planting, and also unsuitable temperature might be lead to maximum possible damage. It is reported that mature plants are more resistant to disease than young plants of fenugreek (Canova 1959; Rossi et al. 1990; Schneider et al. 1976; Vloutoglou and Kalogerakis 2000; Weiland and Koch 2004). The sensitivity of mature plants/leaves to the fungal pathogen has not been identified until now, but passive mechanisms such as a decrease in the thickness of the epicuticular wax layer in mature leaves (Tewari and Skoropad 1976) or an increase in stomatal density in mature leaves (Gwinn et al.

1987; Paul and Munkvold 2005) easies the entry of pathogens into plants. Udaya (2018) stated that more severe infection was determined in the vaccine applied to sensitive genotype regardless of seed yield loss. However, the resistant genotype showed a lower correlation between capsule infection and loss of seed yield. It was predicted that high leaf drop leads to loss of high yield, but it was noted that difference between the leaf growth and the development stage of the plant has been found insignificant. This may be due to the small size and lightness of fenugreek leaves that have no major impact on loss of yield. Other researchers reported that (Hasan et al. 2016; Khan et al. 2009; Pundhir and Mukhopadhyay 1987; Tedford et al. 2018) the vaccine concentration also affected disease severity along with other parameters.

In a separate study on several fenugreek varieties tested under *C. traversiana* disease, Mishra et al. (2011) therapeutic agents (Bavistin, Vitavax, Thiram, Griseofulvin, Dithane m-45, and Blitox) were used at different concentrations (0, 100, 250, 500, and 1000 ppm). Maximum inhibition of *C. traversiana*'s radial growth (90.5%) was exhibited by Bavistin at 50 ppm concentration, followed by Vitavax (78.2%), Thiram (72.2%), Griseofulvin (58.5%), Dithane m-45 (41.5%), Blitox (41.3%), Sulfamethoxazole (35.8%), and Streptocycline (24.4%). It was also observed that the leaf spot in the crop was reduced when the seeds were treated with these therapeutics before planting. It has been reported that leaf spot diseases of some other plants are controlled by seed treatment (Khunti et al. 2002; Saxena and Tripathi 2006).

14.3.2 Powdery Mildew

Fenugreek powdery mildew is caused by especially *Leveillula taurica* and *Erysiphe polygoni* (Fig. 14.5). The most prominent feature of dust mold disease comes out as white flour spots on both sides of the leaves and on the pods. The fungal progress is

Fig. 14.5 *Erysiphe polygoni* in our experimental area

also seen on stems, flowers, and pods. Since this disease causes severe infection, it causes serious losses in yield (Wiese 1987). So far, there has been no report on the inheritance of powdery mildew resistance in fenugreek. However, there are few studies on the resistance of powdery germplasm lines to powdery mildew. In a hereditary study against this powdery mildew in fenugreek, the experimental material consisted of the two parents (viz., UM·305 and RMt-143), F1 and F2 of the cross viz., UM-305 x RMt-143 and the check varities (RMt 1 and local check). As a result of the research, the parent UM-305 is resistant to powdery mildew, whereas the other parent, viz., RMt-143 and the check varieties (RMt-1 and local check) are susceptible to powdery mildew (Raje et al. 2002). Thus, reaction of parents and F1's indicated that the susceptibility to powdery mildew was a dominant character, and resistance was the recessive character. Similar findings for powdery mildew resistance have been recorded in pea (Singh et al. 1983; Janila et al. 2001).

Carbendazim fungicide is known to affect mitosis and cell division in powdery mildew. Previous studies have demonstrated that application of carbendazim fungicide suppresses spindle microtubules aggregation, disrupts chromosomal alignment in the metaphase plate and microtubule–kinetochore interaction (Yang et al. 2011). The efficacy of propiconazole against *E. polygoni* has been previously reported by Biju (2000). Triazole fungicides, which are propiconazole, hexaconazole, and diphenoconazole, have been reported to prevent germination of spores of *L. taurica*. Efficacy of trisol group fungicides against green gram powdery mildew (*E. polygoni*) (Venkatrao 1997; Khunti et al. 2002), powdery mildew (*Sphaerotheca pannosa*), powdery mildew (*E. cichoracearum*) in fenugreek (Chovatiya 2010) was reported by various researchers (Nane and Thapliyal 1993).

The potentiality of carbendazim has been reported to inhibit spore germination of Oidium erysiphoides of carbendazim potential, *E. Polygoni* (Nawaz and Narayanasamy 1983; Kunkalikar 1989), f. sp. ziziph (Sataraddi 1994), and *E. cichoracearum* (Hiremath 1996). Thiophanate methyl 70 WP and tebuconazole 25 EC were found to have moderate (57.13 and 50.98%) spore germination inhibition, respectively. In addition, powdery mildew was observed as a second disease in fenugreek leaves, but it was stated to be at a low level. Alike, Sharma and Gupta (1994) the germination of *Podosphaera leucotricha*, which causes dust mold in apples, inhibited using thiophanate methyl 70 WP. Also, picoxystrobin 25 EC fungicide was determined as the least effective fungicide (32.12%).

Dhruj et al. (1996) indicated that propiconazole, penconazole, hexaconazole, tridemorph, triadimefon, dinocap, and sulfur used avert the powdery mildew (*Leveillula taurica* and *Erysiphe polygoni*) disease in fenugreek. All fungicides have been found to remarkably decrease the disease compared with control. It has been noted that the best powerful fungicide is penconazole, followed by hexaconazole and propiconazole, respectively. Rekha et al. (2016) conducted a study in Rajasthan, studied that Wettable sulfur, Hexaconazole, Dinocap, Propiconazole, Tridemorph, Difenoconazole, Azoxystrobin, Mancozeb, and Carbendazim were used against powdery mildew in field conditions. It was determined that all fungicides significantly reduced the powdery mildew density

compared with the control. Among these fungicides, hexaconazole increased 63.08% seed yield and increased maximum (84.68%) disease control.

Nine fungicides (systemic fungicides, difenoconazole, hexaconazole, penconazole, propiconazole, non-systemic fungicides, wettable sulphur, mancozeb and dinocap) have been studied against powdery mildew disease in 20 fenugreek genotypes. Among these genotypes, it was stated that there were differences in disease resistance and the resistance of 4 genotypes was quite good. In addition, the most effective fungicide was found as diphenoconazole, followed by penconazole fungicide (Chovatiya 2010). As a result, many studies should be conducted about fenugreek powdery mildew, because of causing serious yield losses (27–33%) particularly throughout the flowering and pod formation stage of the crop. Therefore, disease resistant genotypes should be developed, and studies with organic products should be supported that do not harm nature.

14.3.3 Downy Mildew

During its cultivation, fenugreek is assaulted by fungal and bacterial diseases, out of which downy mildew causes maximum crop loss. Uppal et al. (1935) reported the occurrence of downy mildew from Bombay for the first time and Sharma and Munjal (1977) recorded this disease from Himachal Pradesh. However, it was accepted as a minor disease, and since then there have been no enough studies about this disease despite the serious damage.

Downy mildew symptoms are in the form of white to dark gray mycelium, and it appears on the upper leaf surface. The disease occurs the presence of conidia and conidiophores of the pathogen and causes stunted growth of the plant. Conidiophores were hyaline, dichotomously branched 6–10 times, and 270–510 (average: 432) μm long. The adaxial surfaces of the leaves showed minör chlorotic spots frequently at the leaf margins, while the abaxial surfaces showed a grayish violet (Mirzaee and Doostali 2015).

According to the formula described by Rooney-Latham et al. (2009), a suspension of sporangia was sprayed on the leaves of healthy fenugreek plants and the inoculated plants were incubated for 2 days in a humid room at 18–20 °C and 80–90% relative humidity, then moved to a greenhouse. 12 days after vaccination, typical mold symptoms developed and not performed in vaccinated plants but in mold controls and performed Koch's postulates.

Shekhawat et al. (2016) reported that organic adjustment of soil by adding neem cake was seen as effective control of the powdery and downy mildew in fenugreek. Also, Chhata and Verma (2010) indicated that the use of *Trichoderma viride*, *Pseudomonas fluorescens, a*nd neem cake inhibited the powdery and downy mildew diseases and aphid. In addition, they indicated that the application of neem cake increased the multiplication of aerobic microorganisms in field condition. Thereby, these microorganisms might have fixed the available nitrogen and utilized it for decomposition of organic matter. Similarly, Azcon (1989) and Muthulaxmi et al. (2010) noted that the use of Trichoderma can induce growth of plants but alleviate

the pathogenic symptom in leguminous crop. When carbon monoxide concentration increased in the soil, aerobic activity of microorganism also increases, and inhibits the growth of pathogen, helps to build up the crop health. These microorganisms also release some enzymes, which help to improve the crop health and check the growth of pathogenic fungi (Anonymous 2002).

Although it has been noted that fungicides such as chlorothalonil, mancozeb are effective for downy mildew, their use should be approved in countries such as the USA, Canada (Latin and Rane 1999). Against downy mildew, the best powerful products are fluopicolide, famoxadone+cymoxanil, cyazofamid, and zoxamide. To prevent or delay the development of new resistant strains, it is recommended that eradicant fungicides mixed with a protectant fungicide in tank (Colucci and Holmes 2010; Keinath 2015).

As a result, an effective way to prevent disease is to remove moisture from the cultivated areas and surrounding areas. In the cultivated areas, air circulation can be improved by applying a ground irrigation, such as drip irrigation system. Reducing moisture in indoor environments such as in the home or greenhouse will also help.

14.3.4 Damping Off

Damping off is a serious disease that affects germinal seeds and young seedlings, and is caused by several soil-borne fungi, especially *Fusarium solani* (Yangui et al. 2008). Although fungicides are used to increase efficiency, it has been stated that their long-term use is negative on the environment. Many factors, especially environmental factors, are effective in the occurrence of the disease. Among these factors, it can be listed interaction between host and pathogenic fungi, soil types, soil moisture content, planting distance, differences in planting dates, used agricultural practices, and varieties (Roy 1997).

When compost and compost extracts are applied to the soil, the organic matter content of the soil increases and also increases the water holding capacity and provides macro- and micronutrients to plant growth and soil quality (Scheuerell and Mahaffee 2004 and Heather et al. 2006; Craft and Nelson 1996). Many researchers have stated that compost and compost extracts are one of the richest sources to suppress microorganisms that cause disease in field. The use of compost and compost extracts has greatly reduced the incidence of initial and advanced damping off, and has improved on fenugreek plants grown in the presence or absence of *F. solani*. The use of compost and compost extract is very useful as an environmentally friendly application, and can be used as an alternative for inorganic fertilizers/fungicides to increase plant growth and reduce the incidence of the disease and therefore provides higher yields (Shrestha et al. 2011; Scheuerell and Mahaffee 2004; Litterick et al. 2004; Carpenter-Boggs 2005). Fenugreek seedlings have low resistance to damping off, even most seedlings may be die. However, when plants have mature leaves and a well-developed root system, they can naturally better resist fungal or mold causing damping off (Yangui et al. 2008). To prevent damping off in seedlings, the containers to be planted should be disinfected by soaking in 10%

household bleach solution for 30 minutes. Also, before planting in the open field, the soil should be expected to reach the optimum temperature for germination. Likewise, seeds should be sown in a sunny location, well-draining soil, and fertilizer should be applied after the true leaves emerge (Svendsen 2016).

14.3.5 Fusarium Wilt

Fusarium wilt of fenugreek is one of the major diseases that cause moderate to large damage to the product. The pathogen remains in both soil and fenugreek seeds (Bansal and Gupta, 2000; Hashmi and Thrane 1990; Komaraiah and Reddy 1986; Pierre and Francis 2000). The pathogen causing wilt disease is reported to be *Fusarium oxysporum* Schltdl, which belongs to Ascomycetes member (Borg, 1936; El-Bazza et al. 1990; Hashmi 1988). The micelles of *F. oxysporum* are pale white to pink in color, often characterized by a purplish hue and are rarely abundant. It is stated that this fungus produces three spores called micro- and macroconidia and chlamydospore (Agrios 1997; Smith et al. 1988). The spores of the pathogen spread with the seeds, soil, and plant parts that they invade (Pierre and Francis 2000).

Wilt symptoms firstly appear as small openings in the veins on the outside of the young leaflets, eventually accompanied by the drooping of the mature leaves. When the wilting occurs at the seedling stage, the plant can immediately become pale and die. Wilting causes to sag down the leaf in mature plants, stunting continues with yellowing of the lower leaves and then fading of the leaves and young stems. This is followed by marginal necrosis of infected leaves, rapid leaf fall, and death of the plant (Agrios 1997). These symptoms become more pronounced in mature plants in the period between flowering and fruit ripening (Smith et al. 1988).

Trichoderma harzianum is used against plant fungal diseases as biological control agents. However, there are not enough studies on the mechanisms by which fungi protect. In a study conducted by Abdel-Monaim (2013), trichoderma species which were isolated from their natural environments and their mechanisms in plant protection were investigated. Overall, it has been stated that natural isolates inhibited *Fusarium wilt* disease in plant growth and yield parameters under in vitro and in vivo conditions. Therefore, *T. harzianum* may be chosen to be the most promising biocontrol agent for *F. oxysporum* f. sp. lycopersici. So, the biocontrol agents of plant diseases might be exploited for sustainable disease management programs to save environmental risk (Hwang et al., 1994; Mazzolla et al. 1992; Shanahan et al. 1992).

14.4 Conclusion and Prospects

In conclusion, there is an urgent need to apply for economic and eco-friendly alternative strategies for the management of fungal diseases and abiotic stress. Fenugreek germplasms have a wide range of genetic variability and diversity. Hence, researchers who used resistant varieties could be used for the management

diseases of fenugreek are trying to come up with new resistant varieties and cultivars using the conventional breeding method and by advanced plant biotechnological methods. On the other hand, biocontrol agents such as organic and eco-friendly products should be developed to prevent fungal diseases in fenugreek. Furthermore, fenugreek breeding programs should be developed in order to develop resistant fenugreek genotypes to abiotic stress (drought, salt stress, heavy metal) conditions. Therefore, the method of characterization of genetic resources should be the researchers' favorite topics to develop higher yielding plants to biotic and abiotic stress conditions.

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Heavy Metals Induced Stress and Metabolic Responses in Fenugreek (*Trigonella foenum-graecum* L.) Plants

15

Mohd. Kashif Kidwai and Sanju Bala Dhull

Abstract

Human activities such as unsustainable chemical based agriculture practices, industrialization, urbanization, improper waste disposal, etc. have resulted in the release of enormous quantities of toxicants, i.e. xenobiotic compounds such as agrochemicals which includes fertilizers, pesticides, industrial waste, agricultural waste, and domestic waste having metals, metalloids, etc. in different ecosystems causing various types of pollution in all the spheres of this planet. Both anthropogenic and natural pollutants affect diverse life forms including humans thereby causing various types of stress in them. Abiotic components of an ecosystem such as release of Heavy metals due to anthropogenic processes induce oxidative stress in different agricultural crops all over the globe. Fenugreek (*Trigonella foenum-graecum* L.) is a significant annual leguminous crop having both food as well as medicinal importance. Heavy metal such as Copper (Cu), Mercury (Hg), Arsenic (As), etc. induce oxidative stress in fenugreek adversely affecting growth parameters, i.e. reduction in seed germination, reduction in vegetative growth, adversely affecting photosynthetic machinery causing inhibition in induction of pigments such as chlorophyll and carotenoid. Cytotoxic affects such as reduction in cell division, mitosis, chromatin structure are also associated with heavy metal induced oxidative stress along with generation of reactive oxygen species (ROS) and induction of antioxidant enzymes, super oxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), etc. in response of heavy metal induced oxidative stress.

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Keywords

Fenugreek · Heavy metals · Reactive oxygen species · SOD · CAT · APX

15.1 Heavy Metals

Heavy metals are one of the important natural resources mainly present in the outer earth crust and required by many organisms for their various metabolic and physiological purposes; however, heavy metal induced pollution is one of the current issues pertaining to the environment all over the planet.

The characteristic features of heavy metals are the atomic mass of more than 20 along with the density higher than 5 $g \cdot cm^{-3}$ (Li et al. 2019). Heavy metals are crucial environmental pollutants widely studied for their toxicity related issues in a variety of ecosystems all over the planet. The origin of heavy metals in the environment are related to industrial operations such as mining smelting etc., indiscriminate use of chemicals in agricultural practices in post-green revolution era, improper waste disposal, domestic waste discharge of metals in different spheres of the environment, etc. the natural sources of heavy metals which are originated in earth's crust by undergoing the process of weathering on a time scale and the concentration of heavy metal depends on the type of rock, whereas anthropogenic sources includes modern agricultural processes involving agrochemicals, industrial activities, i.e. mining, anneries, smelting, metal plating, automotive textiles, petroleum combustion plants, etc., improper discharge of untreated domestic and industrial effluents, faulty sewage disposal methods, release in atmosphere due to smoke, gases, automobile exhaust, etc. gets accumulated in soil for very long period (Gupta et al. 2009; Pattnaik and Reddy 2011; Lorestani et al. 2012; Srinivas et al. 2013; Pinto et al. 2015; Arif et al. 2016; Kumar et al. 2016; Ghori et al. 2019; Li et al. 2019; Mahapatra et al. 2019; Fatima et al. 2020). On the basis of requirement, heavy metals are characterized in two broad categories, i.e. (I) essential heavy metals such as Zinc (Zn), Copper (Cu), Nickel (Ni), Iron (Fe), etc. (II) and nonessential heavy metals such as Lead (Pb), Cadmium (Cd), Mercury (Hg), etc. (Pattnaik and Reddy 2011; Emamverdian et al. 2015; Pinto et al. 2015; Arif et al. 2016; Rai et al. 2016; Kumar et al. 2016; Ghori et al. 2019).

Heavy metals include Lead (Pb), Cadmium (Cd), Nickel (Ni), Cobalt (Co), Iron (Fe), Zinc (Zn), Chromium (Cr), Mercury (Hg), Arsenic (As), Silver (Ag), etc. (Pinto et al. 2015; Arif et al. 2016; Rai et al. 2016; Kumar et al. 2016; Li et al. 2019). Each of the heavy metal is reported to pose variety of risks affecting the overall biotic community in different ecosystem. However, heavy metals such as Cadmium (Cd), Mercury (Hg), Arsenic (As), Lead (Pb), etc. are not essential and cause toxicity symptoms in diverse living organism including humans even when available in very low concentrations. Cadmium (Cd) is one of the most mobile heavy metals, whereas Lead (Pb) is reported to have very poor mobility therefore it retain in soil for very longer period of time (Houri et al. 2020). Heavy metals are transferred through food chains which include organisms representing successive trophic level such as plants

as producers, animals including humans as consumer and microorganisms as decomposers. The heavy metals are reported to get accumulated in the tissues of different organism through the process of bioaccumulation (Srinivas et al. 2013; Pinto et al. 2015; Pattnaik and Reddy 2011; Neilson and Rajakaruna 2015; Houhou et al. 2018). Heavy metals are considered a toxicant if it occurs where it is not required or in a form or concentration and pose detrimental effects to different life forms of the environment (Pinto et al. 2015). Metals are easily absorbed by plants and gets bioaccumulated in their tissues. Plants inherit the property to accumulate heavy metals from heavy metal contaminated soil and water used for irrigation (Dang et al. 1990).

Heavy metals are non-biodegradable in nature and persist in soil for a long time, they bioaccumulate in the food chains of various ecosystems through the uptake at initial tropic level and subsequently via consumption at secondary and tertiary levels involving the diverse life forms of different ecosystems (Pattnaik and Reddy 2011; Pinto et al. 2015; Kumar et al. 2016; Li et al. 2019; Fatima et al. 2020).

Metals are required by plants for their various processes needed for growth and productivity, some of the metals are required in lesser quantity, whereas some in higher quantity. The issue of plant toxicity is surfaced when the undesired or nonessential metals for e.g. Cadmium (Cd) available in soil is absorbed by plants in high concentration through roots as well as leaves resulting to cause various types of toxicities (Houri et al. 2020). However excess amount of metals available in soil bioaccumulate in various parts of the plants which potentially induces variety of adverse effects at multiple level due to the oxidative action of metals.

Soil encountering heavy metal pollution is a global environmental issue that tempted the global scientific community focusing on various risks associated with cultivation of agricultural commodities including different biotic components of agro-ecosystem. Approximately five million sites all over the globe have been identified for soil pollution from different sources which amount to approximately five hundred million ha of agricultural land. From economic point of view heavy metal induced pollution in soil pose adverse impact amounting to be more than US $10 billion annually (Li et al. 2019). Heavy metals are reported to adversely affect about 12% of the global yield of agriculturally cultivated crops (Arif et al. 2016). Heavy metals are widely reported to initiate different responses in plants which ranged from anatomical, physiological, biochemical including photosynthesis and respiration, molecular, crop yield, etc. Heavy metals are toxicants which can impair cellular metabolism when available in high concentration causing mitodepressive effect. Some of the physiological processes which get affected are intracellular and extracellular enzymatic processes, protein structure, water balance, cell division, morphogenesis, etc. (Sinha et al. 2007; Dubey 2011; Elleuch et al. 2013; Srinivas et al. 2013; Neilson and Rajakaruna 2015; Dziubanek et al. 2015; Pinto et al. 2015; Kumar et al. 2016; Sidhu et al. 2016; Arif et al. 2016; Rai et al. 2016; Ghori et al. 2019; Fatima et al. 2020). Heavy metals induced pollution is one of the prominent cause of leaf senescence leading to the death of cell (Houri et al. 2020) ultimately causing retardation of plant growth. Heavy metals are reported to adversely affect the

soil microbiological populations thereby affecting soil parameters (Neilson and Rajakaruna 2015).

Water and food are the unavoidable pathways through which humans get exposed to toxic substances. i.e. xenobiotics including heavy metals. Heavy metals are reported to be transferred in the food chain depending on the mobility of the heavy metals and their bioavailability in soil. Heavy metals which are non-essential for plants enter into plant system through the entry route of essential metals. The processes of uptake and accumulation of heavy metals by plants are influenced by several soil properties such as pH, clay content, soil organic matter (SOM) content, redox potential, cation exchange capacity (CEC), temperature, soil moisture aeration, etc. (Neilson and Rajakaruna 2015; Kumar and Aery 2016; Kumar et al. 2020).

On the basis of the potential for accumulation of heavy metals plants are categorized into three broad categories (I) Excluders, (II) Accumulator, and (III) Indicator plant species (Kumar and Aery 2016). Among edible plants vegetables consumed as leafy vegetables such as spinach (*Spinacia oleracea* L. spinach), lettuce (*Lactuca sativa*), coriander (*Coriandrum sativum)*, fenugreek (*Trigonella foenum-graecum*), etc. are reported to accumulate high amount of heavy metals in leaves, fruits, roots, etc. due to the process of translocation and in some cases plants get aerial deposition which enhances their capacity to uptake heavy metals (Neilson and Rajakaruna 2015) (Table 15.1).

Among different heavy metals such as Lead (Pb), Nickel (Ni), Zinc (Zn), and Cadmium (Cd), Cadmium is the most toxic element for fenugreek and onion followed by Ni, Pb, and Zn recorded by (Dang et al. 1990).

15.2 Fenugreek

Fenugreek (*Trigonella foenum-graecum* L.) popularly known as Methi in north India is an annual herb of Fabaceae family (Alaraidh et al. 2018; Houhou et al. 2018; Wani and Kumar 2018; Dhull and Sandhu 2018; Mikkey et al. 2019) regarded as a multi-purpose crop cultivated in different parts of world, i.e. west Asia, Middle East, Russia, Latin America, North Africa, Some parts of Europe, USA, Canada, United Kingdom including semi-arid regions of India. Fenugreek is popularly cultivated as forage crop, spice crop, nutraceutical and pharmaceutical crop, green manure crop, cover crop, etc. (Ahmad et al. 2009; Talukdar 2011; Joglekar et al. 2012; Allue et al. 2013; Talukdar 2013; Kapoor and Pande 2015, Zandi et al. 2015; Lahuta et al. 2018; Sadak 2019; Mickky et al. 2019; Dhull et al. 2020a, 2020b; Kumar et al. 2020; Xalxo and Keshavkant 2020). In addition, the diverse use of different parts of fenugreek for various purposes such as flavoring agents, stabilizers, adhesives for cosmetics, paper, paints industry, etc. is reported by many researchers (Lahuta et al. 2018; Mikky et al. 2019) as given in Fig. 15.1. Fenugreek is considered a suitable crop for rainfed agriculture when cultivated as green manure crop, forage crop, spice crop, medicinal crop, etc. (Zandi et al. 2015). Due to its strong flavor and aroma, fenugreek is one of such plants whose leaves and seeds are widely consumed as a

Table 15.1 Crops reported for having exposure of different heavy metals

S. No	Crop	Heavy metal	Authors
1	Mung bean (*Vigna radiata* L. Wilczek)	Cobalt	Jaleel et al. (2009), Arif et al. (2016)
		Lead	Choudhary and Singh (2000), Ashraf et al. (2015)
		Cadmium	
		Chromium	Shanker et al. (2004), Karuppanapandian et al. (2006), Suthar et al. (2014)
2	Bean (*Vicia faba* L.)	Lead	Wang et al. (2010), Pourrut et al. (2011), Shahid et al. (2014), Arif et al. (2016)
3	Pea (*Pisum sativum* L*)*	Chromium	Tripathi et al. (2015), Arif et al. (2016)
		Lead	Devi et al. (2013)
		Cadmium	Lozano-Rodrigerez et al. (1997), Chaoui and Ferjani (2005), Agrawal and Mishra (2009)
		Copper	
4	Mustard (*Brassica juncea* L.)	Cadmium	Minglin et al. (2005), Pinto et al. (2009), Gill et al. (2011), Bauddh and Singh (2012)
		Copper	Arif et al. (2016), Yusuf et al. (2016)
5	Common bean (*Phaseolus vulgaris* L.)	Copper	Bouazizi et al. (2010)
		Chromium	Zeid (2001)
		Arsenic	Talukdar (2011), Talukdar (2013)
		Lead	Geebelen et al. (2002)
		Cadmium	Padmaja et al. (1990), Somashekaraiah et al. (1992)
		Nickel	Samarakoon and Rauser (1979)
		Cobalt	
6	Pepper (*Capsicum annum* L.)	Copper	Diaz et al. (2001)
7	Radish (*Raphanus sativus* L.)	Cadmium	Rivetta et al. (1997)
		Lead	Biteur et al. (2011)
8	Rice (*Oryza sativa* L.)	Cadmium	Ross et al. (1992), Herawati et al.(2000), Rizwaan et al. (2016), Rizwaan et al. (2018)
		Arsenic	Arif et al. (2016), Dixit et al.(2016)
		Lead	Yang et al. (2016)
		Copper	Herawati et al.(2000)
		Nickel	Ross et al. (1992);
9	Maize (*Zea mays* L.)	Lead	Seregine et al. (2004), Wang et al. (2007), Gupta et al. (2009), Kaur et al.(2015), Singh et al. (2015)
		Chromium	Singh et al. (2015)
		Cadmium	Lozano-Rodrigerez et al. (1997), Seregine et al. (2004), Wang et al. (2009), Nocito et al. (2008), Wang et al. (2016)
		Mercury	Lipsey (1975)
		Aluminum	Pineros and Kochian (2001)

(continued)

Table 15.1 (continued)

S. No	Crop	Heavy metal	Authors
10	Wheat (*Triticum aestivum* L.)	Lead	Kaur et al. (2012)
		Nickel	Arif et al. (2016)
		Mercury	
		Cadmium	Lin et al. (2007), Ahmad et al. (2009)
		Copper	Navari-Izzo et al. (1998)
11	Pigeon pea (*Cajanus cajan* L.)	Nickel	Arif et al. (2016), Rao and Sresty (2000)
		Cadmium	Sheoran et al. (1990)
12	Barley (*Hordeum vulgare*)	Cadmium	Sharma et al. (2004)
13	Soybean (*Glycine max* L.)	Cobalt	Jayakumar et al. (2008), Arif et al. (2016)
		Copper	Sanchez-Pardo et al. (2012)
		Aluminum	Cakmak and Horst (1991)
		Cadmium	Shamsi et al. (2008)
14	Chickpea *Cicer arietinum* L.	Arsenic	Gunes et al. (2009)
15	Spinach (*Spinacia oleracea* L.)	Chromium	Vikram et al. (2011)
16	Tomato (*Lycopersicum esculentum* mill.)	Cadmium	Dong et al. (2006), Sbartai et al. (2012), Pattnaik and Reddy (2011)
		Lead	
		Copper	
		Zinc	
17	Indian ginseng (*Withania somnifera*)	Copper	Khatun et al. (2008)

spice in food preparations. Fenugreek is reported to be a rich source of calcium, iron, â-carotene, vitamins, etc. (Sadak 2019). The medicinal value of fenugreek seeds is mentioned in traditional Indian scriptures such as ayurveda as well as in Greek and Latin scripture, i.e. pharmacopeia. The seeds of fenugreek are reported to be rich in metabolites having therapeutic importance such as galactomannans and biochemicals such as nicotinic acid, saponins, fenugreekine, sapogenins, coumarin, phytic acid, scopoletin, trigonelline, flavonoids, and mucilaginous fiber (Ramesh et al. 2001; Lahuta et al. 2018; Dhull et al. 2019; Dhull et al. 2020a, 2020b). Lysine- and L-tryptophan-rich proteins are reported to be present in fenugreek seeds. Fenugreek is traditionally used in treatment of some metabolic disorders, bronchitis, arthritis, ulcer, digestive issues, cancer, etc. (Amin et al. 2001; Shabbeer et al. 2009; Prabhu and Krishnamoorthy 2010; Alsemari et al. 2014; Aher et al. 2016; Lahuta et al. 2018; Wani and Kumar 2018; Sadak 2019).

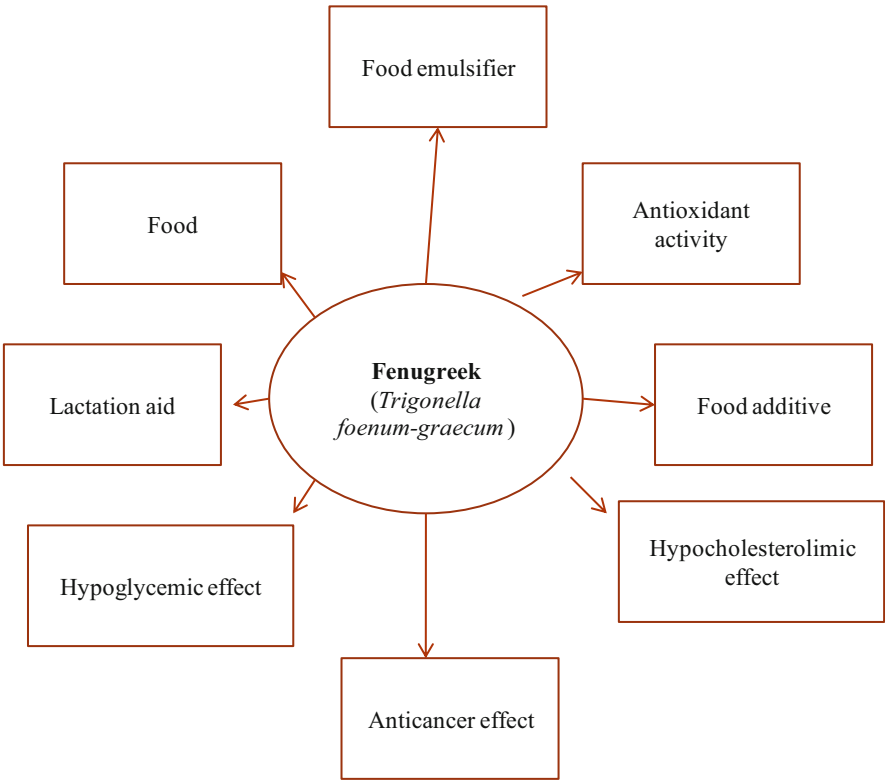

Fig. 15.1 Different uses of Fenugreek (*Trigonella foenum-graecum* L.)

15.3 Responses of Plants in Heavy Metal Contamination and Defense Mechanisms

Among all the types of plants, plants cultivated as agricultural crops suffer the most as presence of heavy metals based contamination in both water and soil negatively influences their diverse processes adversely affecting the growth parameters, i.e. germination, plant length, etc., metabolic processes adversely affecting mitochondria, biochemical processes such as photosynthesis, retardation in transport of sugars, gas exchange process, induction of Reactive Oxygen Species (ROS) like H_2O_2 causing oxidative damage in lipids, nucleic acids proteins, etc., chromosomal aberrations, abnormal mitosis, inhibition of cell division, resulting to cause retardation in growth leading to cell death (Kehrer 2000; Pattnaik and Reddy 2011; Kaur et al. 2014; Kaur et al. 2015; Sidhu et al. 2016; Farid et al. 2017; Rizwaan et al. 2017; Alaraidh et al. 2018; Mahapatra et al. 2019; Fatima et al. 2020).

In some cases due to the process of hormesis plant undergoes growth promotion effect in presence of low concentration of heavy metals (Kumar and Aery 2016)

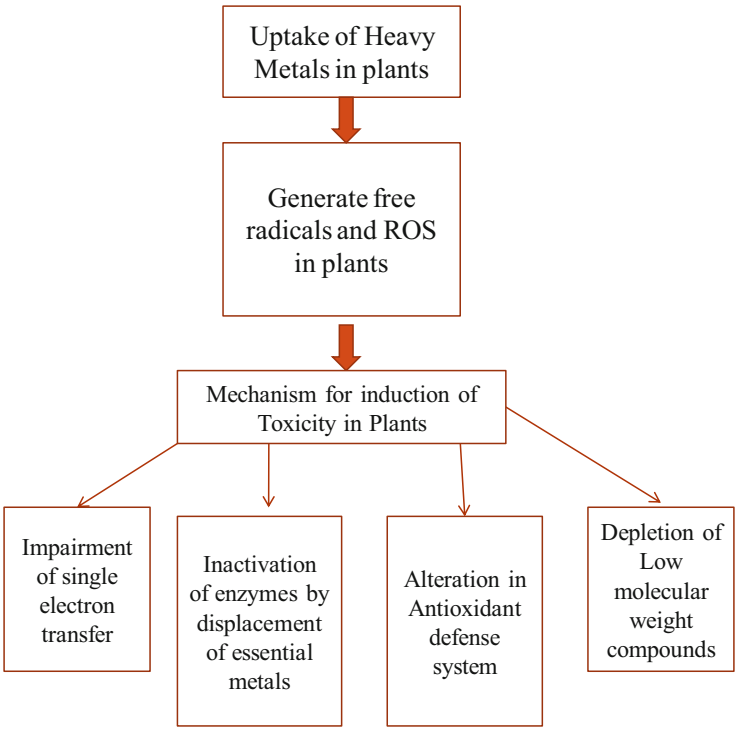

Fig. 15.2 Summary of heavy metal induced toxicity and mechanisms in plants

however plants facing high concentration of different heavy metals develop toxicity symptoms due to heavy metal induced oxidative stress as given in Fig. 15.2. To overcome the heavy metal mediated oxidative stress plant adopts different strategies for the survival but at the expense of energy which results for spoor or stunted growth. The strategy depends on the plant species and the metal content present in the soil. Roots of plants exposed to heavy metal contaminated sites develop various defense mechanisms to protect the plants from excessive uptake and further translocation of metal within plant.

Due to the process of evolution with the passage of time plants for their survival have developed various adaptive and detoxification strategies to overcome heavy metal induced oxidative stress. Some of the strategies are metal chelation, immobilization, uptake reduction, metals pumping, cell wall binding, sub-cellular metal compartmentation, sequestration in vacuoles, production of specific ligands (Alaraidh et al. 2018; Fatima et al. 2020). Plants undergoing heavy metal mediated stress induce several genes and resistance proteins to overcome the stress condition for their survival (Fig. 15.3).

Toxicities induced by heavy metals adversely affect the binding ability of metals to different types of ligands present in biological system such as carboxylate ion, imidazole, sulfhydryl group, aliphatic amine, etc. Absorption, translocation, and

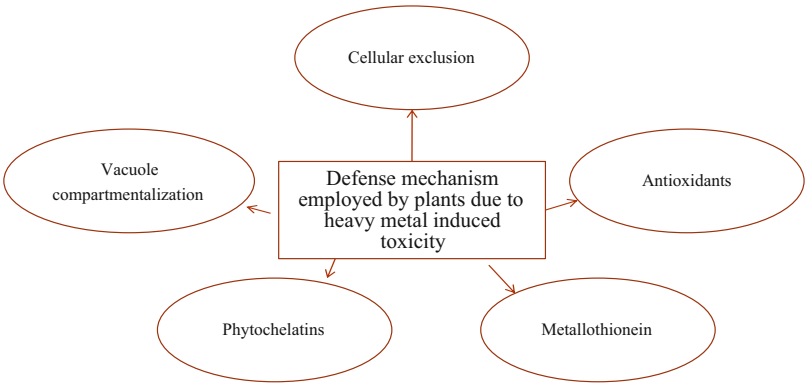

Fig. 15.3 Defense mechanism employed by plants in response to heavy metal induced toxicity (adapted from Pinto et al. 2015)

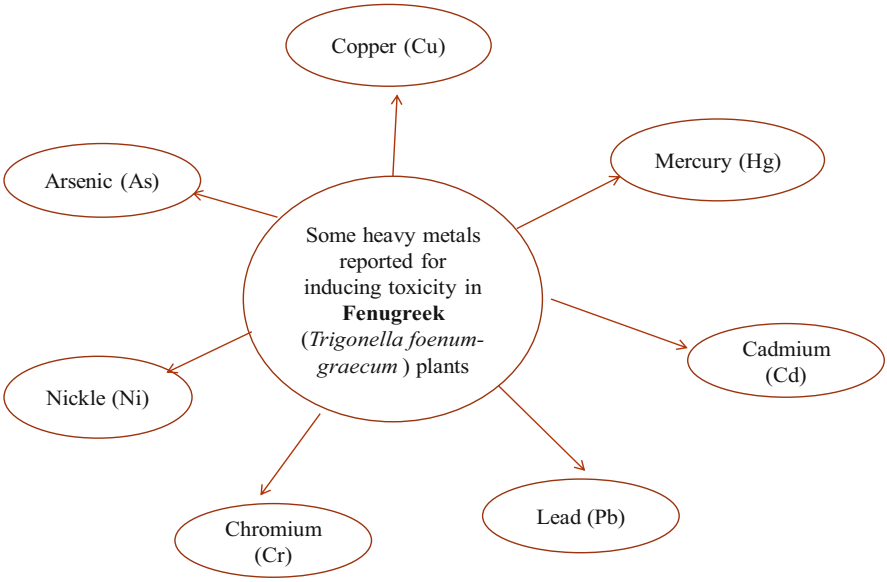

Fig. 15.4 Heavy metals reported to induce phytotoxicity in fenugreek plants

transportation of heavy metals within the plant tissue depend on plant species, concentrations, and oxidation state of heavy metals.

Heavy metals adversely affect the process of photosynthesis by negatively influencing the photosynthetic apparatus. Heavy metals influence plants by affecting the PS I and PS II and indirect effect on photosynthesis. Heavy metals impair the photosynthetic apparatus in plants thereby reducing the formation and accumulation of photosynthetic pigments. As exhibited in Figs. 15.4 and 15.5 different heavy metals affect the fenugreek plants ultimately retarding the growth parameters and

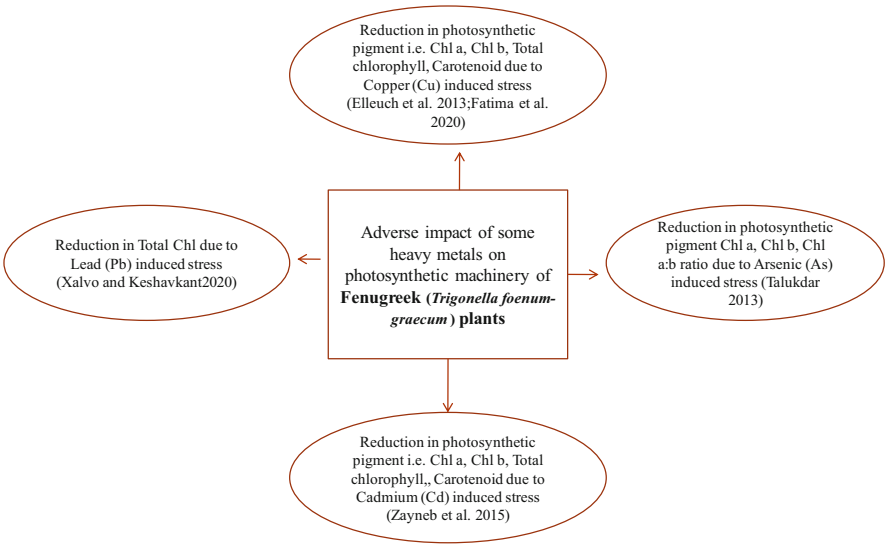

Fig. 15.5 Effect of heavy metals on photosynthetic process in fenugreek plants

yield. Some of the effects of heavy metals reported on photosynthesis are decreased Rubisco activity, inhibition of PSI and PSII, increased lipoxygenase activity, change in composition of lipid in thylakoid membrane, inhibition of assimilation of carbon dioxide, interference in oxygen evolving complex, decrease in Chl a: b ratio, reduction in number of chloroplast, change in light harvesting protein complex, inhibition in hill reaction, alteration in pigment protein complex, etc. are reported in various plant species, i.e. Pigeon pea (*Cajanus cajan* L.), Fenugreek (*Trigonella foenum-graecum* L.), Wheat (*Triticum aestivum* L.), Pea (*Pisum sativum*), Rice (*Oryza sativa* L.), Maize (*Zea mays* L.), Mustard (*Brassica juncea* L.), Common Bean (*Phaseolus vulgaris* L.), *Nicotiana plumbaginifolia*, *Pogonatherum crinitum,* etc. (Padmaja et al. 1990; Sheoran et al.1990; Somashekaraiah et al. 1992; Navari-izzo et al. 1998; Ahmad et al. 2009; Wang et al. 2009; Zaghdoudi and Carpenteir 2011; Elleuch et al. 2013; Talukdar 2013; Bashri and Prasad 2015; Zayneb et al. 2015; Kumar and Aery 2016; Rai et al. 2016; Houhou et al. 2018; Fatima et al. 2020; Xalxo and Keshavkant 2020).

15.4 Defense Mechanism in Plants

Once the heavy metal is entered in plant system, evolutional processes have enabled the plants to employ various defense mechanisms for uptake, accumulation, and translocation of different heavy metals as exhibited in Fig. 15.3. These mechanisms detoxify the heavy metals by excluding the free ionic forms from the cytoplasm.

Metallothioneins are metal-binding, low-molecular-mass cysteine rich proteins widely reported for detoxification of heavy metals. Metallothioneins bind heavy

metal ions through the cysteinyl groups which are present in their structure in abundance (Cobbett and Goldbrough 2002; Pinto et al. 2015; Kumar and Aery 2016).

Vacuole is commonly considered as the main storage cell organelle for metals in plant confirmed by several studies that the vacuole is the site for the accumulation of heavy metals. Heavy metals are transported into vacuole, after removal from the cellular compartments such as cytosol. Hyperaccumulator plants store heavy metals in vacuoles present in leaf cell, whereas non hyperaccumulator plants sequester heavy metal in vacuoles of root cell (Pinto et al. 2015; Kumar and Aery 2016; Sharma et al. 2016).

Phytochelatins are small polypeptides rich in cysteine reported to possess the potential of complex formation with heavy metals in cytosol later on transporting to vacuole thereby countering the toxicity induced by heavy metals (Cobbett and Goldbrough 2002; Kumar and Aery 2016).

All the heavy metals enter in plants through apoplastic and symplastic pathways. Cellular exclusion of heavy metals is another strategy adapted by various plants to overcome the toxicity induced by heavy metal. A large fraction of heavy metals are found in the apoplastic space in plant roots (Kumar and Aery 2016).

Malondialdehyde (MDA) is a resultant product of polyunsaturated fatty acids existing in membranes, and considered as an important indicator of oxidative stress (Yadav et al. 2009; Yadav 2010; Pinto et al. 2015). Proline is reported to play an active role in chelation of heavy metals in cytoplasm as a defense strategy (Kumar and Aery 2016).

One of the most popular mechanisms employed for entrapment of heavy metals in the apoplasm by binding with organic acids or different anionic groups present in cell wall prevent the uptake of heavy metals through plant root cells (Pinto et al. 2015). Heavy metals are reported to enter in plant through root cells where they encounter defense mechanism by amino acids, metal-binding peptides, sequestration in vacuoles, etc. Other defense strategy commonly adopted by the plants having exposure of heavy metal leads to activation of antioxidant machinery which counteract the oxidative stress. Lipid peroxidation is one of the prominent effects induced by the heavy metal mainly causing the biomembrane degradation.

With the ongoing process of evolution, plants acquired different defense strategies as discussed earlier and accumulation of heavy metals in apoplast, epidermal cells, endodermis are some of the recorded sites, it is pertinent to mention that more than 80% of the accumulated heavy metals bind with cell wall (Wang and Greger 2004) and one of the basic reasons for high concentration of heavy metals in root zone however some of the accumulated heavy metal is translocated in shoot zone further metal uptake proteins and metal efflux protein facilitate the translocation and detoxification of heavy metals in plants (Kumar and Aery 2016).

15.5 Morpho-Physiological Responses in Plants

Morpho-physiological parameters in plants such as fenugreek such as germination percent seedling length, seedling vigor index, etc. are negatively influenced due to the oxidative stress induced by heavy metals (Ahmad et al. 2009; Srinivas et al. 2013), salinity (Mickky et al. 2019), solar U V (Sharma et al. 2019) acid rain (Zaghdoudi and Carpenteir 2011), pesticides (Mahapatra et al. (2019), etc., whereas inhibition of photosystems I and II in plants of fenugreek under salt stress was reported by Zaghdoudi and Carpenteir (2011) and Srinivas et al. (2013).

Growth parameters of fenugreek, i.e. germination percent, plant length, and fresh weight were negatively affected by Copper, Lead, Cadmium, Chromium. Root length was comparatively more inhibited than the shoot length as absorption of heavy metals is more through roots affecting the root cells (Talukdar 2011; Elleuch et al. 2013; Fatima et al. 2020) and due to presence of more defense proteins in root zone; however, the effect is different according to the metals as cadmium pose the most inhibitory effects than chromium followed by lead (Muhammad et al. 2008; Alaraidh et al. 2018). Copper when present in high concentration, produces phytotoxicity symptoms adversely affecting various physio-biochemical, metabolic processes and morphological attributes of plants along with the report of accumulation in plant tissues. Copper available in excessive amounts in plant cells bind with proteins causing alteration in function and structure leading to cell death causing poor and stunted growth of plants. Heavy metal like Copper is reported to hinder the process of intake of various essential nutrients such as magnesium, potassium, etc., ultimately reducing the uptake of nutrients by plant roots required for growth and development of plants and is one of the prime reason for reduced growth of plant species like fenugreek (Azooz et al. 2012; Elleuch et al. 2013; Fatima et al. 2020).

As presented in Fig. 15.5, heavy metals such as Copper, Arsenic, Cadmium, Lead, etc. are reported to induce oxidative stress which inhibits the production of photosynthetic pigments in fenugreek plants due to alteration in mesophyll cells of leaf thereby causing oxidative damage with the increase in MDA, EC and H_2O_2 contents, etc. (Elleuch et al. 2013; Talukdar 2011; Talukdar 2013; Zayneb et al. 2015; Xalxo and Keshavkant 2020; Fatima et al. 2020). In case of fenugreek plants exposed to cadmium and lead in different concentrations the density of stomata and its length and width were declined under metal stress at all the stages of development. Both cadmium and lead negatively influenced the foliar parameters such as leaves number, area of leaf, number of branches per plants. The density of trichome, proportion of pith and cortex, density of vessel, length and width of xylem fibers, dry weight, plant length were reduced in plants after having exposure of high concentration of heavy metals such as cadmium and lead (Ahmad et al. 2009).

15.6 Antioxidant Responses in Plants

Plants experience various toxicity symptoms after having the exposure of heavy metals. Heavy metals are reported to induce the production of Reactive Oxygen Species (ROS) as presented in Fig. 15.6 which are toxicological intermediates generated in plants causing oxidative stress resulting into enhanced lipid peroxidation, degradation of biological macromolecules, alteration in membranes, ion leakage, cleavage of DNA strands, etc. (Pinto et al. 2015). Oxidative stress is an outcome of excessive generation of ROS or free radicals both intracellular as well as extracellular producing cytotoxicity (Gill and Tuteja 2010). chemical compounds having heavy metals are one of the potential sources having the capability of catalyzing Haber–Weiss reaction and Fenton reaction. Haber-Weiss and Fenton reactions are the established mechanisms considered to be responsible for the generation of highly reactive hydroxyl ion radical in living organisms.

Redox heavy metals such as Copper and Chromium catalyze the formation of harmful free radicals (ROS) through Fenton and Haber–Weiss-type reactions thereby inducing lipid peroxidation, whereas non-redox metals like Cadmium, Lead, Mercury, etc. produce ROS indirectly, mostly by causing depletion of glutathione and distracting the electron transport chain (ETC) in plants (Arif et al. 2016). It is also reported that heavy metals alter the stomatal conductance in plants thereby reducing the carbon dioxide level and induce formation of ROS. Once the ROS i.e. free radicals are generated in any biological system negatively influence the cellular functioning leading to oxidative burst in different cellular sites impairing the synthesis and function of lipids, proteins, DNA, alteration in signal transduction pathways, etc. (Kehrer 2000; Arif et al. 2016). The generation of ROS is reported to occur in peroxisomes, endoplasmic reticulum, mitochondria, cell wall, etc. (Kehrer 2000; Gill and Tuteja 2010; Das and Roychoudhary 2014; Arif et al. 2016). Different types of reactive oxygen species are presented in Fig. 15.6. Among all types of reactive oxygen species (ROS), i.e. Superoxide radical ($O_2^{\cdot -}$), Singlet oxygen ($^1O_2$),

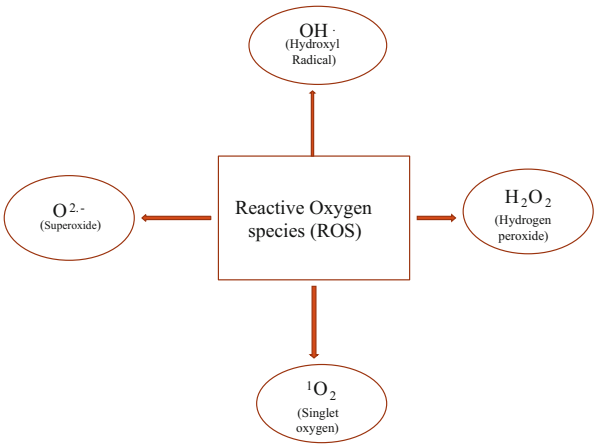

Fig. 15.6 Reactive oxygen species

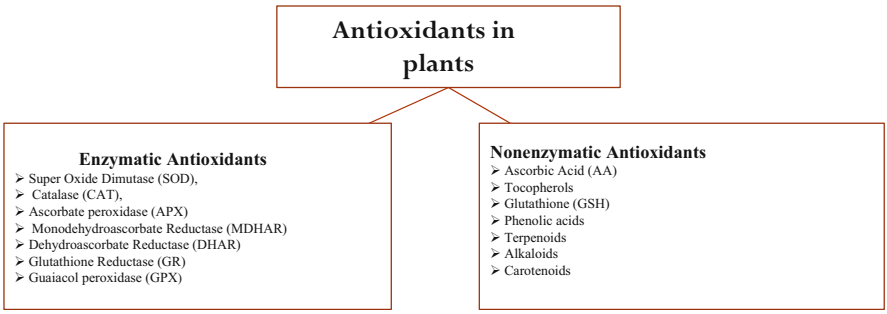

Fig. 15.7 Types of antioxidants in plants

Hydrogen Peroxide (H_2O_2), and Hydroxyl radicals (OH^-), Hydroxyl radicals (OH^-) are considered as the most reactive oxygen species and dangerous to the metabolic and physiological functioning of plants and if produced in excess amount leads to death of cell (Gill and Tuteja 2010).

Among different defense strategies investigated in plants as exhibited in Fig. 15.7 plants are equipped with antioxidant system for detoxification and scavenging of ROS. Plants have innate capacity to produce different phytochemicals such as antioxidants. Antioxidants are biological reducing agents which avert the oxidation of significant biomolecules and regulate the generation of free radicals (Dhull et al. 2016; Kaur et al. 2018; Dhull et al. 2020c). Due to the presence of heavy metals such as Copper, Cadmium, Chromium, Lead, Mercury, Arsenic, Nickel, etc. in excessive quantities plants employ their anti-oxidative system which include enzymatic anti-oxidant i.e. Superoxide dismutase (SOD) Catalase (CAT), Guaiacol peroxidase (POD), Peroxidase (APX), Glutathione reductase, Guaiacol peroxidase (GPX) and non-enzymatic antioxidants, i.e. Ascorbic acid, Tocopherols, Carotenoids, Terpenoids, Glutathione, Alkaloids, Phenolic Acids, Anthocyanins (Elleuch et al. 2013; Karmakar 2014; Bashri and Prasad 2015; Arif et al. 2016; Sidhu et al. 2016; Alaraidh et al. 2018; Mahapatra et al. 2019; Sharma et al. 2019; Fatima et al. 2020; Xalxo and Keshavkant 2020). The antioxidant capacity in a plant indicates its potential to tolerate heavy metal induced oxidative stress.

Superoxide dismutase (SOD) reduces the superoxide anion into H_2O_2 causing initial defense against the reactive oxygen species (ROS). CAT and POD further detoxify H_2O_2 into H_2O and O_2 thus causing overall defense against the heavy metal induced ROS in various plants like Rice, Radish, Wheat, Pea, bean, fenugreek, etc. under oxidative stress conditions caused by various abiotic components present in an ecosystem such as Heavy metals, Pesticides, Salinity, etc. (Pattnaik and Reddy 2011; Kapoor and Pande 2015; Arif et al. 2016; Mahapatra et al. 2019; Fatima et al. 2020; Xalxo and Keshavkant 2020). Polyphenol oxidase (PPO) an antioxidant enzyme scavenges H_2O_2 in chloroplasts and plays an important role in lignin biosynthesis, whereas ascorbic acid, a non-enzymatic antioxidant scavenges the H_2O_2 radicals. However when plant undergoes high exposure of heavy metals the production of antioxidant enzymes like SOD, CAT etc. are required in adequate quantity for

scavenging of ROS generated in plants inducing heavy metals toxicity (Arif et al. 2016; Houri et al. 2020; Xalxo and Keshavkant 2020).

Influence of heavy metals on fenugreek plants is investigated by various researchers as presented in Table 15.2 and 15.4 and some of the adverse effects of heavy metals such as Copper (Cu), Lead (Pb), Mercury (Hg), Arsenic (As), and Cadmium (Cd) are as follows:

15.7 Effect of Copper (Cu) on Fenugreek Plants

In case of high exposure of copper in fenugreek plants large amount of copper binds with cellular proteins causing adverse effects on structure and function of cell leading to cell death which results for reduction in growth as presented in Tables 15.2 and 15.3. Copper is also reported to interfere in nutrient uptake such as magnesium (Mg) and potassium (K) reducing the uptake adversely affecting plant growth. The reduction in photosynthetic pigment affects the mesophyll of fenugreek leaf cell. Exposure of Arsenic induced the production of various ROS which leads to the synthesis of Antioxidants, i.e. APX, SOD, DHA, ASA in fenugreek plants (Elleuch et al. 2013; Fatima et al. 2020).

15.8 Effect of Arsenic (as) on Fenugreek Plants

Arsenic is globally known as a toxicant. Arsenic (As) is a nonessential heavy metal and toxic element reported for variety of plants. Arsenic gets accumulated in roots in comparison of shoots. Generally, plants uptake arsenic as As(V) and translocate it via the xylem along with water and minerals as As(III)-S compound Arsenic induce oxidative stress when present at high concentration results in reduction of plant growth due to alteration in diverse processes as presented in Tables 15.2 and 15.3. The reduction in Chl a and Chl a/b ratio affects the photosynthetic activity in fenugreek plants. It is reported that plant exposure to As leads to ROS generation by the conversion of arsenate As (V) into arsenite (As (III) which is reported for its high toxicity (Wang et al. 2002; Kumar et al. 2016). The excessive production of H_2O_2 is increased to several folds due to the accumulation of Arsenic in roots. Accumulation of MDA and electrolyte leakage is also recorded due to accumulation of Arsenic. Antioxidants, i.e. APX, SOD, DHA, ASA were enhanced, whereas CAT was reduced. Roots of fenugreek are reported to have high concentration of Arsenic than shoots, pods, and seeds (Wang et al. 2002; Talukdar 2011, 2013).

15.9 Effect of Cadmium (Cd) on Fenugreek Plants

Cadmium is a nonessential heavy metal for plants. Cadmium has been ranked seventh among the top toxic metals due to its high solubility in water and has high toxicity. Cadmium toxicity is easily identifiable in the form of abnormal seed

Table 15.2 Toxic effects of heavy metals on fenugreek plants

S. No	Heavy metal	Toxic effect	Authors
1	Copper (cu)	Reduction in seed germination, retardation in growth parameters, chlorosis, reduction in synthesis of chlorophyll	Elleuch et al. (2013), Fatima et al. (2020)
2	Arsenic (as)	Reduction in rate of germination, delayed growth, retardation in growth parameters at high concentration	Talukdar (2011), Talukdar (2013)
3	Lead (Pb)	Reduction in fresh weight and dry weight, decrease in crop yield. Increase in proportion of pith and vasculature, decrease in stem cortex, reduction in dry weight, number and size of stomata. Reduction in growth, oxidative stress, poor synthesis of chlorophyll	Dang et al. (1990), Alaraidh et al. (2018), Xalxo and Keshavkant (2020)
4	Cadmium (cd)	Reduction in fresh weight and dry weight, decrease in crop yield, decrease in proportion of pith and vasculature, increase in stem cortex, reduction in dry weight, number, and size of stomata, reduction in growth and pigment, induction of ROS	Dang et al. (1990), Bashri and Prasad (2015), Zayneb et al. (2015), Alaraidh et al. (2018)
5	Nickel (Ni)	Reduction in fresh weight and dry weight, decrease in crop yield interveinal chlorosis	Dang et al. (1990), Parida et al. (2003)
6	Chromium (Cr)	Reduction in rate of germination, reduction in shoot and root length	Alaraidh et al. (2018), Dheri et al. (2007)
7	Mercury (hg)	Adverse impact on growth parameter reduction in cell division	Karmakar (2014)

germination, stunted growth, chlorosis, browning of root tips leading to the death of plants as presented in Tables 15.2 and 15.3. Cadmium is reported to inhibit the cell division and alters the chromosome causing physiological damages and genotoxicity. Cadmium is reported to cause inhibitory effect on seed germination leading to decline of growth of fenugreek seedlings due to the enhanced concentration of cadmium in both root zone as well as shoot zone. Due to accumulation of cadmium in fenugreek plants different ROS are generated as a result causing decrement in the process of photosynthesis. Cadmium is reported to adversely affect the photosynthetic machinary in plants adversely affecting the production of photosynthetic pigments like Chlorophyll a and Chlorophyll b causing senescence in leaves which results in increased Chl a/b ratio and is reported to interfere with growth hormone auxin by initiating the production of auxin degrading enzyme IAA

Table 15.3 Antioxidant response in Fenugreek (*Trigonella foenum-graecum* L.) plants in presence of heavy metals

S. No	Heavy metal	Antioxidants	Authors
1	Cadmium (cd)	SOD, APX, CAT, Polyphenol, Flavonoids	Bashri and Prasad (2015), Zayneb et al. (2015), Alaraidh et al. (2018)
2	Lead (Pb)	SOD, CAT, POD	Alaraidh et al. (2018), Xalxo and Keshavkant (2020)
3	Arsenic (as)	SOD, APX, CAT	Talukdar (2013)
4	Copper (cu)	APX, SOD, CAT, POD	Elleuch et al. (2013), Fatima et al. (2020)
5	Mercury (hg)	POD, CAT, SOD	Karmakar (2014)
6	Chromium (Cr)	CAT, POD, APX	Alaraidh et al. (2018)

oxidase in fenugreek plants. Cadmium induced stress causes an increased production of H_2O_2 and O_2 which enhances the activity of SOD and CAT (Aery and Sarkar 1990; Dang et al. 1990; Aery and Sarkar 2012; Bashri and Prasad 2015; Zayneb et al. 2015; Kumar et al. 2016; Alaraidh et al. 2018).

15.10 Effect of Lead (Pb) on Fenugreek Plants

Lead (Pb) is a commonly occurring heavy metal in different types of ecosystem. It enters in the plant system through soil induces toxic effects in fenugreek plants such as impairment of plant growth such as affecting root elongation, seed germination, seedling development, transpiration, photosynthesis, and cell division (Kaur et al. 2015) as presented in Tables 15.2 and 15.3**.** Lead is reported to bind with membranes causing separation of the granular material from the plasma membrane, thus inhibiting growth of polysaccharide chains that ultimately lead to disorientation of cellulose microfibrils. Fenugreek plant growth was adversary affected at high concentration of lead causing decrease in dry matter (Tanwar et al. 2013). The translocation of Cadmium in stem and leaves is reported to be comparatively lesser than roots in fenugreek plants. The production of antioxidants like SOD, CAT, POD, etc. was reported by some earlier researchers (Alaraidh et al. 2018; Xalxo and Keshavkant 2020).

15.11 Effect of Mercury (Hg) on Fenugreek Plants

Fenugreek plants encounter oxidative stress in presence of mercury such as $HgCl_2$ which resulted in the anatomical abnormalities in seedlings at different time intervals (Karmakar 2014). Due to the mercury mediated stress parameters such as

Table 15.4 Studies conducted on toxic effects of heavy metals on fenugreek (*Trigonella foenum-graecum* L.)

S. No	Title of study	Authors
1	Physiological responses of fenugreek seedlings and plants treated with cadmium	Zayneb et al. (2015)
2	Arsenic-induced changes in growth and antioxidant metabolism of fenugreek	Talukdar (2011)
3	Physiological aspects of cadmium and lead toxic effects on higher plants cadmium application	Seregin et al. (2004)
4	Influence of nickel-contaminated soils on fenugreek (*Trigonella corniculata* L.)growth and mineral composition	Parida et al. (2003)
5	Biomass partitioning and morphological parameters of *Trigonella foenum-graecum* submitted to sulfur deficiency	Houhou et al. (2018)
6	Efficacy of fenugreek plant for ascorbic acid assisted phytoextraction of copper (cu); a detailed study of cu induced morpho-physiological and biochemical alterations	Fatima et al. (2020)
7	Effect of cd, Ni, Pb, and Zn on growth and chemical composition of onion and fenugreek	Dang et al. (1990)
8	Response of fenugreek (*Trigonella foenum-graecum* L.) seedlings under moisture and heavy metal stress with special reference to antioxidant system	Karmakar (2014)
9	Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenum-graecum* L.)	Sadak (2019)
10	Morpho-anatomical responses of *Trigonella foenum-graecum* Linn. To induced cadmium and lead stress	
11	Indole acetic acid modulates changes in growth, chlorophyll a fluorescence and antioxidant potential of *Trigonella foenum-graecum L.* grown under cadmium stress	Bashri and Prasad (2015)
12	Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge: Involvement of antioxidants	Sinha et al. (2007)
13	Alteration of antioxidant gene expression in response to heavy metal stress in *Trigonella foenum-graecum* L.	Alaraidh et al. (2018)
14	Fractionation of chromium in tannery sludge-amended soil and availability to fenugreek plants	Allue et al. (2013)
15	Morphological and biochemical behavior of fenugreek (*Trigonella foenum-graecum*) under copper stress	Elleuch et al. (2013)
16	Accumulation and mobility of heavy metals in fenugreek (*Trigonella foenum-graecum* L.) and tomato (*Lycopersicum esculentum* mill.) grown in the field amended with urban wastes, and their composts and vermicomposts	Pattnaik and Reddy (2011)
17	The effects of heavy metals on seed germination and plant growth on Coccina, Mentha and *Trigonella* plant seeds in Thimmapuram, E.G. district, Andhra Pradesh, India	Srinivas et al. (2013)
18	Growth and antioxidant responses of *Trigonella foenum-graecum* L. seedlings to lead and simulated acid rain exposure	Xalxo and Keshavkant (2020)

plant length, dry weight, fresh weight and mitotic index were negatively affected the growth and cellular activity of plants having mercury. Alteration in mitosis and abnormality in vascular bundles are recorded and the root growth is investigated to be sensitive for mercury. Fenugreek seedlings exhibited low level of antioxidant activity revealing the failure of free radicals scavenging activity (RSA) under the exposure of mercury. However induction in SOD, CAT, and POD activity along with induction of MAD was recorded (Karmakar 2014). Mercury causes a significant decline in catalase activity compared to PEG. Decline in catalase activity under mercury toxicity indicates delay in removal of H_2O_2 and toxic peroxide mediated by catalase a key enzyme involved in removal and decomposition of H_2O_2. Mercury induces intense oxidative stress causing inactivation of membrane bound proteins with the increase in permeability of membranes. Mercury based phytotoxicity clearly affects the quenching ability of the antioxidant to DPPH radical (Karmakar 2014) resulting in low yield followed by death of plants (Table 15.4).

15.12 Conclusion

Heavy metal alter the significant physiological processes in fenugreek plants even in presence of various defense mechanisms, i.e. production of Phytochelatins, Metallothionein, sequestration of Heavy metals in vacuoles, anti- oxidant systems reflects the ability of fenugreek plants to survive the oxidative stress induced by various heavy metals even then heavy metals are reported to hinder major biochemical processes including the process of photosynthesis resulting to produce a variety of phytotoxic symptoms ranging from chlorosis, retarded growth and decrease in yield. Fenugreek plants have been reported to have the ability to accumulate the heavy metals in root and shoot cells as a strategy but the long term intake of high concentration of accumulated heavy metals in plant tissues is an alarming issue for diverse life forms surviving in agro-ecosystems adversely affecting the ecological balance including the valuable ecosystem services needed for the effective functioning of the ecosystem. Fenugreek plants products such as plant leaves as vegetable and seeds as spice are popularly included in the diet of humans consumed for their medicinal and dietary properties involves human food chain which may induce acute and chronic toxicity health issues like occurrence of kidney disorder, liver disorder, bone and neural disorder, disruption of immunological processes, various types of cancer in humans. As the nutritional value of heavy metal contaminated edible plants including fenugreek is reduced it is the need of the hour to investigate the responses of plants to metal toxicities in totality and evaluation of adverse effects in agro-ecosystem based food chains. Metal pollution index (MPI) is an important parameter for monitoring metal pollution level in contaminated medium. Increased metal pollution index of edible plants reflects the high concentration of heavy metals posing negative effects on human health in long duration.

Plants such as fenugreek possess the ability to accumulate the heavy metals may be used in phytoremediation of metal contaminated land provided the plant products may not be incorporated in food chain. Keeping in view of the sustainable

development goals, i.e. Goal 3: (Good health and well-being) and Goal 12: (Responsible consumption and production). The cultivation of fenugreek needs monitoring of various sources of heavy metal contamination for such heavy metals present in agricultural soil, irrigational water, presence of sanitary landfill site, etc. Fenugreek plant products are widely reported to be consumed for their various medicinal and nutraceutical properties. Genetically modified fenugreek plants may be developed in near future which can resist the heavy metal induced oxidative stress along with the potential to detoxify the heavy metals. Both agriculturist and consumers must have awareness regarding the heavy metal accumulating ability of fenugreek plants. Ecofriendly concept of sustainable agriculture focuses on judicious use of agrochemicals must be practiced and rule of law must prevail for strict implementation of regulatory guidelines regarding the use of xenobiotic compounds having heavy metals. This chapter is an attempt to present the overview of heavy metal induced toxicity in plants, causes of oxidative stress and defense responses in fenugreek plant against toxicity induced by different heavy metals.

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Genomics, Transcriptomics, Proteomics and Metabolomics Approaches

16

Spandan Chaudhary, Pooja Chaudhary, and Shiv Patel

Abstract

Trigonella Foenum-graecum, commonly known as fenugreek, is documented from fifteenth century for its use as spices and smelling agent. This dicotyledonous plant of Fabaceae family (subfamily Papilionaceae) is widely used for numerous medicinal applications all over the world. Adaptability to diverse atmospheric conditions, temperatures, and soils makes this plant cultivable in different habitats. More than 260 species of fenugreek are diffused in more than 20 countries of Asia, Europe, Africa, America and in some areas of Australia. Medicinal and clinical properties of fenugreek belong to its bio-active secondary metabolites such as alkaloids, flavonoids, steroids and saponins. There are many literature available describing cultivation, ecophysiology and traditional uses, medicinal properties, phytochemical and nutrient contents of fenugreek. In the last two decades, fenugreek has caught attention from scientific community due to its one of the most valuable components—Diosgenin. The Omics approaches genomics, transcriptomics, proteomics, and metabolomics are very important to understand systems biology. This chapter summarizes the major research work conducted on fenugreek by aforementioned omics approaches and its applications. Authors have tried to touch upon the understanding of omics approaches, their methodology, analytical techniques involved and theirs usage and output along with the researches conducted on fenugreek plant.

Keywords

Genomics · Transcriptomics · Proteomics and Metabolomics · Fenugreek

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16.1 Introduction

Genomics, transcriptomics, proteomics, and metabolomics are four different terminologies of scientific studies which together can be called as Omics (Fig. 16.1). These techniques together provide a holistic view of any biological specimen whether it is a cell, tissue or organism. Integration of aforementioned Omics approaches can collectively also be referred as systems biology (Horgan and Kenny 2011). Fundamental aspects of Omics approach are to study any complex systems as a whole. Traditional scientific approaches are driven by hypothesis, whereas omics approaches generate hypothesis based on generated data, which can be further validated through more studies (Kell and Oliver 2004). Omics techniques are useful in decoding physiological processes, essential pathways leading to important metabolites and screening, diagnosis, and prognosis of disease. It will also be helpful in developing good varieties with desired traits as it can be used to reveal host–pathogen interaction and developing bio-marker for trait of interest. Omics approaches have proven advantages in developing new varieties with desired traits in less time than the traditional breeding approaches. In eukaryotes, identification of metabolic pathways, dissection of molecular mechanisms and detection of key genes responsible for desired trait is strenuous due to complex mechanisms. Globally, rapid advances in functional genomics research have been observed. Breakthrough research in technology advancement has made Omics tools more powerful which has provided an edge to the scientist all over the world.

Genomics is the study of complete genome of organisms by decoding the genetic codes of the genetic material. Genomics research aims to understand genes at genome level, whereas transcriptomics research identifies genes involvement in specific functions. Proteomics studies include the systemic analysis of proteins which carry out various functions of the cells, tissues or organs whereas metabolites are the end product of the complete cellular process. "Metabolites" are the reflection of changes in the biological systems due to any external or internal factors. Omics approaches help in understanding the genotype–phenotype relationship, identifying

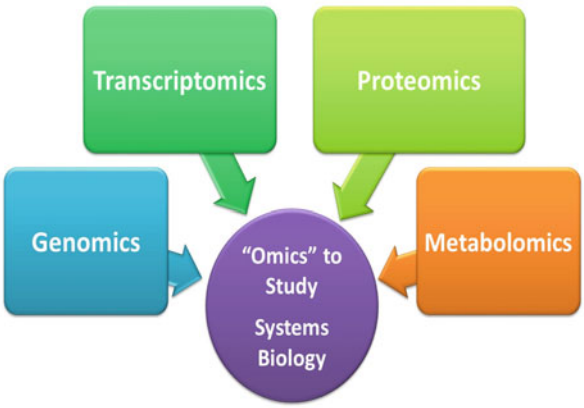

Fig. 16.1 Omics at glance

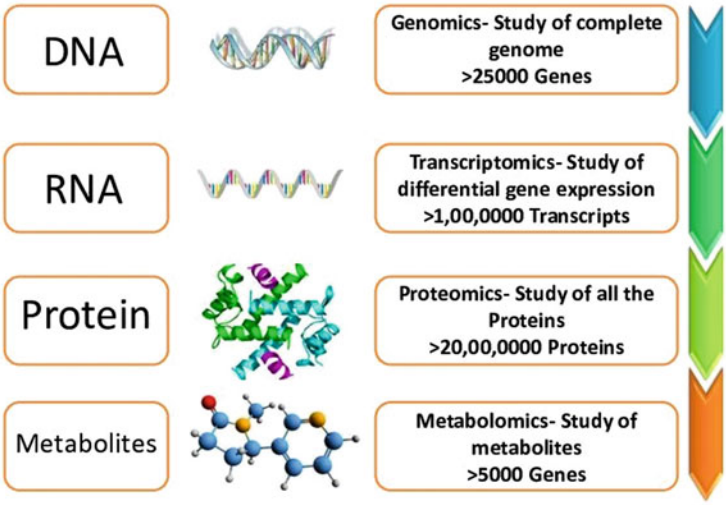

Fig. 16.2 Figures mentioned here express approximate quantity at each level. DNA is converted to RNA through transcription process, RNA is converted to protein through translation, protein is converted to form various metabolites by multiple biological processes

genes and their interactions, involvement of genes in metabolic pathways of interest. This helps in improving quality, productivity, and overall plant breed with desired traits (Agrawal et al. 2015). Omics studies have numerous applications, from understanding basic concepts and pathways of various physiological functions to generating genetically edited supreme plants. Omics approaches flow biological material from DNA to metabolites (Fig. 16.2).

Trigonella foenum-graecum, a plant with intense medicinal applications and very popular as species in many Asian and European countries, is also known for its use for its pivotal secondary metabolites (Chaudhary et al. 2018a). This plant belongs to Fabaceae family and commonly known as Fenugreek. During decade, this plant has attracted attention of researchers globally due to its capability of producing important secondary metabolites like alkaloids, flavonoids, steroids, and saponins (Jani et al. 2009; Murlidhar and Goswami 2012; Vaidya et al. 2013). In particular, diosgenin, a steroidal saponin, has been investigated for its medicinal uses and fenugreek has been reported as source of raw material for the production of steroidal hormones (Chaudhary et al. 2015, 2018b). Omics approaches studied on fenugreek till date are comparatively less but have provided promising results. Major genomics, transcriptomics, proteomics, and metabolomics studies conducted on fenugreek plant are covered in this chapter with its potential future scope.

16.2 Genomics of *Trigonella Foenum-graecum*

Genomics is defined as the study of whole genome means entire DNA. For studying genomics of any living organism, the first step is the estimation of genome which means estimating the total amount of DNA present in particular organism. Genome estimation is determined by flow cytometry and reflected as C-value. Flow cytometry analyses relative fluorescence intensity, and hence relative DNA content. In flow cytometry, the genome size of an unknown sample is determined by comparing it with nuclei of a reference standard, whose genome size is known (Doležel and Bartoš 2005). The C-value of fenugreek was determined as 0.7, which was 1.5-fold higher than the values of model legumes *Lotus corniculatus* L. var. *japonicus* Regel [syn. *Lotus japonicus* (Regel) K. Larsen] and barrel (*Medicago truncatula* Gaertn.). The genome size of both these species was around 470 Mbp, whereas the genome size of fenugreek is around 685 Mbp (Vaidya et al. 2013; Young et al. 2003). Despite being very important medicinal plant whole genome sequencing of this plant is yet not conducted. Based on few data available on fenugreek karyotype and genome, it has been identified that the somatic chromosome numbers of *Trigonella* taxa are 2n = 14, 16, 30, and 46 along with B chromosomes (Martin et al. 2011). Molecular characterization of fenugreek plants using bio-markers such as RAPD, SSR, AFLP, RFLP have been conducted by many researchers across the world. Majority of varieties have found to be closely related to each other except Kasuri methi (*Trigonella corniculata*) (Sundaram and Purwar 2011). Kasuri methi is popular in India and Pakistan for its use as spice due to its characteristic fragrance. It is phenotypically also different from other fenugreek varieties in its leaf size and height. Scientists have also discovered its genetic difference from other fenugreek varieties at gene expression level (Spandan and Pooja 2019). Genome sequencing can help in discovery of genes and molecular markers associated with diverse agronomic traits, genes related to adaptation to environmental and biotic stresses, genes responsible for yield and growth parameters, etc. which in turn can significantly helpful for crop improvement. There is strong need to carry out genome sequencing of this amazing plant so that it can be utilized at full potential like other medicinal plants.

16.3 Genomics Methodology

Though genome sequencing of Fenugreek is pending but its methodology is well established as it has already been used for many bigger genome sequencing projects like human genome project, Arabidopsis genome project, Rice genome sequencing, etc. Genome sequencing uses combined approaches like mate-pair, paired end, shotgun, etc. but to provide basic information about the methodology, paired end approach is described in the flowchart (Fig. 16.3). First step of the genomics is the high quality DNA isolation. Isolated DNA is subjected to fragmentation by mechanical shearing method. This produces DNA fragments having blunt and sticky ends. Fragmented DNA is subjected to end-repair process which includes mixture of three

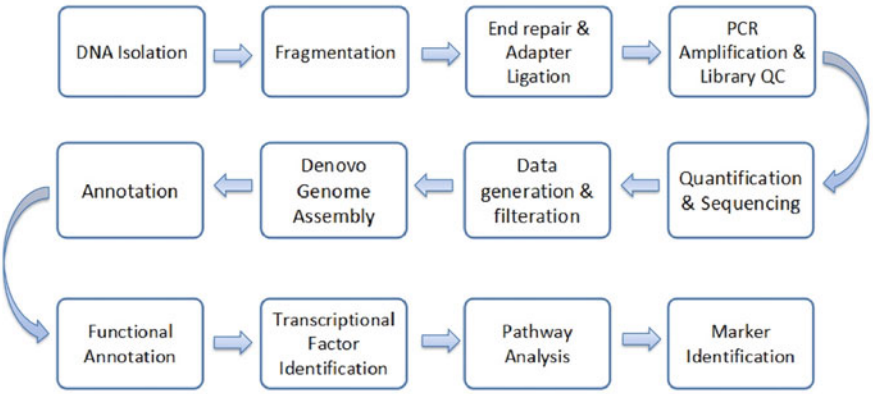

Fig. 16.3 Flowchart of whole genome analysis. Steps mentioned in this flowchart are common steps involved in shotgun/paired-end genome sequencing library preparation and its bio-informatics analysis

kinds of enzymes: DNA polymerase, Klenow-DNA polymerase, and Exonucleases. DNA polymerase synthesizes in 5′ to 3′ direction, Klenow-DNA polymerase synthesizes in 3′ to 5′ direction whereas exonuclease removes single ended DNA. Mixture of mentioned three enzymes coverts all the fragments into blunt ended form. Sequencing technology specific adapters are ligated followed by amplification of the DNA using adapter specific PCR primers. Prepared pool of randomly amplified DNA fragments is called library which is than subjected to quality check and quantification. Very small amounts (in nMol; or pMol concentrations) of library is used for sequencing and data generation. Generated data is analyzed by bio-informatics approaches where data is subjected to filtration in order to remove low quality reads and adapter sequences. High quality reads are used for preparing genome assembly by scaffolding and gap filling processes using various software and tools. Next step after Assembly is annotation for gene prediction using available databases. Last step of analysis is functional annotation of data against different databases like NR, Pfam database, KOG, Uniport, etc. In case of hybrid approach, data is generated separately for each method using the same DNA and is assembled together at assembly step of analysis, it is also called as hybrid assembly approach.

Complex denovo genome sequencing requires hybrid sequencing approach which means different types of data generation, i.e. shotgun sequencing along with long read sequencing. Long read sequencing usually generates 5–10 kb of reads whereas shotgun generates 150 bp–300 bp of reads (Illumina platforms). Long reads helps in assembly when used along with high quality shotgun sequencing reads. Currently Illumina sequencing along with PacBio Sequel is used for bigger genome sequencing projects. At initial stages, plant genome sequencing projects like Theobroma cacao genome, Apple genome, Grape genome, etc., were conducted

using Sanger sequencing and Roche 454 sequencing which generates 400 Mbp of single-read data with 300–500 bp of read lengths (Edwards and Batley 2010).

16.4 Transcriptomics of *Trigonella Foenum-graecum*

Fenugreek plant is known for its role as spices and medicinal properties since last 500 years (Chaudhary et al. 2018a). Fenugreek has been used to treat epilepsy, paralysis, gout, dropsy, chronic cough, diabetes, piles, sinus, lung congestion, inflammation, infections, as well as in hair treatment, breast enhancement, etc. in Indian and Chinese traditional medicine (Chaudhary et al. 2015; Mullaicharam et al. 2013). It is also reported to possess anti-microbial, anti-parasitic, anticancer, anti-fertility, anti-aging, galactagogue, and hypocholesterolaemic effects (Chaudhary et al. 2015). Such pronounced medicinal properties of this plants are virtue of pivotal biochemicals compounds and its secondary metabolites like saponins which are diosgenin, yamogenin, tigogenin, neotigogenin and gitogenin etc. Out of all the secondary metabolites reported till date form fenugreek, Diosgenin is the most important compound as it is used as precursor for synthesizing more than two hundred steroidal drugs such as contraceptives, testosterone, progesterone, and glucocorticoids (Zhou et al. 2019).

Advancement in next generation sequencing technologies have paved way large-scale sequencing of plants genome and transcriptome. For discovering new biosynthetic pathways, efficient methods are required to capture expression of all the transcripts varying with variable levels of target compounds. RNA-seq is a cost-effective, ultra-high-throughput deep sequencing technology providing a revolutionary advancement in transcriptome-scale sequencing. RNA-seq is competitive and in some aspects superior to Microarray technology especially in case of unavailability of genome sequencing data. Short reads generated as a result of direct sequencing of cDNAs are assembled into a transcription profile. For qualitative and quantitative characterization of transcriptomes and discovering new exons and splicing variants RNA-seq is a proven significant tool (Shi et al. 2011; Chikara et al. 2014).

16.5 Transcriptome Sequencing (RNA Sequencing) Methodology

RNA-seq methodology includes total RNA isolation from any part of plant system followed transcripts selection by Poly-A RNA selection or ribosomal RNA depletion. In eukaryotes poly-A tail is added as a part of post-transcriptional modification but in prokaryotes this process does not occur hence in eukaryotes any one of the methods can be used. Enriched mRNA will then be enzymatically fragmented and converted to cDNA using reverse transcription reaction. This process generates double stranded cDNA which is than subjected to adapter ligation and amplification by sequencing chemistry specific primers to generate RNA-seq library. After quality check and quantification process, library is subjected to sequencing and data

generation using fully automated sequencer. Generated raw data is than filtered and trimmed using bio-informatics tools to get high quality reads. These reads are assembled by denovo or reference-based approach to get final transcript assembly which is than subjected to functional annotation and pathway analysis. Reference based approach can be used in case of availability of genome sequence data but for fenugreek, denovo approach is used as it lacks genome sequencing data. Flow chart of RNA sequencing and analysis is available in Fig. 16.4. Generated transcripts from RNA-Seq data, were subjected to Gene ontology assignment and pathway mapping using Kyoto Encyclopedia of Genes and Genomes (KEGG) automatic annotation server of Genes and Genomes (KEGG). The KEGG Automatic Annotation Server (KAAS) provides functional annotation of genes by basic local alignment search tool (BLAST) comparisons against the manually curated KEGG GENES database. After this, all the contigs are assigned with the unique Enzyme Commission (EC) numbers based on the similarity hit against KEGG database using BLASTX (default threshold bit-score value). Distribution of contigs under the respective EC number is used to map them to the KEGG biochemical pathways.

16.6 RNA-seq of Fenugreek

In recent time, many transcriptome, i.e. RNA sequencing studies have been conducted to decode the complete set of genes involved in diosgenin biosynthesis (Vaidya et al. 2013; Mehrafarin et al. 2010) (Vaidya, Mehrafarin). Until, RNA sequencing was used, no much information was available about steroid (Diosgenin and other compounds) biosynthesis pathways except the fact that cholesterol and sitosterol are their cycloartenol-derived precursors. In 2010, Mehrafarin et al. have reported role of eleven important enzymes and probable pathway of cholesterol biosynthesis from acetyl-coA. Further investigating for the same, Vaidya et al. in 2013 have tried reporting complete steroid synthesis pathway by RNA sequencing approach. It was inferred that three pathways, namely glycolytic pathway, mevalonate pathway, and steroid biosynthesis pathway are involved in diosgenin biosynthesis. They concluded that diosgenin may be synthesized from squalene 2,3-oxide in two ways, (1) from lanosterol via the formation of cholesterol and (2) from cycloartenol via the formation of sitosterol. This was the first attempt to define the diosgenin biosynthesis pathway. They used SOLiD 4 Genome Analyzer for generating 42 million high quality reads from fenugreek plant from which 18,333 transcripts were functionally annotated.

16.7 Diosgenin Biosynthesis Pathway

Scientists have tried to map complete biosynthesis pathway of diosgenin through RNA-seq approach. Vaidya et al. got mapping of 27 transcript contigs on the glycolytic pathway from glucose to acetyl-coenzyme A and 17 of the contigs on the mevalonate pathway, which connects the glycolytic and steroid pathway (Vaidya

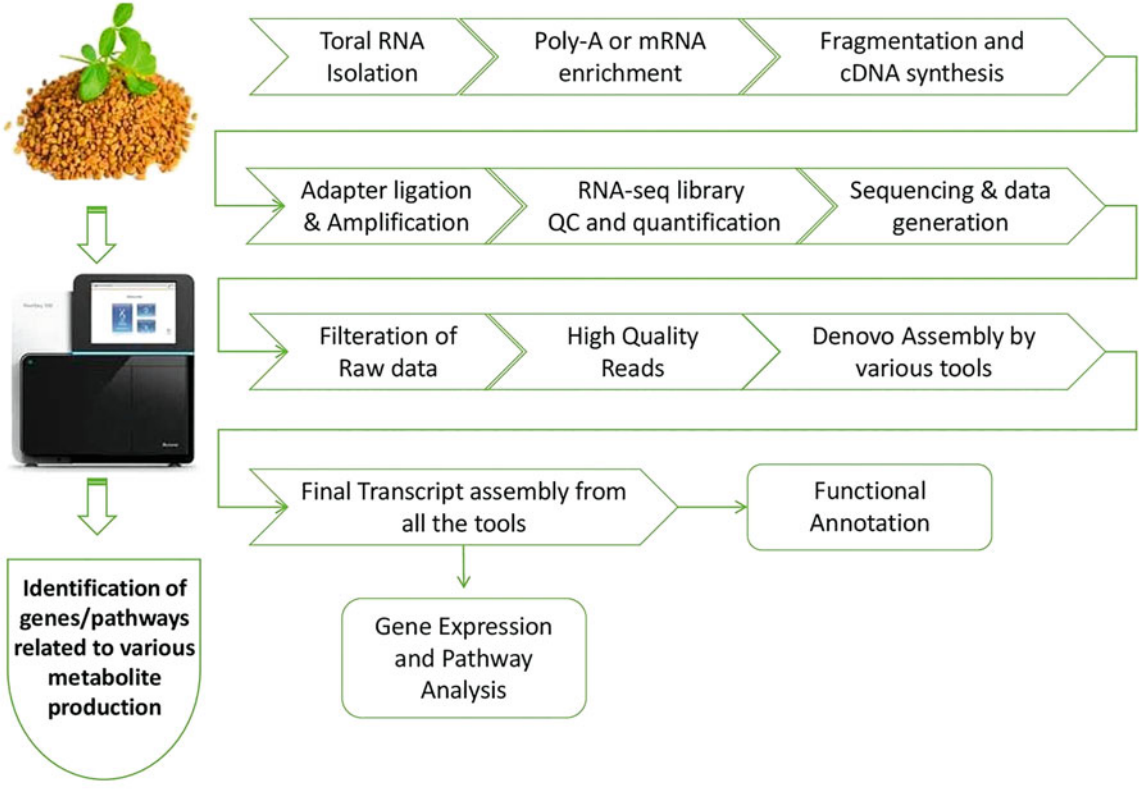

Fig. 16.4 Flowchart of RNA-seq analysis. This figure illustrates general steps involved in RNA-seq library preparation and its bio-informatics analysis. Picture of illumina sequencer shown is used just for representation of sequencing technology

et al. 2013). Based on the identified genes in studies reported, complete diosgenin biosynthesis pathway was mapped by Chaudhary et al. in 2015 (Chaudhary et al. 2015). They hypothesized the role of few important key regulator enzymes based on the RNA-seq data and confirmed the same through gene expression experiments. They targeted two important regulatory genes: *3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and sterol-3-glucosyl transferase (STRL)* and found their upregulation impacting on diosgenin yield confirming their role in diosgenin biosynthesis pathway. *HMGR* is key regulatory enzyme for isoprenoid or mevalonate biosynthetic route in plants that catalyzes the irreversible conversion of 3-hydroxy-3-methylglutaryl-CoA (HMG) to mevalonate whereas *STRL* plays important role in synthesis of cycloartenol to diosgenin via sitosterol. They reported up-regulation of both the genes from 3-fold to >20-fold and also the natural content of diosgenin from 0.5–0.9% to 1.1–1.8%, proving the hypothesis of possible pathway (Chaudhary et al. 2015). Further investigating the pathway in depth, Ciura et al. in 2017 revealed the intermediate steps of diosgenin synthesis from cycloartenol via cholesterol which are missing in earlier studies. They aimed at dissecting the fact about the diosgenin synthesis from squalene-2,3-oxide in two ways, (1) from lanosterol via the formation of cholesterol and (2) from cycloartenol via the formation of sitosterol, which was established in previous studies (Vaidya et al. 2013; Chaudhary et al. 2015; Mehrafarin et al. 2010). However, they could not obtain transcripts related to lanosterol synthase-an enzyme essential in this biosynthesis beginning from lanosterol from their RNA-seq data. Reason for this may be the similarity in the structure of the two enzymes, namely Cycloartenol synthase (CAS) and lanosterol synthase. These enzymes were found to carry 77–79% identical amino acid sequences in Arabidopsis thaliana (Suzuki et al. 2006; Umate 2015). They eliminated the possibility of the synthesis of diosgenin from lanosterol keeping only cycloartenol in pathway. By combining all the studies referred in above write up, Fig. 16.5 can be used to illustrate so far defined pathway of diosgenin biosynthesis. There can be still many steps involved in the biosynthesis pathway which may be revealed completely only if genome sequencing of the plant is available.

Diosgenin is pivotal product due to its medicinal applications and usage in pharma industry, it is commercially very important. Currently, certain wild species of Mexican yam (*Dioscorea* spp.) are used for diosgenin production. This kind of yams needs more time (at least 3 years) to accumulate high diosgenin levels (Rosser 1985) while fenugreek has can be a good alternative due to shorter growth cycle and low cost of production (Chaudhary et al. 2015). Therefore researchers all over the world, trying to enhance the natural diosgenin content of fenugreek to make it most effective by up-regulating important genes of the biosynthesis pathway. With the knowledge of the diosgenin biosynthesis pathway available through RNA-seq application, scientists have used external stimulator to enhance diosgenin content (Chaudhary et al. 2015; Ciura et al. 2017a). Both the studies have used Methyl jasmonate (MeJA) as external elicitator. Role of jasmonic acid, in elicitor-induced signal transduction pathway, was first described by Gundlach et al. It was first isolated from the plant pathogenic fungus *Lasiodiplodia theobromae* as plant growth inhibitor. It triggers plant defence mechanism by activating cascade of intracellular

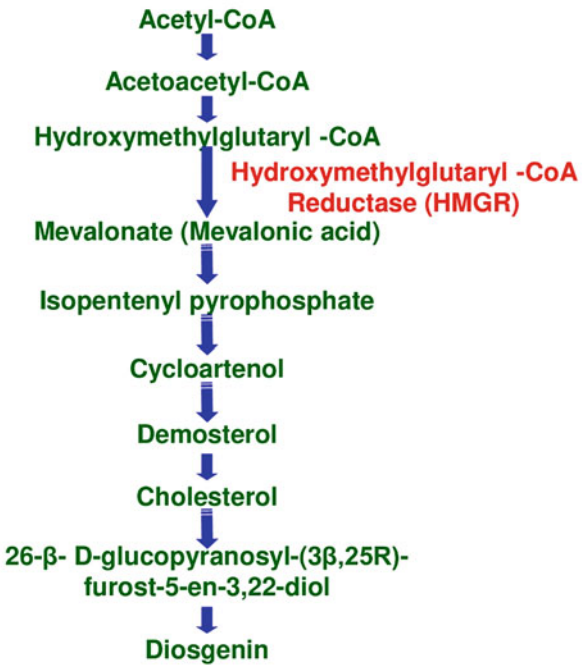

Fig. 16.5 Diosgenin biosynthesis pathway. This pathway depicts only few important steps of complete diosgenin biosynthesis pathway. This is schematic representation of complete pathway which includes many intermediate steps, compounds, and enzymes. This pathway is modified from the manuscript of Chaudhary et al. (2015) and modified on the bases of other studies and their outcomes. Broken arrows indicate multiple steps involved

signals. It also initiates de novo transcription of genes as phenylalanine ammonia-lyase (PAL), the key enzyme of the phenylpropanoid pathway leading to accumulation of anti-microbial phytoalexins (Gundlach et al. 1992). Apart from MeJA, cholesterol and squalene were also used to enhance the diosgenin content as both are intermediate product of the pathway (Ciura et al. 2017a). Adding cholesterol to suspension cultures of fenugreek has resulted positively, whereas treating hairy root cultures of fenugreek with cholesterol did not work (Khanna et al. 1975; Merkli et al. 1997).

16.8 Proteomics of *Trigonella foenum-graecum*

Systemic analysis of protein population in a confined environment like tissue, cell or sub-cellular compartment is called Proteomics studies. Proteins are important components of major signaling and biochemical pathways, proteomics studies are essential to reveal molecular mechanisms underlying plant growth, development, and interactions with the environment. Recent advancement in technologies have provided immense opportunities for high-throughput proteomics studies which have paved way beyond simple protein identification to analyzing various functional aspects like quantification, PTM, sub-cellular localization, and protein–protein interactions. Proteomics helps in studying highly complex and dynamic biological systems in simpler form. Qualitative and quantitative measurements of large

numbers of proteins have direct impact on cellular biochemistry which is possible to study by Proteomics. This can provide accurate analysis of cellular state or system changes during growth, development, and response to environmental factors. Though Genomics and transcriptomics are important to identify early-stage transmission from genome to cellular machinery mRNA levels varies with the abundance of cognate proteins. Though mRNA codes for proteins and some idea about their expression can be derived but it will not be accurate because of various alternate splicing and mRNA processing, one mRNA can produce many different proteins (Ideker et al. 2001). Proteins are present in a vast range of concentrations and their functions are defined by sub-cellular sorting, interaction with other molecules, and regulation by cellular and environmental signals. Proteomics research has reached to advanced levels in model micro-organisms such as *Escherichia coli* and yeast and in mammals such as humans but it is far behind in plants. But now plant proteomics is progressing rapidly due to availability of genome sequences of model plants like Arabidopsis, rice, and Poplar and the availability of enormous ESTs and gene indices for at least 31 plant species (http://www.tigr.org).

16.9 Methodology

Plants contain high amount of proteases, polysaccharides, starch, cell wall polyphenols, lipids, and various secondary metabolites, etc. which make them more complex and difficult for Proteomics studies compared to other organisms and mammals. Some important proteins are present in high concentration in some specific parts like ribulose bisphosphate carboxylase/oxygenase (Rubisco) in leaves and storage proteins in seeds leading to suppress other protein profiles. There is no universal procedure for all the proteins due to the diversity of protein abundance, size of proteins, charge, hydrophobicity, post-translational processing and modifications, and complexation with other molecules. Basic protocol used for protein precipitation is by combining TCA and acetone but it has its own drawbacks like pellet dissolving issue, hydrolysis of proteins by TCA, etc. (Damerval et al. 1986; Watson et al. 2003). Another method which has been shown to generate high quality protein extracts from a variety of plant species is phenol extraction method followed by precipitation with methanol and ammonium acetate (Hurkman and Tanaka 1986). This method is more time consuming and the pellet can be difficult to resolubilize. Over the period of time, various strategies have been developed to fractionate proteins into subproteomes based on biochemical, biophysical, and cellular properties. This has enhanced the coverage and detection of certain groups of proteins such as membrane proteins and low abundant proteins (Chen and Harmon 2006). A complied matrix of complete proteomics methods and techniques have been presented in Table 16.1 which is derived from a review article by Chen and Harmon (2006).

Table 16.1 Proteomics matrix. Major steps involved and analytical techniques/technologies used and their application in each step is mentioned

Steps involved	Method/tools/technologies used and their application
Sample preparation	1. TCA and acetone based method
	2. Phenol chloroform based method followed by precipitation with methanol and ammonium acetate
Protein separation	1. Electrophoresis: 1-D SDS-PAGE: commonly used 2-DE (IEF/SDS-PAGE): used for high-resolution profiling of proteins
	2. Liquid phase separation a. HPLC—most often used for peptide fragments b. MUDPIT—multidimensional protein identification technology
	3. Mass spectrometery a. MALDI-TOF MS—measures the masses of peptides derived from trypsinized parent protein b. ESI-MS/MS—useful for protein quantification c. MALDI-TOF/TOF and MALDIQTOF—used to generate high quality PMF data as well as MS/MS data
Protein identification	1. MALDI-TOF PMF—preferred method for protein identification because of the advantages of high-throughput, tolerance to salt, and low cost 2. HPLC ESI-MS/MS—used for routine protein identification 3. Nanoflow HPLC-MS—A new popular technique for plant proteomics
Protein quantification	1. ICAT (isotope-coded affinity tag)—most commonly used method 2. iTRAQ (isobaric tags for relative and absolute quantification): used for quantification of up to four samples simultaneously

16.10 Proteomics for Diosgenin

Proteomics studies on fenugreek plant were conducted mainly for diosgenin aspect. In a study conducted on fenugreek, plants were treated with methyl jasmonate (as an elicitor) and cholesterol considering it a precursor of steroids and steroidal saponins. This study was aimed at analyzing the proteome stress and its effect on steroidal saponin (diosgenin) biosynthesis. Proteins were separated by two-dimensional electrophoresis (2-DE) followed by identification using MALDI-ToF/ToF and database searches using Mascot search engine. Researchers found total 63 and 41 protein spots were deferentially expressed due to methyl jasmonate and cholesterol treatment, respectively. Identified proteins were classified into seven groups: photosynthesis, energy, metabolism, protein metabolism, secondary metabolism, stress and defense, and other. They also concluded that 18 proteins expressed in treatment-specific manner, whereas nine proteins were responsive to all treatments. Results showed over expression of proteins related to oxidative stress, defense, and secondary metabolism. Defense reaction at the proteome level as a response to stress was found to be elicitated by MeJA and cholesterol treatment. Authors found elevated levels of protease inhibitors, thioredoxin, pathogenesis-related protein, monodehydroascorbate reductase, hairpin-binding proteins, lectins, and chalcone isomerase in fenugreek plant treated with MeJA which finally led to enhanced

diosgenin synthesis while cholesterol treatment did not increase diosgenin content (Ciura et al. 2017b).

Apart from diosgenin related studies, proteomic approach can also be used to provide profiling and characterization of protein expression in diseased state or in response to drug treatment. Scientists explored protective effects of trigonelline in isoproterenol (ISO)-induced myocardial dysfunctions in adult rats and by proteomic approach to understand its mechanism of action. They used different concentrations of trigonelline on ISO-induced rats and investigated different indices including cardiac marker enzymes (creatine kinase-MB (CK-MB), glutamate pyruvate transaminase (SGPT), and lactate dehydrogenase (LDH)), lipid peroxidation, antioxidants, cardiac histology, and electrocardiogram. Proteomic analysis revealed the potential of trigonelline to prevent ISO-induced oxidative stress primarily through downregulation of two proteins, namely Hsp27 and aB-crystallin along with other pathways like, CaMKII inhibition (Panda et al. 2013). All the studies proved that proteomics can be very useful tool to study the systems biology but it needs support of transcriptomics and metabolomics studies to provide conclusive results.

16.11 Metabolomics of *Trigonella foenum-graecum*

Metabolomics is the study of metabolite profiles in a system (cell, tissue, or organism) under a given set of conditions. Diverse chemical nature of metabolites and in particular plant systems making metabolomics very challenging. Metabolites are the result of the interaction of the system's genome with its environment. They are not merely the end product of gene expression but also form part of the regulatory system in an integrated manner. Metabolomics can enable greater understanding of a biological system when studied in combination with proteomics, transcriptomics.

Fenugreek majorly contains secondary metabolites like saponins, flavonoids, and alkaloids and few free amino acids like 4-hydroxy lecucine and some volatile compounds like heptanoic acid, n-hexanol, dihydroactiniolide, dihydrobenzofuran, tetradecane, a-muurolene, b-elemene, and pentadecane. Fenugreek seeds contains 35% alkaloids, 17–50% Galactomannans, 10% flavonoids (100 mg/g of fenugreek seeds), 4.8% saponins, 0.02–0.05% volatile compounds and 0.2–0.9% diosgenin (Vaidya et al. 2013). Alkaloids, along with some other volatile compounds, are mainly responsible for the bitter taste and typical aroma of fenugreek (Chaudhary et al. 2018a). Most medicinal properties of fenugreek are related to its phytochemicals and secondary metabolites. Due to less research data available on fenugreek plants and complexity of the metabolome with a wide variety of chemically diverse compounds such as lipids, organic acids, carbohydrates, amino acids, nucleotides, and steroids, among others, complete metabolome profiling has not yet identified and reported.

In metabolomics, NMR (nuclear magnetic resonance) spectroscopy and MS (mass spectrometry) techniques are the most frequently used analytical tools. In addition, FTIR (Fourier transform infrared) approaches have been successfully used to investigate chemical response to plant inter species. For profiling phenolic

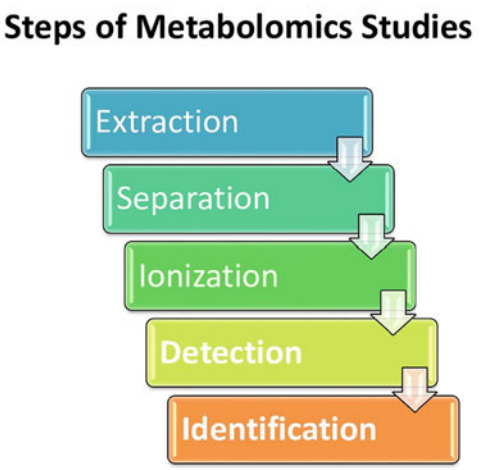

Fig. 16.6 Schematic representation of steps involved in Metabolomics studies

compounds in lignin biosynthesis, HPLC-UV (high-performance liquid chromatography) method along with statistical grouping was implemented (Rochfort 2005). MS can identify and quantify metabolites even at very low concentrations (femtomolar to attomolar) with high resolution, sensitivity, and dynamic range. MS based studies include sample preparation, extraction, capillary electrophoresis (CE), and/or chromatographic separation, introduction of sample for ionization process (charged molecules), and detection of possible metabolites on the basis of their mass-to-charge ratio (m/z) (Nalbantoglu 2019). Pictorial representation of metabolomics methods is given in Fig. 16.6.

16.12 Process of Metabolomics

Sample extraction: Based on hydrophilic and hydrophobic nature of the compounds, optimized methanol-water-chloroform combinations are applied for extraction procedure. Extraction process is usually done in two phases: first phase involves extraction with aqueous solvent (e.g., methanol-water) followed by extraction with a non-polar solvent (e.g., chloroform) of the centrifuged pellet in second phase. Solvent-free sample preparation/extraction method "solid-phase micro extraction (SPME)" is used for extraction of VOCs, which enables extraction of organic compounds from gaseous, aqueous, and solid materials.

Sample separation: Sample separation techniques like liquid chromatography (LC) and gas chromatography (GC) are used coupled with MS systems like GC-MS, HPLC-MS, UPLC-MS. Sometime, direct injection techniques are also used which includes direct infusion MS and direct analysis in real-time MS (DART-MS). For separation of non-polar compounds Reversed-phase LC using C18 columns is applied, while hydrophilic interaction chromatography (HILIC) is used for separation of polar compounds (Tolstikov and Fiehn 2002). For separation

of volatile organic compounds (VOCs) such as fatty acids and organic acids, GC-MS is widely used. Two important separation techniques LC/MS and GC-MS have different sensitivities from recovery and coverage point of view. For less polar bio molecules like: alkylsilyl derivatives, eicosanoids, essential oils, esters, perfumes, terpenes, waxes, volatiles, carotenoids, flavonoids, and lipids, GC-MS is preferred. Polar bio-molecules like: organic acids, organic amines, nucleosides, ionic species, nucleotides, and polyamines etc can be analyzed by LC-MS. Both the methods can be applied for alcohols, alkaloids, amino acids, catecholamines, fatty acids, phenolics, polar organics, prostaglandins, and steroids (Wilson and Walker 2010).

Ionization of metabolite: Post chromatographic separation step, samples are pumped through MS capillary to obtain positive or negative electrically charged ions in gas phase. Various ionization sources include electron ionization (EI), chemical ionization (CI), electrospray ionization source (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photo-ionization (APPI), and matrix assisted laser desorption ionization (MALDI) are applied based on polarity of the metabolite (Nalbantoglu 2019).

Detection of metabolite: Ionized bio-molecules create high-resolution mass spectrum composed of mass-to-charge (m/z) ratios of fragment ions which is detected by MS at sub-femtomole levels. Mass analyzers include time of flight (TOF), quadrupole time of flight (QTOF), quadrupole, ion trap, and orbitrap (Nalbantoglu 2019).

Data analysis and metabolite identification: For identification of metabolite of interest, large amounts of complex raw data involving specific metabolic signals are extracted from MS is analyzed in specialized bio-informatics methods and software. These methods can automatically perform processing of peak selection, assessment, and relative quantification, etc. Raw data is processed for background spectral filtering (noise elimination), retention time correction, appropriate peak assignment for the same compound (identification of matching m/z and assigning adducts appropriately), peak detection, peak alignment (matching peaks across multiple samples) and peak normalization (adjusting peak intensities and reducing analytical drift), and chromatogram alignment. After data is processed, data interpretation, and metabolite identification from mass spectrum can be used for functional interpretation, enrichment analysis, pathway analysis, and metabolite pathway networks mapping, etc.

16.13 Important Metabolites of Fenugreek

Galactomannans and amino acids: Galactomannans or mucilaginous fibers are predominantly hemicellulosic polysaccharides found in seeds of fenugreek and represent approximately 17–50% (Raghuram et al. 1994; Kochhar et al. 2006) of dry seed weight. Their function is to thicken the surface and they make major part of the cell walls of endosperm. Basic structure consists of a principal chain of 1,4-linked mannose (b-D-mannopyranosyl) units (Man) which are substituted by single galactose (D-galactopyranosyl) unit (Gal) 1,6-alinked at C-6 oxygen position n (Dilokpimol 2006; Scheller and Ulvskov 2010; Wang et al. 2012).

Galactomannans possess hydrophilic properties making them water-soluble molecules. Due to its high water binding capacity this molecule has better accessibility to enzymatic degradation in comparison to cellulosic microfibrils. Water retention capacity of galactomannan makes it extensively useful as a gum or gelling factor (Dilokpimol 2006). The varying proportion of Gal to Man units in the galactomannan gums in different fenugreek species may be possibly linked to the genotypic or environmental components (Dea and Morrison 1975) which also imparts various chemical characteristics such as water holding, thickening, gelling, emulsifying, suspending, binding, and production of films. Afore mentioned properties of galactomannans make them largely used multi-purpose materials in different industries (Srivastava and Kapoor 2005). Galactomannas are also assumed to be responsible in proper restrain and relief of diabetes (type 2) in both animals and humans (Vats et al. 2003). Galactomannan from fenugreek seeds is comparatively more soluble because of high degree in the weight ratio of d-galactosyl to d-mannosyl (galactose weight content—50%) compared to guar gum (33–40%), tara gum (25%) and locust bean gum (17–26%), causes the in comparison to the others (Reid and Meier 1970; Brummer et al. 2003).

16.14 Diosgenin

Diosgenin is the most widely used compound of pharmaceutical and nutraceutical industries. It is produced as steroidal saponin in fenugreek from cholesterol. Its molecular weight is 414.621 g mol^{-1} and is made up of 27 carbon, 42 hydrogen, and three oxygen atoms. Its scientific name is (3β,25R)-spirost-5-en-3-ol, $C_{27}H_{42}O_3$. Diosgenin is used as a precursor raw material for the production of hormones like testosterone, glucocorticoids, and progesterone and steroidal drugs (Raghuram et al. 1994). It has proven effect in the treatment of hypercholesterolemia and show anticancer and anti-aging activities, as well as cardioprotective and contraceptive properties. Apart from the few listed properties, diosgenin has been scientifically reported to have many other medicinal and clinical properties which are published in literature (Chaudhary et al. 2018a).

16.15 Fenugreek Metabolomics

There are limited studies conducted for fenugreek metabolomics with some focusing purely on saponins and some targeting complete metabolome. Major studies used fenugreek seeds as starting material but few of them used various other parts like leaves, stem, roots, etc. For extraction purpose, plant material is dried and grounded to fine powder followed by homogenization with methanol or ethanol in presence of sulfuric acid. The mixture is then subjected to hydrolysis at temperature $>120°$. Thus prepared hydrolysate is extracted thrice with petroleum ether or n-hexane. Dry residues are dissolved in suitable solution and also filtered in case of impurities. Analysis of the extracted metabolite is done through various techniques like HPLC,

UPLC-CID-MS/MS, GC-MS, etc. depending on the requirement. Usually many kinds of saponins are extracted together but some studies have reported methods for obtaining highly pure diosgenin along with its quantification methods (Chaudhary et al. 2018b; Trivedi et al. 2007).

Large-scale metabolomics-based MS experiments were conducted to reveal for compositional differences in *Trigonella* species. In results, scientists found a total of 93 chromatographic peaks from the examined samples pertaining diverse phytochemical classes, viz. flavonoids, steroid/triterpene saponins, nitrogenous dipeptides, phenolic acids, and fatty acids. Authors reported that the differences mainly originate from the content of C-flavonoids and saponins relevant secondary metabolite classes of *Trigonella* seed chemical composition which may be responsible for various traits of fenugreek (Farag et al. 2016).

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Part IV

Medicinal and Clinical Approaches of Fenugreek

Medicinal Properties of Hulba (Fenugreek) in Unani System of Medicine

17

Mohammad Zakir, Safiya Khanam, and Munawwar Husain Kazmi

Abstract

Hulba consists of the dried ripe seeds and/or whole herb of fenugreek (*Trigonella foenum-graecum* L. (Fabaceae). When seeds are used it is called Tukhm-i-Hulba and when whole plant is used it is known as Hulba or Methi. It is also known as Bahubeeja, Goat's horn, Greek hay, Menthi, Shamlit, Shamlid, etc. It is indigenous to the Mediterranean region, India, Indonesia and China and commonly cultivated in these countries. It is used as vegetable herb and its seeds used as spices in such countries. It is an annual aromatic herb, with a well developed taproot, leaves alternate and trifoliolate, flowers whitish, fruits are linear pods, glabrous and seeds oblong rhomboidal, yellowish brown to reddish brown in colour. It contains pharmacological active constituents like mucilage (25–45%), small amount of essential oil (0.01%) and a variety of secondary metabolites, like alkaloids and flavonoids. Recent studies have proved that it possesses anti-hypercholesterolemia activity, anti-hyperglycaemic activity and antioxidant activities. According to traditional medicine literature it is used to treat abdominal colic, bronchitis, indigestion, impotence, liver disorders, wounds, common cold, etc. As per Unani medicine it possesses several important actions like *Mundij* (concoctive), *Muhallil-i-Waram* (resolve inflammation), *Muqawwi-i-A'sab* (nervine tonic), etc. and used to treat *Falij* (hemiplegia), *Laqwa* (facial palsy), *Waja'al-Zahr* (backache), *Waram al-Tihal* (splenitis), etc.

Keywords

Fenugreek · Hulba · Medicinal properties · Tukhm Hulba · Unani · Waram al-Tihal

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17.1 Introduction

According to the Unani Medicine *Mizāj* (temperament) is a specific quality produced by action and reaction of opposite qualities of elements in a particular drug which are broken up in small particles in order to facilitate their mixing. When these components interact among themselves, a condition is produced, which is found in equal proportion in all the particles of the compound. This new formation is known as *Mizāj* (temperament) of the drug (Ibn Sīnā 2014), e.g. if we mix hot water into cold water it becomes moderate. Hot property and cold property act against each other and a new 'intermediate' property emerges that will be neither so hot nor so cold in such extent as it was earlier. This intermediate property is *Mizāj* (temperament) of the drug and represents the final property of the particular drug. Unani drugs have been divided into four *Mizāj* (temperament), i.e. *Hārr Yābis* (hot and dry), *Hārr Ratb* (hot and wet), *Barid Yābis* (cold and dry) and *Barid Ratb* (cold and wet) temperament (Razi 1991; Kabir al-Din and Muhammad 2014; Ahmad 1983).

Sihhat (health) is a state of the body where the *Mizāj* (temperament) of every organ is moderate in all aspects. *Akhlat* (humours) are fluids of the body that serve the functions of nutrition, growth and repair of the organs. *Khilt* (humour) is singular of *Akhlat* (humours) and there are four humours in the body. Every humour has its own temperament which is specific and unique to it. Dominance of a humour or disturbance in the normal proportion of the humour causes derangement of the temperament of the organ or body and leads to the deficiency or disease (Ibn Sīnā 2014; Nafis 1916; Arzani 1924, 1992). Morbidity of humour is caused by derangement of either its quantity or quality. Evacuation of morbid material accumulated in the body is necessary to restore health. To expel morbid material *Nudj* (concoction) procedure must be applied. The concoction is a process by which the morbid material is made fit/able to be expelled out or evacuated out of the body; the drugs used for this purpose are called *Mundij* (concoctive). *Ishāl* (purgation) is a process of purging or free evacuation of faeces by some drugs which are known as *Mushil* (purgative) (Ibn Sīnā 2014; Razi 1991; Anonymous 2012). In the management of any disease or to maintain homeostasis of the body single drug or compound formulation has been used since centuries. Hulba is an important Unani drug used internally as well as externally to treat many diseases. It is used as a single drug or in many compound formulations as an important ingredient.

17.2 Unani Concept of Treatment of Disease by Drugs

The history of the treatment of disease began with the use of herbs, and this fact is verified by the descriptions of various herbs in oldest available Materia Medica for the treatment. The first authentic Materia Medica was written by Greek physician Pedanios Dioscorides (*Disquridus*) in the first century AD. He has described nearly 600 drugs in this book. The famous Arabian physician Ibn Baytār (1190–1248 AD)

Table 17.1 Human temperament (*Mizāj Insānī*)

Mizāj (temperament)	*Dominance of humour*	*Kayfiyāt* (qualities)
Damwī al-Mizāj (sanguineous temperament)	*Dam* (sanguine)	*Hārr Raṭb* (hot and wet)
Balghamī al-Mizāj (phlegmatic temperament)	*Balgham* (phlegm)	*Bārid Raṭb* (cold and wet)
Safrāwī al-Mizāj (bilious temperament)	*Safra'* (yellow bile)	*Hārr Yābis* (hot and dry)
Sawdāwī al-Mizāj (melancholic temperament)	*Sawdā'* (black bile)	*Bārid Yābis* (cold and dry)

has written a Materia Medica '*Kitab-al-Jame al-Mufradat al-Advia wa al-Aghzia'* describing 1400 single drugs in details (Rafiq al-Din 1985).

The Unani system of medicine diagnoses and treats the patient as a single unit looking into their overall physical, mental and spiritual aspects. The basic fundamentals, diagnosis and treatment procedures of the system are based on scientific principles and holistic concepts of health and healing. The fundamental concept of the four elements (*Arkān Arba'a*), which are considered pillars of the life and known as elements is unique to the Unani system of medicine (USM). The fire (*Nār*), air (*Hawā'*), earth (*Arḍ*) and water (*Pānī*) are four basic categories of elements which are essentials for any living matter, i.e. plants and animals. These elements have specific qualities (*Kayfiyat*), i.e. hot and dry, hot and moist, cold and dry and cold and moist, respectively (Ibn Sīnā 2014; Qarshi 2014; Jurjani 2008).

Every plants, animals and human beings have their own temperament which is specific and unique for them. The temperament (*Mizāj*) is defined as a new state of matter having a quality different from that present in the elements before coming into combination. The Mizāj is a resultant uniform state or state of equilibrium or homeostasis emerging after the combination of more than one element. The temperament can be divided into two major categories, i.e. human temperament (*Mizāj Insānī*) and temperaments of the drugs (*Mizāj Advia*) (Ghani 2011; Ibn Rushd 1987).

According to the humoral (*Akhlat*) theory, human body contains four *Akhlat* (humours), *Dam* (sanguine), *Balgham* (phlegm), *Safra'* (yellow bile) and *Sawdā'* (black bile). These humours are collectively called *Akhlat Arba'a*. The temperament of human being depends upon the dominant humour (*khilt*) and is divided into four types, i.e. *Damwī* (sanguineous), *Balghamī* (phlegmatic), *Safrāwī* (bilious) and *Sawdāwī* (melancholic) temperament. Sanguineous temperament is the result of dominance of sanguine, likewise phlegmatic, bilious and melancholic temperament are due to the dominance of phlegm, yellow bile and black bile in the body, respectively. Sanguineous, phlegmatic, bilious and melancholic temperaments have specific qualities (*Kayfiyat*), i.e. hot and wet, cold and wet, hot and dry and cold and dry, respectively (Ibn Sīnā 2014; Qarshi 2014; Jurjani 2008) (Table 17.1; Fig. 17.1).

The temperament imparts distinctive qualities in the human being which are unique to the person and its homeostasis is required to maintain health. The

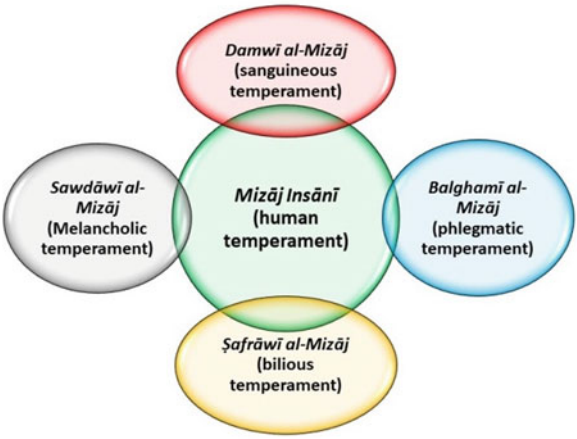

Fig. 17.1 Human temperament (*Mizāj Insānī*)

impairment in the temperament leads to the pathological changes in the body and results in development of the disease. According to the involvement of humours, diseases are classified into four categories, i.e. sanguineous ailments (*Amrād Damwiyya*), phlegmatic ailments (*Amrād Balghamiyya*), bilious ailments (*Amrād Safrāwiyya*) and melancholic ailments (*Amrād Sawdawiyya*). The restoration of the equilibrium of humours and rectification of deranged temperament can be done by various modes of treatments. The accepted modes are regimenal therapy (*'Ilāj bi'l-Tadb r*), dietotherapy (*'Ilāj bi'l-Ghidā'*), pharmacotherapy (*'Ilāj bi'l-Dawā'*) and surgery (*'Ilāj bi'l-Yad*) (Kabir al-Din and Muhammad 2014; Nafis 1916; Ibn Rushd 1987; Qarshi 2014; Anonymous 2016). It is essential that overall effect or combined qualities of the humours are in accordance with the temperament (*Mizāj*) of the individual for the maintenance of the health. The temperament has great importance in diagnosis and treatment and played a vital role in selection of suitable diet or drugs for effective management of a particular disease (Anonymous 2012).

The interaction of various elements present in the drug produces temperament of the drug, which determines the pharmacological activity of that drug in the human body. Out of four qualities, i.e. heat, coldness, moistness and dryness, the combination of two qualities exists in each temperament. Two qualities are primary, while other two are secondary qualities, i.e. heat and coldness are primary, while moistness and dryness secondary. The combination of a primary and a secondary quality exists in a particular temperament, e.g. hot and moist, hot and dry, cold and moist and cold and dry. During interaction between two elements if opposite qualities are equal in magnitude, then the resultant quality will be moderate in hotness and coldness and called moderate temperament. If we mix hot water into cold water it becomes moderate. Hot property and cold property act against each other and a new 'intermediate' property emerges that will be neither so hot nor so cold in such extent as it was earlier. The drugs can be broadly divided into five categories on the basis of the temperament, i.e. *Mu'tadil* (moderate), *Hārr Raṭb* (hot and moist), *Hārr Yābis* (hot

Table 17.2 Temperament of drugs (*Mizāj Advia*)

Mizāj (temperament)	*Kayfiyāt* (qualities)	Net quality
Mu'tadil	Hotness = coldness	Moderate
	Wetness = dryness	
Hārr Raṭb	Hotness > coldness	Hot and wet
	Wetness > dryness	
Bārid Raṭb	Coldness > hotness	Cold and wet
	Wetness > dryness	
Hārr Yābis	Hotness > coldness	Hot and dry
	Dryness > wetness	
Bārid Yābis	Coldness > hotness	Cold and dry
	Dryness > wetness	

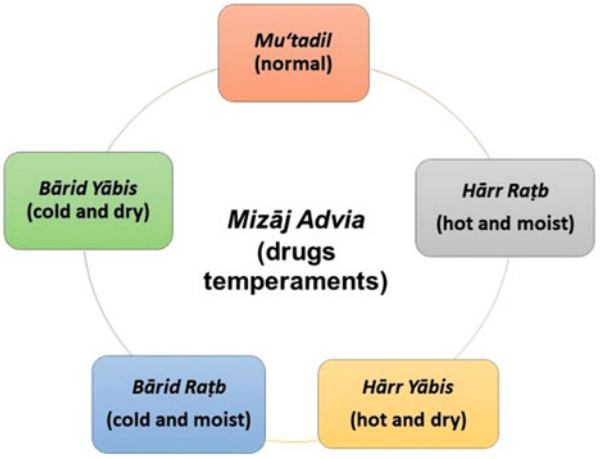

Fig. 17.2 Temperament of drugs (*Mizāj Advia*)

and dry), *Bārid Raṭb* (cold and moist) and *Bārid Yābis* (cold and dry) (Ghani 2011; Ibn Rushd 1987; Qureshi 1995) (Table 17.2; Fig. 17.2).

The categories of different temperaments of drugs are further classified into four grades (darjat) according to the severity of the basic qualities (*Kayfiyāt*).

1. *Darja Awwal* (first degree/imperceptible): The drugs of this degree, after entering the body and getting affected by its heat, produce heat (*Harārat*), coldness (*Burūdat*), moistness (*Rutūbat*) and dryness (*Yubūsat*) above normal, but their effects are not visible externally. However, when these drugs are used several times or in a higher dose, the resultant heat, coldness, moistness and dryness can be felt by the body.
2. *Darja Dom* (second degree/perceptible): the drugs of this degree, after entering the body produce an effect which can be felt even after first administration, albeit, it is not that severe to affect the daily routine in an adverse manner.
3. *Darja Som* (third degree/powerful): The drugs of this degree affect the daily routine adversely even after first administration and if used for several times or in

a higher dose, it may cause damage to the body. However, these drugs are not toxic in a strict sense.

4. *Darja Chaharum* (fourth degree/very powerful): The drugs of this category derange the body functions after administration. The drugs of this category must be used with caution. Generally these drugs are used after doing detoxification (Tadbīr) process to make them safe, detoxified drugs are called Mudabbar drugs (Ghani 2011; Ibn Rushd 1987).

In the treatment of a disease the drugs having qualities opposite to the prevailing one in the diseased organ are used on the principle of *'Ilāj bi'l Didd* (heteropathy). While treating the diseases caused by the morbidity of hot humours, drug possessing cold temperament must be used. In this case it is advised to start with single drugs having some nutritive values. Initially first degree drug should be used, if no response only, then switch to next level (Ghani 2011; Ibn Rushd 1987).

The temperament of Hulba seeds and leaves is hot and dry (*Hārr Yābis*); therefore, it is not recommended to those people who have hot temperament. Some Unani physician placed Hulba in second degree (*Darja Dom*), while some placed it in third degree (*Darja Som*) as per their experience. As it is placed in third degree so its long term use may cause severe adverse effects. Excessive eating of Hulba leaves is harmful for gonads and also produces headache. The knowledge of correct temperament with its category of a particular drug is necessary to prevent or counter the adverse effect in human body. The basic knowledge of temperament of the disease and drug is utmost necessity for safe and effective treatment of a disease (Ghani 2011; Ibn Rushd 1987).

17.3 Hulba (Fenugreek) in Unani System of Medicine

Seeds and leaves of Hulba are generally used in Unani system for the management of many disease conditions either internally or by external application. Some actions are common for both parts of the plant, while some actions and uses are different from each other. It is an annual aromatic herb, up to 60 cm high with a well developed taproot, leaves alternate and trifoliolate; flowers whitish, solitary, 12–15 mm long; fruits are linear pods, glabrous and seeds oblong rhomboidal, 3–5 mm long and 2–3 mm wide yellowish brown to reddish brown in colour (Anonymous 2001, 2009).

17.3.1 Geographical Distribution

It is indigenous to the Mediterranean region, India, Indonesia and China and cultivated in these countries. Moreover, this species is globally distributed in the Mediterranean regions, Europe, Asia, South Africa and Australia and introduced into America. In India the crop is cultivated throughout the country for culinary and medicinal purposes, as well as for fodder (Anonymous 2001, 2009; ENVIS 2020).

17.3.2 Description

The herb is a commonly used as a vegetable in India. It is green in colour, slightly bitter and aromatic herb. The whole plant and its seeds are used as a medicine in the USM (Ghani 2011; Rafiq al-Din 1985; Kabir al-Din 2007; Fazlullah 1877; Hasan 1865; Zaki 1890). Mucilage obtained from the seeds is also used as a medicine (Ghani 2011). It is nearly half metre tall, with blackish green leaves and white flower. It has orange colour triangular seed with sharp smell with bitter taste (Rafiq al-Din 1985; Fazlullah 1877; Ali 1860; Hasan 1865; Qarshi 1870; Zaki 1890). Shelf life of leaves and seeds is one year and three years, respectively (Rafiq al-Din 1985).

17.3.3 Selected Vernacular Names

Hulba (Ghani 2011; Kabir al-Din 1955), Methi, Fenugreek, Methi Dana (Ghani 2011), Shamleet (Kabir al-Din 1955), Shamleez (Kabir al-Din 1955; Ali 1860), Tukhm Shamleet, Bazrul Hulba, Tarah Shamleet, Baqlatul Hulba (Fazlullah 1877; Hasan 1865; Zaki 1890) (Fig. 17.3).

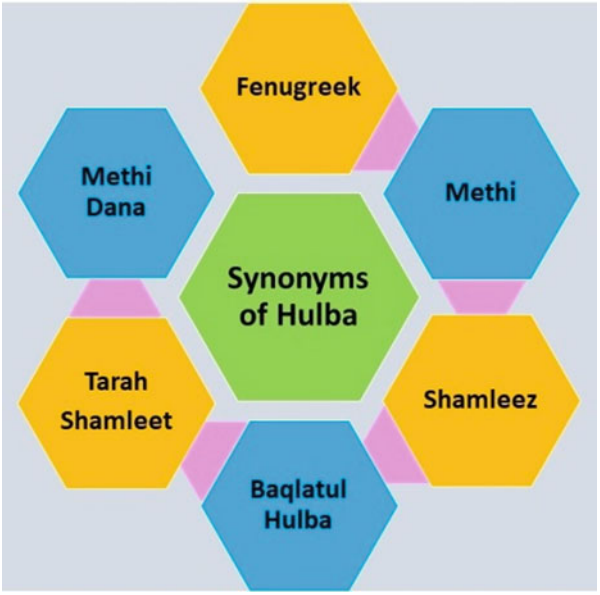

Fig. 17.3 Vernacular names of Hulba

17.4 Medicinal Properties of Hulba (Fenugreek) Leaves in Unani Medicine

17.4.1 *Mizāj* (Temperament)

Hārr Yābis in 2^0 grades (Ghani 2011; Kabir al-Din 1955; Fazlullah 1877; Hasan 1865; Zaki 1890), *Hārr Yābis* in 3^0 grades (Rafiq al-Din 1985) (Fig. 17.4).

17.4.2 *Afa'al* (Therapeutic Actions) of Hulba Leaves

Hulba leaves possess various pharmacological actions, i.e. *Dafi'a Amraz Barida* (cure diseases of cold temperaments), *Mundij* (concoctive), *Muhallil-i-Waram* (resolve inflammation) (Ghani 2011; Kabir al-Din 1955; Rafiq al-Din 1985; Fazlullah 1877; Ali 1860; Hasan 1865; Zaki 1890), *Jali* (detergent), *Muqawwi-i-A'sab* (nervine tonic) (Kabir al-Din 1955; Rafiq al-Din 1985), *Muqawwi-i-Badan* (general tonic) (Kabir al-Din 1955), *Muqawwi-i-Mi'da* (Stomachic), *Munaffith-i-Balgham* (Expectorant) (Kabir al-Din 1955; Rafiq al-Din 1985; Zaki 1890), *Kasir-i-Riyah* (Carminative) (Kabir al-Din 1955; Rafiq al-Din 1985; Fazlullah 1877; Zaki 1890), *Muqawwi-i-Bah* (Ghani 2011; Kabir al-Din 1955), *Muharrik-i-Bah* (stimulate libido) (Rafiq al-Din 1985), *Mulayyin* (laxative) (Fazlullah 1877; Ali 1860; Hasan 1865), *Mudirr-i-Bawl* (Diuretic) (Rafiq al-Din 1985; Ali 1860; Fazlullah 1877; Hasan 1865; Zaki 1890) and *Mudirr-i-Hayd* (Emmenogogue) (Ghani 2011; Kabir al-Din 1955; Ali 1860; Zaki 1890).

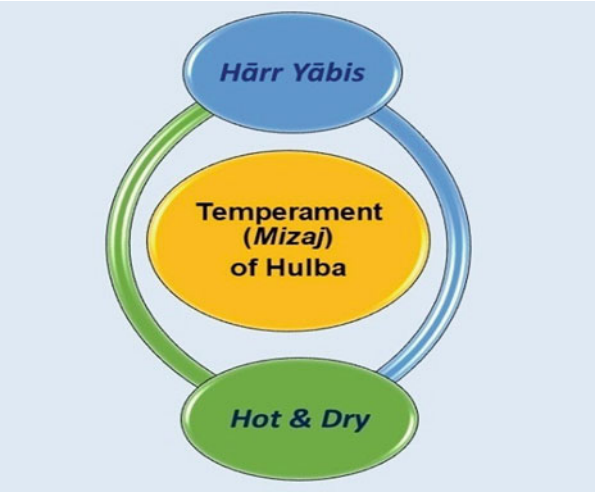

Fig. 17.4 *Mizāj* (temperament) of Hulba

17.4.3 *Nafa'e Khas* (Main Action)

The main action of Hulba seed is *Dafi'a Amraz Barida* (cure diseases of cold temperaments) due to its hot temperament (Ghani 2011; Kabir al-Din 1955; Rafiq al-Din 1985).

17.4.4 Therapeutic Uses of Hulba Leaves

Hulba leaves produce *Nudj* (concoction) in *Mādda* (matter) and moderate its *qiwam* (consistency) to make it easily extractable. It makes the stool aromatic but urine and sweat become smelly and this is specific action associated with it. It resolves *Riyah* (flatus) in the intestine and *waram* (swelling/inflammation) in the body. It is useful in treatment of *Amraz Barida* (diseases of cold temperaments) like *Sar'a* (epilepsy), *Falij* (hemiplegia), *Laqwa* (facial palsy), *Waja'al-Zahr* (backache), *Waram al-Tihal* (splenitis), *Dard-i Rahim* (spasmodic pain of uterus) and *Burudat-i-Mathana* (coldness of bladder) due to its *HārrMizāj* (hot temperament) (Ghani 2011; Fazlullah 1877; Hasan 1865; Zaki 1890). In cold temperament diseases like *Waja'al-Mafasil* (polyarthritis), *Waja'al-Zahr* (backache) and *Zu'f al-A'sab* (nervine debility), it is used by different methods (Kabir al-Din 1955).

Its external application as *Zimad* (paste) with Sirka (vinegar) resolves *Awrām Batina* (internal inflammation) and *Awrām Zahira* (external inflammation) and swelling of the organs (Ghani 2011; Kabir al-Din 1955; Fazlullah 1877; Hasan 1865; Zaki 1890). It absorbs the excessive secretion of stomach (*Jadhib Rutuba-i-Mi'da*) to protect the stomach from harmful secretions. It is very useful in *Bawāsīr-i badi* (nonbleeding piles). It is used to treat *Surfa* (cough), difficulty in breathing and *Dard-i-Jigar* (pain in liver). It relieves conditions of *Taqtir al-Bawl* (dribbling of urine). If it is taken before food along with honey and *Rai*/black mustard (*Brassica nigra* L.), it induces purgation. If taken with bread (roti) or in full stomach, then purgative action decreases and in some cases it produces *Qabz* (constipation) (Ghani 2011). It is used as an important ingredient in *Muqawwi-i-Bah* (aphrodisiac) compound formulations (Kabir al-Din 1955).

It is also used as external application to remove black heads and other skin pigmentation due to its *Jali* (detergent) action. It is used as *Tilā'* (liniment) alone or with some other suitable drugs to improve complexion. Its mucilage is used in *Dam'a* (epiphora), *Tarfa* (ecchymosis of the eyelids), *Āshob-i-Chashm* (acute conjunctivitis) as *Qutur* (eye drop). It is used as *Joshanda* (decoction) in *Sual* (cough) and *Dama* (bronchitis) with honey to make it sweet. *Joshanda* (decoction) is also used in *Ihtibas al-Tams* (amenorrhea) to remove morbid matters from the uterus. Sitz bath of the *Joshanda* (decoction) is also used in *Ihtibas al-Tams* (amenorrhea), (Kabir al-Din 1955) and *Dard-i Rahim* (spasmodic pain of uterus) (Hasan 1865; Zaki 1890) (Fig. 17.5).

Fig. 17.5 Medicinal properties of hulba (fenugreek) leaves

17.4.5 *Mudir Atharāt* (Adverse Effects)

It is harmful to *Unthayayn* (gonads) (Ghani 2011; Kabir al-Din 1955; Rafiq al-Din 1985; Ali 1860) and unsafe for people having *Hārr Mizāj* (hot temperament) (Ghani 2011; Fazlullah 1877; Hasan 1865; Zaki 1890). If taken for long duration it may cause *Suda* (headache). It may produce *Ghathayān* (nausea) if taken in empty stomach. Its excessive eating is also harmful for *Khusya* (testes) and produces smelly urine (Ghani 2011).

17.4.6 *Muslih* (Corrective)

Muslih are drugs which reduce or counter the adverse effect of a drug and used along with the main drug. Leaves of Kasni (*Cichorium intybus* L.), whole herb of Khurfa (*Portulaca oleracea* L.) and leaves of Palak (*Spinacia oleracea* L.) are used as a

muslih when it is used in people with *Hārr Mizāj* (hot temperament) (Ghani 2011; Kabir al-Din 1955; Rafiq al-Din 1985). Gajar (*Daucus carota* L.) is used for *Suda* (headache) and *Sirka* (vinegar) and *Ābkāma* (special liquid) can be used for *Ghathayān* (nausea). *Sikanjabīn Tursh* (compound formulation), Anisoon (*Pimpinella anisum* L.) and Sweet Anar (*Punica granatum* L.) may also be used in both types of adverse effects as well as for protection of *Unthayayn* (gonads). Roghan Badam Talkh (bitter almond oil) is corrective for protection of *Unthayayn* (gonads). To counter smelly urine *Sharab Rehani* (wine prepared with Rehan (*Ocimum basilicum* L.)) is useful (Ghani 2011). Some physician suggested *Sikanjabīn* (Ali 1860), Turshi (sour items) and Roughan Zard (clarified butter) as a corrective for it (Fazlullah 1877; Hasan 1865; Zaki 1890).

17.4.7 *Badal* (Alternative)

Badal are drugs which can be used in place of main drug if the main drug is not available. *Tukhm-i Methi* (seeds of *Trigonella foenum-graecum* L.) (Kabir al-Din 1955; Rafiq al-Din 1985), Salgham ka saag (leaves of *Brassica rapa* L.) (Fazlullah 1877; Hasan 1865; Zaki 1890) and *Bazr al Katan* (leaves of *Linum utitatissimum* L.) (Ali 1860) are mentioned in literature as alternative to Hulba leaves.

17.4.8 *Miqdar-i Khurak* (Dose)

The dose as mentioned by Unani physician is 3–5 g (Rafiq al-Din 1985), 5 g (Fazlullah 1877; Ali 1860; Hasan 1865) and 7 g (Ali 1860), the minimum dose is 3 g while maximum dose is 7 g.

17.5 Medicinal Properties of Hulba (Fenugreek) Seeds in Unani Medicine

17.5.1 *Mizāj* (Temperament)

Hārr Yābis in 3^0 grades (Ghani 2011; Rafiq al-Din 1985), *Hārr* 2^0 and *Yābis* 1^0 (Kabir al-Din 2007; Qarshi 1870) and *Hārr* 2^0 and *Yābis* 2^0 grades (Fazlullah 1877; Hasan 1865; Zaki 1890).

17.5.2 *Afa'al* (Therapeutic Actions) of Hulba Seeds

Hulba seeds possess the following pharmacological actions, i.e. *Muhallil-i-Waram* (resolve inflammation), *Kasir-i-Riyah* (carminative) (Ghani 2011: Rafiq al-Din 1985; Fazlullah 1877; Ali 1860; Hasan 1865; Zaki 1890), *Mundij* (concoctive) (Ghani 2011; Rafiq al-Din 1985), *Jali* (detergent), *Muqawwi-i-Mi'da* (stomachic),

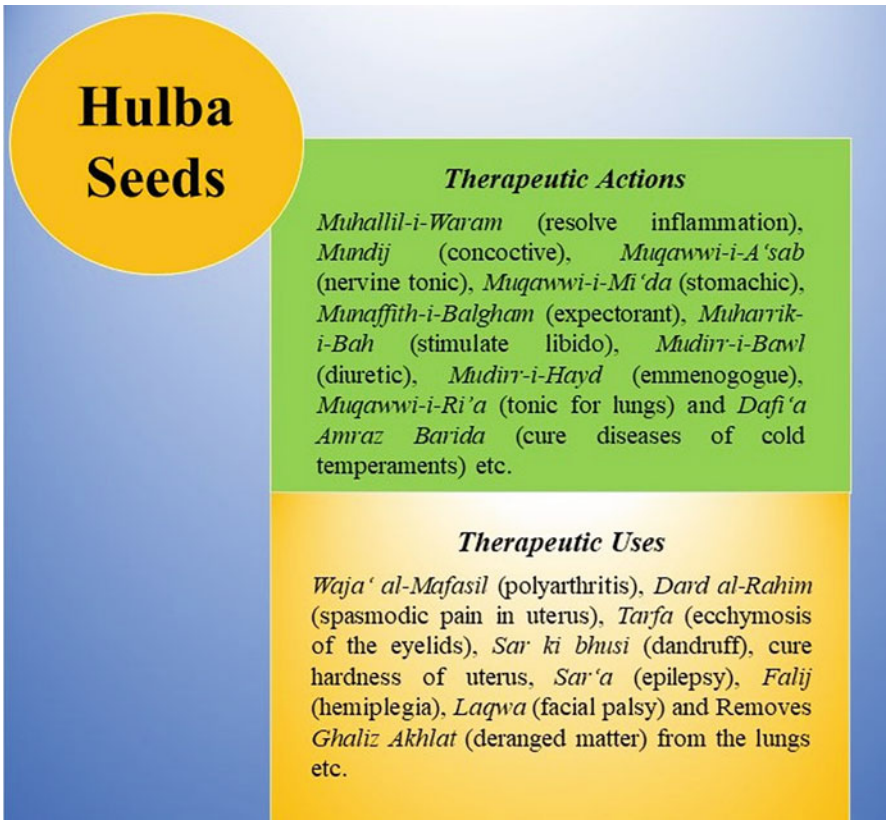

Fig. 17.6 Medicinal properties of hulba (Fenugreek) seeds

Munaffith-i-Balgham (expectorant), *Muharrik-i-Bah* (stimulate libido) (Rafiq al-Din 1985), *Muqawwi-i-A'sab* (nervine tonic) (Ghani 2011; Kabir al-Din 1955; Rafiq al-Din 1985), *Mulayyin* (laxative) (Rafiq al-Din 1985; Hasan 1865), *Mudirr-i-Hayd* (emmenogogue) (Ghani 2011; Kabir al-Din 2007; Fazlullah 1877; Zaki 1890), *Muqawwi-i-Ri'a* (lungs tonic) (Ali 1860), *Mudirr-i-Bawl* (diuretic), *Dafi'a Amrad Barida* (diseases of cold temperaments) (Ghani 2011; Fazlullah 1877; Hasan 1865; Zaki 1890) (Fig. 17.6).

17.5.3 *Nafa'e Khas* (Main Action)

The main action of Hulba seed is *Dafi'a Amraz Barida* (cure diseases of cold temperaments) due to its hot temperament (Rafiq al-Din 1985).

17.5.4 Therapeutic Uses of Hulba Seeds

The seeds of Hulba are very useful in removing inflammation produced by light heat. Its *Joshanda* (decoction) with honey removes *Ghaliz Akhlat* (deranged matter) from the lungs (Ghani 2011; Kabir al-Din 2007; Qarshi 1870). It stimulates *Quwwat-i-Bah* (sexual power) due to presence of *Rutubat Fadliyya* (excessive fluid). It is used in *Tarfa* (ecchymosis of the eyelids) due to its *Jali* (detergent), *Mulayyin* (laxative) and *Muhallil-i-Waram* (resolve inflammation) actions (Kabir al-Din 2007; Qarshi 1870). It is used in treatment of *Sar ki bhusi* (dandruff), *Dard al-Rahim* (spasmodic pain in uterus). It is used in case of blockade of the route of uterus due to *Khushki* (dryness), because it induces *Talayyun* (laxation) and *Irkha* (flaccidity) and removes internal *Mawād* (matter) due to its *Jali* (detergent) and *Mulayyin* (laxative) action (Kabir al-Din 2007). Sitz bath with its *Joshanda* (decoction) is used to relieve *Dard al-Rahim* (spasmodic pain in uterus) and hardness of uterus (Qarshi 1870).

Its *Zimad* (local application of paste) is used to remove swelling and inflammation because of its *Muhallil-i-Waram* (resolve inflammation) action. It also relieves earache when applied locally as *Zimad* (paste) (Ghani 2011; Fazlullah 1877; Zaki 1890). It is applied locally as liniment on checks to improve blood circulation and improve complexion due to its *Jali* (detergent) action (Ali 1860). It is used to treat *Sar'a* (epilepsy), *Falij* (hemiplegia), *Laqwa* (facial palsy) and *Waja'al-Mafasil* (polyarthritis) due to its *Dafi'a Amrad Barida* (cure diseases of cold temperaments) action (Ghani 2011; Fazlullah 1877; Hasan 1865; Zaki 1890). Its *Joshānda* (decoction) with honey is used in *Qabz* (constipation), *Sual* (cough), *Bawasir* (piles) and *Awrām Bātina* (internal inflammation). Its *Joshānda* (decoction) induces vomiting. Its *Zimad* (paste) on breast suppresses the lactation (Ghani 2011).

17.5.5 *Mudir Atharāt* (Adverse Effects)

It is harmful to *Unthayayn* (gonads) (Ghani 2011; Rafiq al-Din 1985; Ali 1860) and unsafe for people having *Hārr Mizāj* (hot temperament) (Ghani 2011; Fazlullah 1877; Hasan 1865; Zaki 1890).

17.5.6 *Muslih* (Corrective)

The leaves of Palak (*Spinacia oleracea* L.) and Khurfa (*Portulaca oleracea* L.) (Rafiq al-Din 1985), *Sikanjabīn* (Ghani 2011; Ali 1860), Anisoon (*Pimpinella anisum* L.) (Ghani 2011; Rafiq al-Din 1985), Berg Kasni (leaves of *Cichorium intybus* L.) (Rafiq al-Din 1985), Roughan Zard (clarified butter) and other Roughan (oil) (Fazlullah 1877; Zaki 1890) are mentioned as corrective to counter the adverse effect of it.

17.5.7 *Badal* (Alternative)

Methi (leaves of *Trigonella foenum-graecum* L.) and *Bazr al-Katan* (seeds of *Linum utitatissimum* L.) are mentioned in literature as alternative to Hulba seeds (Ghani 2011; Fazlullah 1877; Ali 1860; Hasan 1865; Zaki 1890), Iklilul malik (*Trigonella uncata* Boiss.) (Ghani 2011).

17.5.8 *Miqdar-i Khurak* (Dose)

The doses of seeds as mentioned by Unani physician are 3–5 g (Kabir al-Din 1955; Rafiq al-Din 1985), 5 g (Fazlullah 1877; Hasan 1865; Zaki 1890) and 7 g (Ghani 2011; Ali 1860).

17.6 Compound Formulations Containing Hulba (Fenugreek) as an Ingredient

In Unani Medicine, drugs from plant, mineral and animal origin have been used either as single entity or in combination of more than one drug in specific proportion mentioned in Unani Pharmacopoeias and National formularies. If more than one drug is mixed in specific proportion and method, then it is called compound formulation. The following compound formulations contain Hulba (fenugreek) as an ingredient in it. The actions of the formulation and their therapeutic effects are also mentioned with each:

- Shiyaf-i-Kundur has *Mujaffif* (desiccant) action and used to treat *Buthūr-i-Chashm* (eye eruptions) (NFUM 2007).
- Qairooti-i-Arad-e-Baqla has *Muhallil-i-Waram* (anti-inflammation) action and used to treat *Dhāt al-Janb* (pleurisy) (NFUM 2007).
- Zimad-i-Waram Kulya Qawi has *Muhallil-i-Waram* (resolvent of inflammation) action and used to treat *Waram al-Kulya Hādd* (acute nephritis) (NFUM 2007).
- Laooq Hulba has *Mujaffif-i-Balgham* (desiccant to phlegm) action and used to treat *Buhha al-Sawt* (hoarseness of voice) and *Dhīq al-Nafas* (Asthmatic bronchitis) (NFUM 2006).
- Qairooti Arad Karasna has *Muhallil-i-Waram* (resolvent of inflammation) action and used in treatment of *Dhāt al-Janb* (pleurisy), *Dhāt al-Ri'a* (pneumonia), *Dhāt al-Sadr* (pleuritis) and *Dhāt al-'Ard* (Mediastinal pleuritis) (NFUM 2006).
- Majoon Murawwehul Arwah has *Muqawwi-i Badan* (general tonic), *Muqawwi-i-A'sab* (Nervine tonic) and *Muqawwi-i-Bah* (Aphrodisiac) actions and used in treatment of *Zu'f al-Bah* (loss of libido) and *Zu'f al-A'da' Ra'isa* (weakness of vital organ) (NFUM 2008).
- Marham-i-Dakhliyun has *Muhallil-i-Waram* (resolvent of inflammation) action and used to treat *Zu'fal-Rahim* (weakness of uterus), *Waram al-Rahim* (metritis) and *Imtilā' Rahim* (uterine congestion) (NFUM 2008).

- Marham-i-Dakhliyun Murakkab has *Dafi'-i-Waram al-Rahim* (resolvent to metritis), *Muqawwi-i-Rahim* (Uterotonic) actions and used in treatment of *Waram al-Rahim* (metritis), *Salaba al-Rahim* (hardening of uterus) and *Zu'f al-Rahim* (weakness of uterus) (NFUM 2011).
- Safoof Gesu Daraaz have *Mutawwil-i-Sha'r* (hair elongator) and *Munbit-i-Sha'r* (trichogen/hair grower) actions and used in *Intithār al-Sha'r* (ptylosis/hair fall) (NFUM 2011).

17.7 Conclusion

The USM diagnoses and treats the patients looking into their overall physical, mental and spiritual aspects. The fundamentals, diagnosis and treatment procedures of the system are based on scientific principles and holistic concepts of health and healing. The *Mizāj* (temperament) has great importance in diagnosis and treatment and played a vital role in selection of suitable diet or drugs for effective management of a particular disease. Hulba (*Trigonella foenum-graecum* L.) is an important drug of USM and has many important pharmacological actions, i.e. *Dafi'a Amraz Barida* (cure diseases of cold temperaments), *Jali* (detergent), *Kasir-i-Riyah* (carminative), *Mudirr-i-Bawl* (diuretic), *Mudirr-i-Hayd* (emmenogogue), *Muhallil-i-Waram* (resolve inflammation), *Mulayyin* (laxative), *Munaffith-i-Balgham* (expectorant), *Mundij* (concoctive), *Muqawwi-i-A'sab* (nervine tonic) and *Muqawwi-i-Mi'da* (stomachic).

It is commonly used to maintain health and to treat many diseases like *Āshob-i-Chashm* (acute conjunctivitis), *Awrām Bātina* (internal inflammation), *Awrām Zahira* (external inflammation), *Bawasir* (piles), *Dam'a* (epiphora), *Dard-i Rahim* (spasmodic pain of uterus), *Falij* (hemiplegia), *Ihtibas al-Tams* (amenorrhea), *Istisqa'* (ascites), *Laqwa* (facial palsy), *Qabz* (constipation), *Sar'a* (epilepsy), *Sual* (cough), *Tarfa* (ecchymosis of the eyelids), *Waja'al-Mafasil* (polyarthritis), *Waja'al-Zahr* (backache), *Waram al-Tihal* (splenitis) and *Zu'f al-A'sab* (nervine debility) effectively. It is used in natural form so human body accepts, digests and metabolizes it easily without producing adverse effects on different organs of the body. Its pharmacological action can be validated by experimental pharmacology and clinical trial to generate scientific data. Hulba (fenugreek) is used since centuries for the above mentioned purpose effectively without failure, which stressed the efficiency of natural drugs in general and Hulba in particular. The medicinal properties of Hulba (Fenugreek) discussed in this chapter have shown that drugs from natural source can be used for effective and safe management of many diseases. The uses of chemical base drugs have safety concerns and should be avoided as much as possible. The use of natural drugs and diet recommended by Unani can provide an effective and safe alternative to conventional therapy.

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Fenugreek: A Wonder Spice with Versatile Pharmacological Activities and Clinical Applications

18

Pundarikakshudu Kilambi and Priya A. Shah

Abstract

Fenugreek (*Trigonella foenum-graecum*) is commonly known as methi and used as a regular spice and leafy vegetable in India. It attracted the attention of researchers' world over due to the structural diversity of chemicals present in it and a plethora of their pharmacological activities. It has been proved to be effective in treating metabolic disorders, analgesia and arthritis, inflammation, gastric ulcers, and having cytotoxic activity against a number of cancer cell lines. Trigonelline, 4-hydroxyisoleucine, diosgenin, and galactomannans exhibited strong anti-diabetic, anti-hyper-lipidemic, analgesic, and anti-cancer activities. Commercial processes and scale up methods were developed to prepare extracts rich in the above constituents. It is really amazing to note that diosgenin, which has been commercially used as the starting material for the synthesis of various steroid drugs, emerged as a molecule effective on breast, colon, and liver cancers. The analgesic and anti-pyretic activities of fenugreek are worth further studies as very few plants have this type of activity. 4-hydroxyisoleucine is a unique amino acid which is not found in many plants. Its anti-diabetic activity by sensitizing insulin, increasing insulin secretion, is amazing as this would render fenugreek's anti-diabetic activity multipronged. Similarly, trigonelline, the alkaloid present in fenugreek seeds has strong anti-diabetic and analgesic activity. Sugaheal, a standardized fenugreek seed extract (rich in 4- hydroxy isoleucine and Trigonelline) is available in the market as an antidiabetic herbal medicine. Fenfuro, a standardized extract of fenugreek with high concentration furostan saponins is also effective in diabetes. The water soluble fiber rich in galactomannans has anti-diabetic, lipid-lowering activities. Thus every chemical/group of chemicals present in fenugreek and its chemical ingredients have potent pharmacological activities. More focused approach and co-ordinated

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efforts from national and international scientific and medical research community and pharmaceutical entrepreneurs are expected to bring out therapeutically potent products from this spice.

Keywords

Fenugreek · *Trigonella foenum-graecum* · Saponins · Galactomannans · Phenols · Flavonoids · Diosgenin · Trigonelline · 4-hydroxyisoleucine · Diabetes · Hypolipidemic activity · Anti-cancer activity · Anti-oxidant activity · Analgesic activity · Anti-inflammatory activity

Abbreviations

4-HIL	4-Hydroxyisoleucine
5-LOX	5-Lipoxigenase
ABTS	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ASP	Aspartate transaminase
AST	Aspartate amino transferase
CAT	Catalase
Cdk	Cyclin dependent kinase
CFA	Complete Freund's adjuvant
COX	Cyclooxygenase
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylene-diamine-tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
FADD	Fas-associated protein with death domain
FRAP	The ferric reducing/antioxidant power
GH	Growth hormone
GLUT4	Glucose transporter type 4
GPx	Glutathione peroxidase
GSH	Glutatahione
HBA1c	Glycosylated or glycated hemoglobin
HDL-C	High-density lipoprotein cholesterol
HMG-CoAR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
i.p.	Intra-peritoneal
IgE	Immunoglobulin E
IGF-1	Insulin-like growth factor 1
IL	Interleukin
IRS-2 mRNA	Insulin receptor substrate 2 mRNA
JAK	Janus kinase
LDL	Low density lipoprotein

LDL-C	Low density lipoprotein cholesterol
LPO	Lipid peroxidase
MPO	Myeloperoxidase
MTT 3	(4, 5-di methyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B-cell
P2X	Purinoreceptor 2X
PBMN	Peripheral blood mononuclear cells
PCNA	Proliferating-cell nuclear antigen
PGE2	Prostaglandin E2
PI3-K	Phosphatidylinositol-3-kinase
PPAR	Peroxisome proliferator activated receptor
PPARγ	Peroxisome proliferator activated receptor γ
RANKL	Receptor activator of nuclear factor kappa-B ligand
RBC	Red blood cell count
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SGPT	Serum glutamic pyruvic transaminase
SOD	Superoxide dismutage
STAT3	Signal transducer and activator of transcription 3
STZ	Streptozotocin
T2DM	Type 2 diabetes mellitus
TFG	*Trigonella foenum-graecum*
TG	Triglyceride
TNF	Tumor necrosis factor
VLDL	Very low density lipoprotein
WBC	White blood cell count

18.1 Introduction

Fenugreek (*Trigonella foenum-graecum*, Family Fabaceae) commonly known as "Methi" is one of the important spices which has been known to the oldest Egyptian, Greek, and Indian Civilizations. Both the leaves and seeds have been employed in incenses, fumigation, and for expulsion of placenta after child birth (Rouk and Hailu 1963; Rosengarten 1969), as galactogogue and for relief of menstrual cramps (Howard 1987).

In Ayurveda it is being used to mitigate excess of kaph (Phlegm) and Vat (Wind) and recommended for the treatment of cold, influenza, hay fever, sinusitis (Anon. 2013), mouth ulcers, chapped lips, arthritis, spleen, and liver enlargement (Acharya et al. 2006).

India accounts for around 50% of world's fenugreek production of which 90% is consumed domestically (Edison 1995). Morocco, China, Pakistan, Spain, Tunisia, Turkey, Lebanon, Israel, Egypt, Ethiopia, Kenya, Tanzania are other countries that produce fenugreek (Smith 1982; Edison 1995). "Kasuri Methi" is very famous for its

appetizing fragrance and it is used in culinary preparations (Brar et al. 2013). In India it is regularly consumed as a leafy vegetable, in the preparation of rotis, wet and dry snacks, pickles, chutneys, etc.

A lot of work has been done on the multiple therapeutic potential of fenugreek during the last 40 years. It was amazing that this common food spice emerged out to be a power house of structurally divergent chemical molecules which were proved to be effective in a wide array of diseases ranging from anti-microbial activity to anti-cancer activity. Both the leaves and seeds have shown a number of therapeutic activities. Fenugreek has been investigated for its various pharmacological activities in a number of laboratories in different countries the world over. A number of reviews have appeared at regular intervals on the therapeutic potential of fenugreek. Quite a number of methods have been patented to prepare standardized fenugreek extracts with different chemical profiles and therapeutic claims.

An effort was made to analyze and sift through the scientific data available on the chemical composition, pharmacological activities, toxicity studies, and clinical trials of fenugreek. It is difficult to incorporate the data from innumerable number of scientific works which are sometimes contradictory or non-homogenous. This review is an effort to present a brief account of the chemical, pharmacological, and clinical profile of fenugreek.

18.2 Phytochemical Composition of Fenugreek (*Trigonella foenum-graecum*)

Fenugreek can be considered a treasure house of phytochemicals as it is a rich source of vitamins, minerals, primary metabolites like proteins, amino acids, lipids, and polysaccharides and secondary metabolites like alkaloids, flavonoids, saponins, phenols, flavonoids, and flavonoid glycosides.

Fenugreek seeds show the presence of several alkaloids such as trigonelline (Fig. 18.1a) trimethylamine, neurin, choline, gentianine, carpaine, and betaine. The amino acids present in the seed of this plant are isoleucine, 4-hydroxyisoleucine, histidine, leucine, lysine, L-tryptophan, and arginine.

Leaves were reported to contain vitamin C, β-carotene, thiamine, riboflavin, nicotinic acid, and folic acid. The minerals present in leaves include calcium, zinc, iron, phosphorous, riboflavin, carotene, thiamine, niacin, and vitamin C (Rao 2003; Srinivasan 2006).

The primary metabolite content in fenugreek as per US Department of Agriculture (2012) is 23–26% protein, 6–7% fat, and 58% carbohydrates of which about 25% is dietary fiber.

Endosperm was reported to have the highest amount of (4.63 g/100 g) saponins as well as proteins (43.8 g/100 g) that include globulin, histidine, and 4-hydroxy isoleucine (Fig. 18.1b) husk has the highest amount of polyphenols (Madhava Naidu et al. 2011; Işıklı and Karababa 2005).

Aljasass and Al-Jasser (2012) found endosperm to contain 20–30% protein and 35% alkaloids, primarily trigonelline; and many vital vitamins such as vitamin A

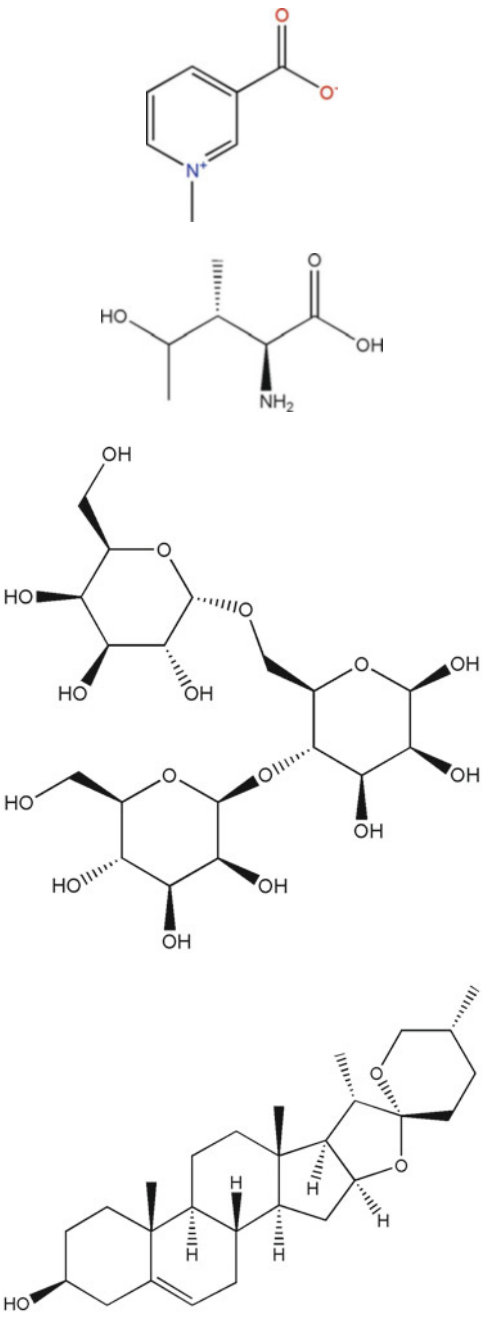
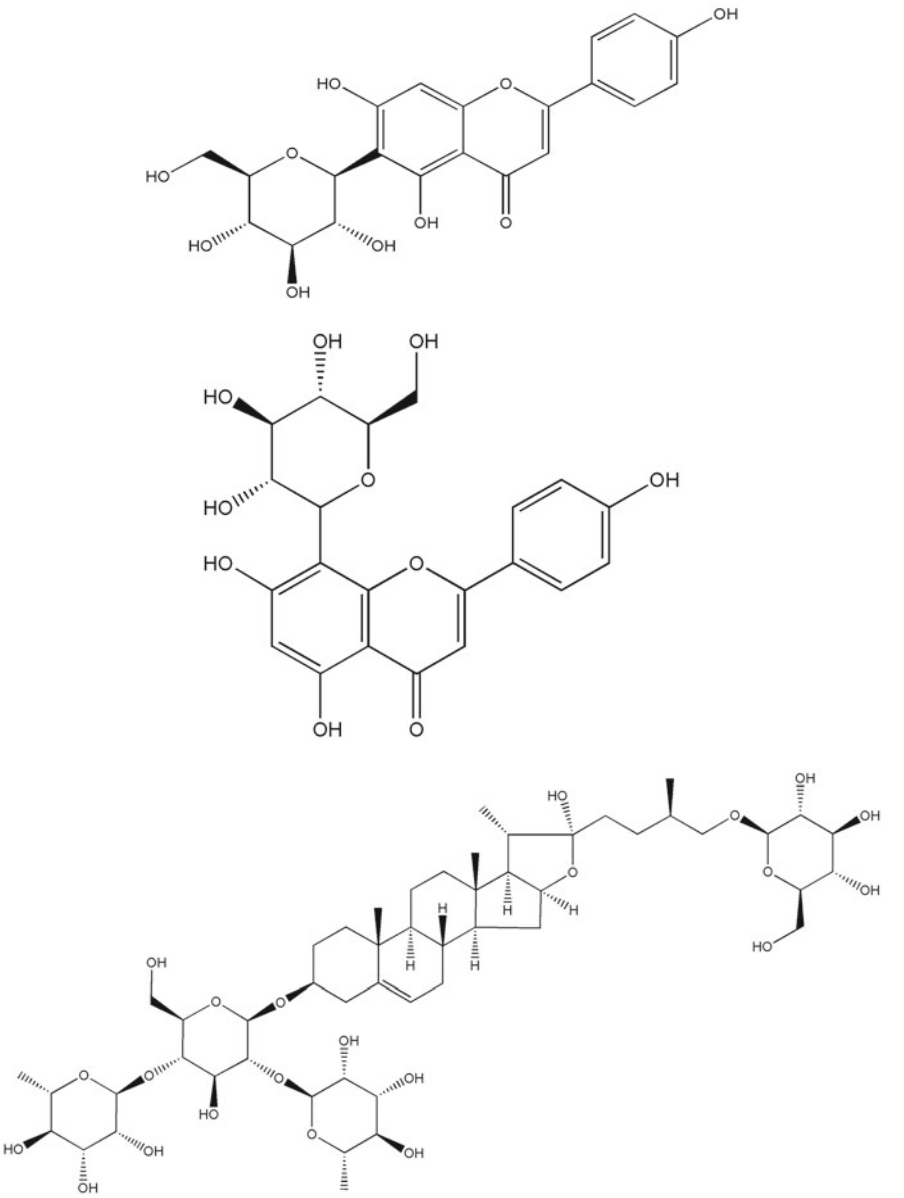


Fig. 18.1 (**a**) Structure of Trigonelline, (**b**) structure of 4-hydroxyisoleucine, (**c**) structure of galactomannan, (**d**) structure of diosgenin, (**e**) structure of isovitexin, (**f**) structure of vitexin, (**g**) structure of protodioscin, and (**h**) structure of trigonelloside C



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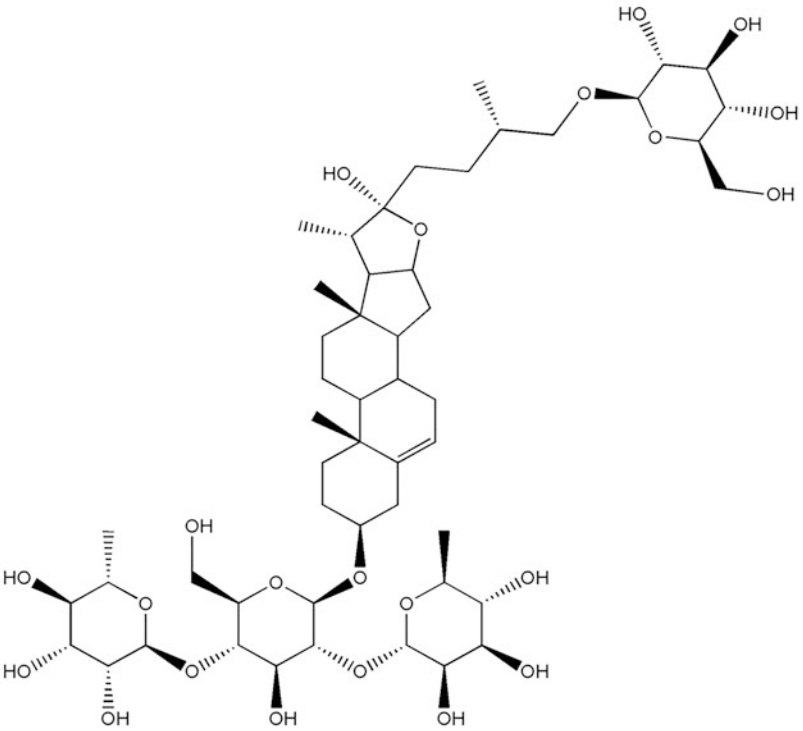

Fig. 18.1 (continued)

(3 ug/100 g), B1 (0.43 mg/100 g), B2 (0.36 mg/100 g), C (12–43 mg/100 g), nicotinic acid (1.1 mg/100 g), and niacin (6 mg/100 g). Reid and Meier (1970) reported that endosperm also contain 7–9% fatty acids, diosgenin, free amino acids, flavonoids, and galactomannans (Fig. 18.1c)

Patil et al. (1997) found the seed to contain 26.2 g% protein, 5.8 g% fat, and 44.1 g% carbohydrate. Yoshikawa et al. (1997) and Petit et al. (1995) isolated many compounds like fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin, trigonelline, diosgenin (Fig. 18.1d), gitogenin, neogitogenin, homorientin, saponaretin, and tigogenin.

Belguith-Hadriche et al. (2013) found the major phenolic compounds in fenugreek seeds to be gallic, caffeic, syringic, p-coumaric, chlorogenic acids, kaempferol, apigenin, naringenin, myricetin, and luteolin.

The total phenolic present in fenugreek seeds are in the range between 1.613 and 2.083 gallic acid equivalents mg/g of extract. The total flavonoids present in the seeds are in the range between 1.847 and 3.778 catechin equivalents mg/g, while the amount of condensed tannins expressed as catechin equivalent was found between 0.730 and 1.051 mg/g (Rahmani et al. 2018).

Fenugreek contains 1–2% flavonoids which include apigenin and luteolin as the main aglycones, and xylose, arabinose, glucose, galactose or rhamnose as C-glycoside (Kamble et al. 2013). He et al. (2015) suggested the major bioactive compounds of the seeds to be rhaponticin and isovitexin (Fig. 18.1e)

The anti-oxidant chemicals reported from fenugreek include N,N′-dicarbazyl, glycerol monopalmitate, stearic acid, β-sitosterol glucopyranoside, ethyl-α-D-glucopyranoside, D-3-Omethylchiroinositol and sucrose, vitexin (Fig. 18.1f), tricin, naringenin, tricin-7-O-β-D-glucopyranoside, and quercetin (Shang et al. 1998a).

Dixit et al. (2005) reported gallic acid, o-coumadin acid, p-coumaric acid, rutin, and caffeic acid. Employing high performance liquid chromatographic (HPLC) analysis, they found the total flavonoids and total phenols in the water extract of 24 h germinated fenugreek seedlings to be between 19.14 and 17.49 mg of quercetin equivalents/g fenugreek powder and 64.61–47.55 mg of gallic acid equivalents/g fenugreek powder, respectively.

Employing gas liquid chromatography, Blank et al. (1997) found many terpene derivatives in the volatile oils that are responsible for the characteristic aroma and flavor of fenugreek. They include olfactometry diacetyl, 1-octene-3-one, sotolon, acetic acid; 3-isobutyl-2-olfactometry diacetyl, 1-octene-3-one, 3-isobutyl-2-methoxypyrazine, butanoic acid, isovaleric acid, 3-isopropyl-2-methoxypyrazine, caproic acid, eugenol, 3-amino-4,5-dimethyl-3, linalool, (Z)-1,5-octadiene-3-one, 4-dihydro-2 (5H)-furanone with characteristic aroma of buttery like, roasty/earthy, metallic, pungent, paprika like, sweaty/rancid, flowery, musty, spicy, etc. Out of all these, volatile compounds, sotolon were reported to be found most predominantly in (5s)-enantiomeric form (95%) in fenugreek.

Pundarikakshudu et al. (2016) got 7% of w/w of petroleum ether soluble extract from fenugreek seed powder which on analysis by gas liquid chromatography (GLC) showed stearic (1.72%), palmitic (9.58%), oleic (33.61%), linoleic (40.37%), and linolenic (12.51%) acids.

Fazli and Hardman (1971) reported 0.83% and 0.92% diosgenin in seeds obtained from Pakistan and Morocco, respectively. But Bakshi and Hamied (1971) reported very low content of diosgenin Algerian (0.35%), Morocco (0.25%), and Indian (0.1%) seeds of *Trigonella foenum-graecum*. The seeds from Kangra (Himachal Pradesh) contain 1.02% of total sapogenins as diosgenin and yamogenin (Puri et al. 1976).

Diosgenin is present as rhamno-rhamno-glucoside called dioscin. Diosgenin was shown to be an important starting material in the production of corticosteroids (Marker et al. 1943). During its conversion to other steroidal drugs, diosgenin is first converted to 16-dehydropregnanolone acetate (16-DPA).

Fenugreek seeds soaked for 72 h in water showed an increase in diosgenin content from 0.4% to 0.8% w/w of dry seeds. Similarly, aerial parts including leaves and stem also contained 0.2–0.4% w/w of diosgenin calculated on dry weight basis (Bhavsar et al. 1983; Pundarikakshudu et al. 1989).

Of all the chemicals present in fenugreek, the water soluble fiber rich in galactomannans, trigonelline, 4-hydroxyisoleucine, protodioscin (Fig. 18.1g), and diosgenin were found to be contributing to most of its pharmacological activities.

The Phytoconstituents found from different parts of *Trigonella foenum-graecum* L. are given in Table 18.1.

18.3 Pharmacological Activities of Fenugreek

Plants of the genus *Trigonella* and particularly of the cultivated species *T. foenum-graecum* (fenugreek) were known and used for different purposes in ancient times, especially in Greece and Egypt (Rouk and Hailu 1963). Leaves of *Trigonella foenum-graecum* commonly known as fenugreek were one of the components of the celebrated Egyptian Incense Kuphi, a holy smoke used in fumigation and embalming rites (Rosengarten Jr. 1969). In the seventeenth century fenugreek seeds were recommended to help expel the placenta of women after giving birth (Howard 1987).

The powders as well as the different extracts of the leaves and seeds exhibited a broad range of activities both in vitro, in vivo, and in clinical studies. However, an analysis of the literature revealed that more than one compound or group of compounds are responsible for the pharmacological activities of fenugreek. A single extract has activity on a number of disease conditions. Discussed below are some of the pharmacological effects exhibited by fenugreek and its extracts or semi-purified or isolated compounds.

18.3.1 Anti-diabetic Activity of Fenugreek (*Trigonella foenum-graecum*)

Many studies confirmed the anti-diabetic activity of fenugreek in both animal experiments and in clinical trials. The crude extracts as well as the isolated compounds exhibited significant glucose lowering activities. Soluble fibers rich in galactomannans, saponins, diosgenin, trigonelline, and 4-hydroxyisoleucine have shown very promising activity in controlling high blood glucose. The extracts and the compounds were found to act by preventing glucose absorption, activating insulin-signaling pathways in adipocytes and liver cells, inhibition of glycogenolysis by liver tissue and liver, increasing insulin secretion, enhancing insulin activity, enhancing insulin sensitivity, and regulating lipid metabolism and enhancing the activity of superoxide dismutase, anti-oxidant enzymes catalase and glutathione peroxidase and decreasing glucose-6-phosphatase and fructose-1,6-biphosphatase enzymes in the liver.

Aqueous extract of fenugreek leaf, upon oral or i.p. administration, exhibited a dose-dependent reduction in blood glucose in both normal and alloxan-diabetic rats. Ethanol extract showed anti-diabetic activity only in diabetic rats and on i.p. administration. LD50 of i.p. and oral administration of the aqueous extract were found to be 1.9 and 10 g/kg, respectively (Abdel-Barry et al. 1997). Sharma (1986) observed that fenugreek leaves have little effect on glycemia.

Table 18.1 The phytoconstituents found from different parts of *Trigonella foenum-graecum* L

Type of material	Type of the chemical constituents	Name of compounds	References
Powder fenugreek seed	Sapogenin	Steroidal sapogenins as diosgenin, gitogenin, tigogenin	Marker et al. (1943)
Powder fenugreek seed	Sapogenin	Mixture contains diosgenin, gitogenin, and trigonella genin	Soliman and Mustafa (1943)
Defatted and powdered seed	Sapogenins	Diosgenin, gitogenin, tigogenin, 25α-spirosta-3,5-diene	Bedour et al. (1964)
Fenugreek seed	Sapogenins	Yamogenin, the 25β-epimer of diosgenin	Fazli (1967)
Fenugreek seed	Spirostene ester	Fenugreekine	Ghosal et al. (1974)
Fenugreek seed	Furostanol glycosides	Trigonelloside C	Bogacheva et al. (1976, 1977)
Fenugreek seed	Saponin	Trigofoenosides A–G as their methyl ethers A1–G1	Gupta et al. (1985a, 1985b)
Fenugreek seed	Six furostanol glycosides, together with two known saponins	Called trigoneosides Ia, Ib, IIa, IIb, IIIa, IIIb along with , trigofoenoside A and its 25-R epimer, glycoside D	Yoshikawa et al. (1997)
Indian fenugreek	Seven new furostanol saponins	Trigoneosides IVa, Va, Vb, VI, VIIb, VIIIb, IX along with the known furostanol saponins, compound C, glycoside F, and trigonelloside C (Fig. 18.1h)	Yoshikawa et al. (1998)
Fenugreek seed	Saponin	Fenugrin B on acid hydrolysis, gave diosgenin and the sugars: glucose, arabinose, and rhamnose	Grangrade and Kaushal (1979)
Fenugreek leaves	Five spirostanolsaponins	Graecunin-B,-C, -D, -E, and -G along with graecunin-A and -F	Varshney and Jain (1979), Varshney et al. (1984)
Acid hydrolysis of the defatted powdered leaf	Steroidal saponins	25α-and 25β-spirosta-3,5-diene and a 1:1 mixture of diosgenin and yamogenin	Fazli and Hardman (1971)
Whole plant	Steroids	β-sitosterol-D-O-glucoside (=daucosterol)	Parmar et al. (1982)
Static cultures of fenugreek	Sterols and sapogenins	Higher levels of β-sitosterol, stigmasterol and of the steroidal sapogenin	Khanna and Jain (1973)
Ethanol extract of the seeds	Six triterpenoids	Lupeol, 31-norcycloartanol, betulin, betulinic acid, soyasaponin I, and soyasaponin I methylester	Shang et al. (1998)
Fenugreek seeds	Flavonoids	Vitexin-2″-O-p-coumarate	Sood et al. (1976)

(continued)

Table 18.1 (continued)

Type of material	Type of the chemical constituents	Name of compounds	References
Fenugreek seeds	Flavonoids	Luteolin, quercetin	Varshney and Sharma (1966)
Fenugreek seeds	Flavonoids	vitexin (8-C-β-D-glucosyl 5,7,4′-trihydroxyflavone), vitexin-7-O-glucoside (afroside), arabinoside of orientin or isoorientin (8-C-/6-C-β-D-glucosyl-arabinosyl-5,7,3′,4′-tetrahydroxyflavone)	Adamska and Lutomski (1971)
Seeds originating from China	Flavonoids	Vitexin, orientin, quercetin, naringenin, tricin, and tricin-7-O-β-D-glucopyranoside	Lin et al. 2000, Shang et al. (1998)
Alcoholic extract of the whole plant	Flavonoids	Luteolin, quercetin, vitexin, isovitexin, and 7,4-dimethoxyflavanone	Parmar et al. (1982)
Leaf extract	Flavonoids	Kaempferol and quercetin	Sood (1975)
Fenugreek stems	Flavonoids	Luteolin, quercetin, and vitexin	Khurana et al. (1982)
Fenugreek stems growing in China	Flavonol glycosides	Kaempferol 3-O-β-D-glucosyl (1→2)-D-galactoside, kaempferol 3-O-β-D-glucosyl (1→2)β-D-galactoside 7-O-β-D-glucoside, kaempferol 3-O-β-D-glucosyl (1→2)-(6″-O-acetyl)-β-D-galactoside 7-O-β-D-glucoside, quercetin 3-O-β-D-glucosyl (1→2)-β-D-galactoside 7-O-β-D-glucoside, and kaempferol 3-O-β-D-glucosyl (1→2)-β-D-galactoside	Han et al. (2001)
36 months old Unorganized seedling callus tissue	Flavonoids	Luteolin, quercetin and vitexin-7-glucoside (afroside)	Uddin et al. (1977)
Leaves and stems	Phenolic compounds	Scopoletin and the lignan γ-schisandrin	Wang et al. (1997)
Whole plant extract	Phenolic compounds	Hymecromone (4-methyl-7-acetoxycoumarin)	Bhardwaj et al. (1977)
Stem	Phenolic compounds	Hymecromone,(E)-3-(4-hydroxyphenyl)-2-propenoic acid (p-coumaric acid) and trigoforin (3,4,7-trimethylcoumarin)	Khurana et al. (1982)

(continued)

Table 18.1 (continued)

Type of material	Type of the chemical constituents	Name of compounds	References
Whole plant extract	Phenolic compounds	Trigocoumarin, 3-(ethoxycarbonyl) methyl-4-methyl-5,8-dimethoxycoumarin together with hymecromone	Parmar et al. (1982)
Seeds	Sesquiterpenes and some oxygenated components	n-hexenol, 2-heptanone, n-heptanal, aniline, phenol, heptanoic acid, 3-octen-2-one, 1,8-cineol, undecane, camphor, 5-methyl-δ-caprolactone, 1-dodecene, methylcyclohexylacetate, dihydrobenzofuran, dodecane, decanoic acid, thymol, 2-hexylfuran, tridecane, γ-nonalactone, eugenol, δ-elemene, 1-tetradecene, tetradecane, calarene, β-ionone, α-muurolene, dihydroactinidiolide, ε-muurolene, β-elemene, β-selinene, γ-elemene, γ-muurolene, calamenene, pentadecane, dodecanoic acid, diphenylamine, 1-hexadecene, hexadecane	Girardon et al. (1985)
Seeds	Amino acids	(2S,3R,4R)-4-hydroxyisoleucine [2-amino-4-hydroxy-3-methylpentanoic acid (2S, 3R, 4R) form]	Fowden et al. (1973), Hardman and Abu-Al-Futuh (1979)

Abor and Abd El Rahman (2014) studied the effect of a 60 day administration of methi powder, its aqueous extract, methanol extract and oil on streptozotocin induced diabetic rats. The elevated levels of blood glucose, HBA1c, fatty acids, ALT, ASP, ALP, creatinine, urea and uric acid, triglycerides, total cholesterol, LDL cholesterol, and VLDL in the diabetic rats were significantly decreased by treatment with the various extracts of fenugreek. Subsequently, an increase in insulin and HDL cholesterol was also noted.

Hannan et al. (2003) noted that the soluble fiber of fenugreek is responsible for improving the metabolic disorders. It improved glucose homeostasis in types 1 and 2 diabetic rats, delayed carbohydrate digestion and absorption, and also increased insulin action. It delayed gastric emptying of carbohydrate, inhibition of intestinal lipase and sucrase activity, and increased gut motility. The fiber fraction was also found to increase both insulin-stimulated and basal glucose uptake in 3T3-L1 adipocytes and augmented liver glycogen content.

It was found that fenugreek seed powder at 1.0 g/kg brought marked improvements in blood sugar, lipid profiles, atherogenic indices, impaired liver functions, oxidative stress in diabetic, and obese rats (Ramadan et al. 2011).

The severe damage caused to islets of Langerhans by alloxan-induced diabetes was prevented or reversed by treatment with fenugreek seed extract (Babu et al. 2010).

Fenugreek seeds contain 25% fiber that can slow down the rate of post-prandial glucose absorption. This may be a secondary mechanism for its hypoglycemic effect (Basch et al. 2003).

Fenugreek seed extract inhibited α-amylase activity in a dose-dependent manner. The extract also suppressed starch digestion and absorption in normal rats, indicating that the hypoglycemic effect is mediated through insulin mimetic effect (Gad et al. 2006)

Fenugreek seed extracts have been reported to exhibit anti-diabetic potential by delaying both gastric emptying time and rate of glucose absorption. They reduced the uptake of glucose in the small intestine mainly due to high fiber content that slows down the metabolism of carbohydrates and lowers blood glucose (Patel et al. 2012).

Baquer et al. (2011) reported that *Trigonella* administration to diabetic rats restored the changed enzyme activities and partially normalized hyperglycemia. They concluded from their experiments that the altered levels of superoxide dismutase, anti-oxidant enzymes catalase, and glutathione peroxidase in liver and kidney of diabetic rats were corrected by treating with insulin, vanadate, fenugreek, and the combined dose of vanadate and fenugreek. It was shown that the activities of glucose-6-phosphatase and fructose-1,6-biphosphatase in the liver and kidneys of diabetic rats are reduced by administration of fenugreek.

Yadav et al. (2004) demonstrated that by the combined treatment of fenugreek and sodium-orthovanadate, activities of nicotinamide adenine dinucleotide phosphate-linked enzymes such as glucose-6-phosphate dehydrogenase, malic enzyme, isocitrate dehydrogenase, and the activities of lipogenic enzymes such as adenosine triphosphate-citrate lyase and fatty acid synthase were decreased significantly in liver and increased in kidney during diabetes as compared to those of control.

Combined administration of the water extract of seeds of *Trigonella foenum-graecum* and *Psoralea corylifolia* in 1:1 ratio reverted the altered carbohydrate metabolic enzymes such as hexokinase, glucose-6-phosphatase, and glucose-6-phosphate dehydrogenase in streptotozocin induced diabetic rats (Bera et al. 2013).

Daily dietary supplementation of 25 and 100 g fenugreek seed powder significantly reduced fasting blood sugar and improved glucose tolerance in diabetic rats. The activity may be due to delay in gastric emptying, carbohydrate metabolism, glucose uptake and absorption (Mooventhan and Nivethitha 2017).

Hydro-alcoholic extract of fenugreek seeds given at different doses (500, 1000, and 2000 mg/kg) to alloxan-induced diabetes rats produced increased body weight and glucose uptake, reduced plasma glucose, glycosylated hemoglobin (HbA1c), liver glucose transport, proinflammatory cytokines, pancreatic enzymes,

and restored depleted glycogen (in muscle and liver) and total protein significantly and dose dependently. It prevented lipid peroxidation and restored glutathione and superoxide dismutase (in liver and pancreas). Moreover, the treatment affected marked improvement in the histo-architecture of liver and pancreas in alloxan-induced diabetic rats (Joshi et al. 2015).

Diabetic rats treated with fenugreek seed extract for 6 weeks exhibited lower triglycerides, total cholesterol, lower blood glucose, and glycated hemoglobin and higher HDL cholesterol in a dose-dependent manner. The activity has been attributed to the galactomannan-rich soluble fiber fraction of seeds (Xue et al. 2007).

Feeding of defatted fenugreek seed powder to dogs was found to lower blood glucose levels, plasma glucagons, and somatostatin levels; carbohydrate-induced hyperglycemia was also found to be reduced (Snehlata and Payal 2012).

Fenugreek seed enhances insulin sensitivity, improves insulin action at cellular level, and recovers the level of HbA1c by utilization of glucose in peripheral tissues thereby maintaining the blood glucose level (Gauttam and Kalia 2013).

The effect of fenugreek seed extract (FSE) on the gene expression of PPARγ in adipose tissue, PPARα in liver and GLUT4 in skeletal muscle in high fat diet fed and low-dose STZ induced T2DM rats was studied by Tharaheswari et al. (2014).

Rats fed with high fat diet and made diabetic with the injection of streptozotocin, exhibited very high blood glucose levels, elevated HbA1C, and reduced levels of insulin. Simultaneous administration of 300 mg/kg of methanol extract of fenugreek seeds for 8 weeks brought about a marked improvement in the above parameters. Similarly, elevated lipids, creatinine kinase, and CK-MD in the high fat diet fed animals and high fat fed diabetic animals were rectified with this concentration of the extract. The reduced concentrations of muscle glycogen and hexokinase activity were significantly enhanced in these diabetic hyper-lipidimic animals on treatment with the extract. These positive effects were further supported by the significant elevation of adipose tissue, PPAR γ, and liver PPARα levels that were otherwise significantly reduced in the diabetic rats.

Bhaskaran S Vishwaraman (2014) patented a method to isolate soluble fiber from fenugreek seeds. The isolated soluble fiber was standardized to contain eleutheroside-C (30%), pinitol (48%), sucrose (6%), raffinose (6.5%), and stachyose (9.5%). It had a LD50 of 2000 mg/kg in mice. This standardized fiber exhibited significant hypoglycemic activity in diabetic animals.

The extract, rich in soluble fiber, when given for 12 weeks to high fat diet fed male C57BL/6 mice at 30, 60, 100 mg/kg dose, reduced insulin resistance by 55–63%, area under curve in oral glucose tolerance test by 22.0%, and area under curve of intra-peritoneal insulin tolerance test (AUC-IPITT) by 42%. The downregulation of mRNA expression of Glut-2, Glut-4, and IRS-2 in the adipose tissue and livers of high fat diet fed mice were significantly upregulated in the soluble fiber treatment group. Similarly, the skeletal muscle IRS-2 mRNA expression in high fat diet fed mice was significantly upregulated as reflected in RT-PCR data. Thus this study by Kandhare et al. (2014) indicates potential of the soluble fiber of fenugreek as a promising prophylactic adjuvant in diabetic obese patients with insulin resistance.

Trigonella seed-derived soluble dietary fiber (SDF) given for 28 days to diabetic rats decreased serum glucose, increased liver glycogen content, and enhanced total anti-oxidant status; without having any effect on insulin secretion (Hannan et al. 2007). In cultured 3T3-L1 adipocytes, glucose transport and insulin action were enhanced by *Trigonella*. These studies suggest that anti-diabetic effect of *T. foenum-graecum* seed-derived SDF is mediated through inhibition of carbohydrate digestion and absorption, and enhanced peripheral insulin action.

Fenugreek galactomannan, exhibited significant and dose-dependent hypoglycemic effect at 150, 300, and 600 mg/kg doses in glucose loaded rats. A reduction of 25%, 28.2%, and 43.78% glucose at the above doses, respectively, was observed. A slight decrease in the lipid peroxidation and increase in the anti-oxidant enzymes like CAT, GL, GPx was also noted. The hemidiaphrams from galactomannan treated rats exhibited enhanced uptake of glucose. In diabetic animals it brought down the glycosylated hemoglobin and reversed the reduced liver glycogen. This points towards another possibility that galactomannan may act through an extra-pancreatic route, such as inhibition of glycogenolysis by liver. Cellular damage caused to the islets of Langerhans was reversed/prevented by galactomannan. Alcoholic extract of fenugreek significantly reduced serum total cholesterol, triacylglycerol, urea, uric acid, creatinine, AST, and ALT levels in diabetic but not in healthy rats (Anwar et al. 2009).

Segments of jejunum and ileum derived from genetically lean and obese rats were incubated with labeled glucose (2 or 32 mmol/L) in the presence of different concentrations of galactomannan ranging from 0.1 to 0.5% (wt/wt). Galactomannan reduced the uptake of glucose in a dose-dependent manner by tissues of both the animals (Srichamroen et al. 2009). This suggests the potential of galactomannan in the management of diabetes.

Raghuram et al. (1994) reported that fenugreek increased erythrocyte insulin reception. In intravenous glucose tolerance test it was noted that fenugreek in the diet reduced the area under the plasma glucose curve significantly and shortened the half-life of plasma glucose by the increased metabolic clearance.

When diabetic rats were treated with fenugreek flavonoids a normalization in the levels of hippuric acid and tryptophan was observed thereby indicating the role of these flavonoids in reducing gluconeogenesis and restoration of amino acid metabolism (Jiang et al. 2018).

Administration of fenugreek seed polyphenolic extract (FPEt) improved insulin sensitivity and tyrosine phosphorylation status in fructose-fed animals compared to metformin treated rats (Kannappan and Anuradha 2009).

Fenugreek seed extract ameliorated the vascular changes that occurred in diabetic rats (Mahdavi et al. 2008).

Xue et al. (2011) reported that aqueous extract of fenugreek improved the functioning of kidney of diabetic rats by protecting them from functional and morphologic injury.

In an in vitro study, the seed extract was reported to have phosphorylated a number of proteins, including the insulin receptor, insulin receptor substrate 1 and

p85 subunit of PI3-K, in both 3T3-L1 adipocytes and human hepatoma cells, HepG2 (Vijayakumar et al. 2005).

These results suggest that fenugreek's effects may be due to activation of the insulin-signaling pathway in adipocytes and liver cells.

Administration of a combination of 0.2 mg/ml vanadate and regular dose of fenugreek powder for 21 days to alloxan-induced diabetic rats was found to be highly effective as blood glucose, pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT) levels returned to near normal values (Sameer et al. 2004).

Madar (1984) attributed the hypoglycaemic effect of fenugreek seeds to the dietary fiber that acts locally in the GIT. Other researchers found other chemical principles present in the seeds act systemically to lower the blood glucose in diabetes (Ribes et al. 1986; Moorthy and Murthy 1989).

Sauvaire et al. (1998) demonstrated that 4-hyroxyisoleucine (4-OHIle) increases glucose-induced insulin release in vitro inhuman and rat pancreatic islet cells. It seems to act only on pancreatic beta cells, since somatostatin and glucagon were not altered in the study.

4-OH isoleucine administered to diabetic rabbits at a dose of 50 mg/kg for 15 days significantly attenuated the glucose tolerance curve and improvement in the glucose-induced insulin response. Upon prolonged administration of this dose to severely diabetic rabbits, a significant lowering of fasting blood glucose and moderate elevation of serum insulin was observed (Puri et al. 2002).

4-OH-isoleucine enhances insulin sensitivity and peripheral glucose uptake in a hyperinsulinemic clamp method used in sucrose—lipid—fed rats. It reduced hepatic glucose output in Zucker fa/fa rats. Acute in vivo injection of 4-OH-Ile also resulted in the insulin receptor substrate 1 (IRS-1)–related activation of phosphatidylinositide 3-kinase (PI3K) in insulin-sensitive tissues (Broca et al. 2004).

In cultured rat muscle cells, glucose uptake and glucose transporter 4 (GLUT4) translocation to the plasma membrane were increased in response to a 16-h exposure to 4-hydroxy isoleucine (4-OH-Ile).

The study also demonstrated that 4-OH-Ile could inhibit both the palmitate induced production of reactive oxygen species and the associated inflammation, as was demonstrated by reduced activation of NF-kB, c-Jun N-terminal kinase isoforms 1 and 2 (JNK1/2), extracellular signal-regulated kinase isoforms 1 and 2 (ERK1/2), and p38 MAPK (Maurya et al. 2014).

Two potential mechanisms were described for insulin sensitizing activity of 4-hydroxy isoleucine (4-OHile): a negative regulation of TNF-α production with an improvement in insulin sensitivity, and increased expression of p-IRS-1 and GLUT4 in the insulin-signaling pathway (Mishkinsky et al. 1967).

4-OHIle stimulates pancreatic islets to secrete more insulin in the presence of high blood glucose. It also has insulin sensitizing effect on muscle, adipose and liver tissues (Avalos-Soriano et al. 2016).

Jetté et al. (2009) reported 4-HIL could reduce insulin resistance (IR) in muscle and/or liver by stimulating phosphoinositide 3 (PI3) kinase activity.

4-hydroxy isoleucine reduced blood glucose in glucose tolerance test in sub-diabetic and moderately diabetic rabbits. This compound was found to be better than tolbutamide in its efficacy (Moorthy et al. 2010).

4-Hydroxyleucine, increased glucose stimulated insulin release in islet cells of mice, rats, and humans. It also increased insulin sensitivity and glucose uptake in peripheral tissues (Randhir and Shetty 2007). It was reported to suppress the progression of type-2 diabetes in a mouse model (Mooventhan and Nivethitha 2017).

4-hydroxyisoleucine prevented the rise in the liver damage markers ALT and AST in fructose fed; insulin resistant streptozotocin induced diabetic rats (Haeri et al. 2009).

Trigonelline was reported to reduce blood glucose concentrations in rats (Mishkinsky et al. 1967; Moorthy and Murthy 1989), protects β-cells of the pancreas, and increases insulin sensitivity index as well as insulin content (Zhou et al. 2012).

It was observed that when trigonellline (150 mg/kg) was administered to high fat diet and low-dose streptozotocin induced diabetes rats for 30 days, blood glucose, glycosylated hemoglobin, AST, ALT, and ALP were significantly reduced. There was an improvement in hepatic and muscle glycogen content in insulin resistant diabetic rats. It reduces insulin resistance and improves impaired glucose tolerance. It was observed that trigonelline augments insulin-stimulated glucose uptake in peripheral tissues. It is believed that trigonelline acts as insulin sensitizer and improves β cell function. The decreased HbA1C level upon trigonelline treatment indicates the beneficial effect of trigonelline in controlling glucose homeostasis (Subramanian and Prasath 2014).

Alkaloids from fenugreek seeds were given at 50 mg/kg (p.o.) dose to streptozotocin induced diabetic rats for 21 days. The treatment brought about a reduction in blood glucose, (from 280 to 141 mg/dl); total cholesterol (from 140 to 107 mg/dl); triglycerides (from 154 to 111 mg/dl); LDL (from 54 to 44.5 mg/dl) and enhanced insulin from 4.17 to 7.27μU/ml and HDL from 35.7 to 47.1 mg/dl. Similarly, the enhanced levels of TBARS and Nitric Oxide in the diabetic rats were also reduced by the treatment with the alkaloid fraction. The vitiated architecture of the kidneys and liver in the streptozotocin treated diabetic rats has shown a tendency to return to normalcy in the treatment group (Helmy et al. 2007).

Glucose uptake was inhibited by diosgenin and trigonelline in vitro (Al-Habori et al. 2001). The seed powder was also found to modulate the expression of glucose transporter 4 (glut-4) in skeletal muscle (Mohammad et al. 2004, 2006).

In β cell dysfunction and subsequent insulin resistance, diosgenin improved pancreatic endoplasmic reticulum (ER) stress in streptozotocin induced type 2 diabetic rats by reducing expression of the C/EBP homologous protein (CHOP) and caspases 12 and 3. Diosgenin also restored normal pancreas morphology, improved serum glucose and insulin concentrations, increased amounts of anti-oxidant enzymes, and enhanced PPARg expression (Tharaheswari et al. 2014).

Diosgenin at the dose of 1 mg/kg, 10 mg/kg, and 50 mg/kg and fenugreek seed powder at a dose of 200 mg/kg were administered orally to high fat diet fed rats for a

period of 7 weeks. At the end of the experiment, precise insulin of insulin-dependent tissues was determined by hyperinsulinemic-euglycemic glucose clamp (HEGC) method. It was observed that hyperinsulinema did not alter the rate of change in the pattern of glucose disappearance. There was no significant change in insulin, insulin-like growth factor-1 (IGF-1), growth hormone (GH), triiodothyronine (T3), thyroxine (T4) when the blood was analyzed by ELISA kits. In fenugreek and diosgenin 1 mg/kg treated rats, an increased metabolic clearance rate of insulin was noted thereby indicating that the body does a balancing act to compensate for hypoglycemia due to enhanced insulin.

Insulin sensitivity index was increased, showing that less amount of insulin was needed to achieve the same rate of disappearance of glucose from blood circulation compared to other groups. The authors also found that in chronic diosgenin and fenugreek treatment the basal level of thyroid hormones, especially T3 significantly decreased, but the consequent IGF-1 elevation in response to hyperinsulinemia in the steady state effectively balances this reduction in the diosgenin groups. The fenugreek treated rats showed a significant elevation of basal T4 levels, but in the steady state period the insulin infusion reduces T4, possibly through stimulating its transformation into T3 due to elevated GH that promotes hypothyroidism (Kiss et al. 2019).

Diosgenin, when administered at 15, 30, and 60 mg/kg for 45 days to diabetic rats exhibited significant decline in blood glucose and increase in the plasma insulin level. It also restored the carbohydrate metabolic key enzymes in muscle and kidneys of diabetic rats to near normal levels (Saravanan et al. 2014).

Thus 4-hydroxyisoleucine, trigonelline, and diosgenin in fenugreek seeds have been proved to be very promising molecules in the treatment of diabetes and have the potential to act as chemical scaffolds for the development of new molecules for treatment of diabetes. The galactomannans present in the soluble fiber have also exhibited potential anti-diabetic activity. Since fenugreek and its various chemical entities have shown good hypo-lipidemic and anti-diabetic activities, the spice is definitely of immense value in managing diabetes, hyper-lipidemia, and liver disorders.

18.3.2 Anti-Hyper-Lipidemic/Anti-Cholesteremic Activity of Fenugreek *(Trigonella foenum-graecum)*

Many studies have conclusively proved that fenugreek powder, its water soluble fiber-containing fractions, the saponins, etc. have lipid-lowering activity in both humans and animals. They mainly act by preventing the accumulation of cholesterol in the liver and ensuring its rapid excretion. Fenugreek also ameliorates oxidative stress in the liver thereby reducing lipid deposition and increasing bile acid output. Diosgenin and 4-hydroxyisoleucine especially exhibited good hypo-lipidemic activities in experimental studies.

When taken post meal, dietary supplementation of fenugreek seed could regulate the production of cholesterol which is attributed to the presence of polyphenols,

saponins, alkaloids, and dietary fiber present in the seeds (Herrera et al. 2018). Fenugreek seeds contain 45.4% dietary fiber of which 32% are insoluble fibers and 13.3% are soluble fibers (Roberts 2011).

Furostanol saponins have been shown to increase food consumption and induce hypocholesterolemia in experimental diabetic rats (Petit et al. 1995).

Fenugreek seed powder, included in the diet, reduced cholesterol levels in mice by around 42–58% (Singhal et al. 1982).

Many researchers attributed the hypo-lipidemic activity of fenugreek seeds to the saponins present in it. These saponins are converted into sapogenins in the gastrointestinal tract which increase biliary cholesterol secretion resulting in reduced serum cholesterol level (Stark and Madar 1993; Petit et al. 1993; Al-Habori et al. 1998; Valette et al. 1984). Terminal ileum is the region from where the saponins are absorbed in the rats (Stark and Madar 1993; Sauvaire et al. 1991).

Ramulu et al. (2011) studied the effect of a 9 week feeding of soluble fiber of fenugreek seed and galactomannan on lipid profiles, HMG-CoAR, fecal bile secretion, and neutral sterols. While treatment with the soluble fibers brought about significant reductions in the triglycerides and total cholesterol, there was an increase in HMG-CoAR activity in the liver microsomes. This point towards the possibility that the fenugreek fiber does not inhibit cholesterol biosynthesis but rather it enhances its excretion. This was evident from the high excretion of bile acids and neutral sterols in the fecal matter in the fiber/galactomannan fed obese animals.

Seventy percent ethanol extract of fenugreek seeds brought down significantly the elevated levels of triglycerides, total cholesterol, LDL-cholesterol in ovariectomy-induced hyper-lipidemia in rats. It significantly ameliorated the oxidative stress in the liver due to ovariectomy and decreased hepatic lipid deposition (Takkella et al. 2019).

C57BL/6J mice were fed either a low fat or a high fat diet with or without fenugreek (2% w/w) (LFFG or HFFG) for 16 weeks. Fenugreek seed supplementation moderately improved glucose tolerance in these mice. There was no significant improvement in insulin tolerance, body weight and body composition, total cholesterol, triglycerides, glycerol or non-esterified fatty acids. Though Fenugreek supplementation to HF-fed mice increased total adiponectin protein expression in subcutaneous inguinal adipose tissue (iWAT) when compared to HF diet alone, it did not affect HF-induced loss of adiponectin mRNA in visceral epididymal adipose tissue (eWAT). These studies indicated that 2.0% W/W fenugreek seed powder supplementation is less effective than four day voluntary wheel running in bringing down the above lipid parameters (Knott et al. 2017)

In a rat everted sac experiment, presence of 400 mg fenugreek seed EtOH extract in the mucosal side reduced bile salt absorption by 84 and 87% for taurocholate and deoxy-cholate, respectively. The absorption of labeled cholesterol was also inhibited. Rats fed with 30 g and 50 g ethanol extract along with high cholesterol diet for 9 weeks exhibited low levels of plasma and liver cholesterol concentrations. It was inferred that this lowering effect may be due to an increase in conversion of cholesterol into fecal bile salts and its excretion (Stark and Madar 1993).

In an experimental model of AlCl3-fed rats, simultaneous supplementation of fenugreek seed powder or extract for 5 months enhanced the levels of LPO in posterior brain, liver, and plasma, along with lactate dehydrogenase (LDH) activities, whereas total cholesterol, TG, and LDL cholesterol levels reversed, suggesting an anti-peroxidative role in the brain which may be attributed to its modulatory effect on plasma lipid metabolism (Belaïd-Nouira et al. 2012).

Raju and Bird (2006) showed that in obese rats dietary fenugreek seed powder supplementation reduced the triglyceride accumulation in the liver, a salient feature of liver steatosis. Fenugreek improved hepatic steatosis and hyper-lipidemia by suppressing the mRNA of lipogenic gene (Mooventhan and Nivethitha 2017).

Changes in the hepatic enzyme activities (3-hydroxy-3-methylglutaryl coenzyme A reductase, cholesterol-7α-hydroxylase, and cholesterol-27-hydroxylase) induced by high cholesterol diet in rats showed significant reversal by feeding fenugreek seed. The supplementation also exhibited anti-lithogenic activity due to the reduction in serum and hepatic cholesterol levels in mice (Lakshmaiah and Srinivasan 2009). Fenugreek supplementation decreased the cholesterol, total protein, glycoprotein, lipid peroxides and cholesterol saturation index in bile, and increased the bile flow rate, and cholesterol nucleation time. All of which are known to regulate cholesterol crystallization (Reddy and Srinivasan 2011).

Hannan et al. (2003) undertook a study wherein fenugreek seed soluble dietary fiber was orally administered at a dose of 0.5 g/kg for 28 days to type II diabetic rats. After the treatment, the fructosamine levels were lowered along with a decrease in triglycerides, cholesterol, and LDL cholesterol. HDL cholesterol increased in the treated animals. No significant effect on insulin levels and platelet aggregation was noted.

It was observed that, on consecutive consumption of fenugreek seeds, serum total cholesterol, LDL and VLDL cholesterol, and triglycerides were significantly reduced but no effect on HDL cholesterol levels was found (Sharma et al. 1990).

An in vitro study of 3T3-L1 adipocytes revealed that diosgenin treatment enhanced adipogenesis and lipid accumulation while also reducing the expression of inflammatory factors (Uemura et al. 2010).

Diosgenin suppressed the accumulation of lipids in HepG2cells by a liver X-receptor-α antagonist like effect. This indicates that diosgenin plays an important role in the therapeutic effect of fenugreek on lipid metabolism disorders in the liver of diabetic obese KK-Ay mice (Uemura et al. 2011).

4-hydroxyisoleucine reduced the total cholesterol level, triacylglycerols, phospholipids, and free fatty acids and increased HDL cholesterol in rabbits (Moorthy et al. 2010).

4-hydroxyisoleucine brought about a decrease of 33%, 22%, and 14% of plasma triglyceride, total cholesterol, and free fatty acids, respectively, in hyper-lipidemic hamsters. A 39% increase in the HDL cholesterol/triglyceride ratio was also noted in these animals (Narender et al. 2006).

Thus, fenugreek seed powder and its various extract showed potent activity in reducing the triglycerides, cholesterol, LDL, and VLDL in various animal experiments. Diosgenin and 4-hydroxyisoleucine seem to be promising chemical

entities for the regulation of lipid metabolism in the body and can serve as good scaffolds for the development of safe drugs for the treatment of lipid disorder.

18.3.3 Anti-cancer Activity of Fenugreek (*Trigonella foenum-graecum*)

Crude extracts of fenugreek and diosgenin exhibited strong anti-cancer activity in vitro in various cancer cell lines. The activity was very striking on cell lines of, breast cancer, colon carcinoma, prostate cancer, liver cancer, and myeloblastic leukemia. The activity on breast and liver cancer cell lines is of great therapeutical significance. The extracts and diosgenin induce apoptosis in these cancer cell lines by DNA fragmentation, increased expression of Caspase 8 or 3, as well as p53 protein, Bax, and PCNA. Diosgenin inhibited TNF-α activated transcription factors such as NF-$\kappa\beta$ and Akt, downregulated the expression of various STAT3-regulated genes such as c-Src, JAK1, and JAK2. Thus the anti-cancer activity of fenugreek seed extracts and diosgenin has been established in a number of in vitro experiments.

Alcoholic whole plant extracts of fenugreek showed in vitro cytotoxicity against different human cancer cell lines such as IMR-32 neuroblastoma cell line, and HT29 cancer cell lines breast cancer cell lines, prostate cancer cell lines, and pancreatic cancer cell lines (Verma et al. 2010; Shabbeer et al. 2009) and in Ehrlich Ascites Carcinoma (EAC) cells induced cancer in Swiss albino mice by showing longer survival time (Prabhu and Krishnamoorthy 2010; Ardelean et al. 2010).

Fenugreek aqueous extract exhibited selective cytotoxicity against cancer cell lines such as T-cell lymphoma (TCP), B-cell lymphomas, thyroid papillary carcinoma (FRO), and breast cancer (MCF7). No cytotoxicity was observed in normal cells (Alsemari et al. 2014).

Ethanol extract of defatted fenugreek seeds inhibited the growth of Ehrlich ascites carcinoma (EAC) in mice. The growth of tumor cells was 86% and 94% less as compared to that of control animals when the extracts were given i.p. for seven days before inoculation with EAC. There was a 70% inhibition of the tumor growth when the extract was injected post-inoculation. There was an increase in the number of cells of the peritoneal exudate and macrophages in the treatment group. The activity is deemed to be indirect by activation enhancement of macrophages (Sur et al. 2001).

Alshatwi et al. (2013) observed that fenugreek methanol extract (FME) induced apoptosis in breast cancer cells was mediated by Fas receptor-independently of either FADD, Caspase 8 or 3, as well as p53.

A chloroform-based fenugreek seed extract could effectively reduce the viability of MCF-7 breast cancer cells through induction of apoptosis associated with increased expression of Caspase 3, 8, 9, p53, Fas, FADD, Bax, and Bak in a time- and dose-dependent manner (Khoja et al. 2011).

Sebastian and Thampan (2007) observed that ethanol extract of fenugreek induced early apoptosis by inversion of phosphatidyl serine, decreasing mitochondrial membrane potential, effecting DNA fragmentation in MCF-7 cells, which is an estrogen receptor positive breast cancer cell line. Cell cycle analysis revealed a

sub-G1 apoptotic population along with cell cycle arrest at G2/M phase in fenugreek extract treated cells. The extract was also found to show apoptosis in the breast cancer in 7,12-dimethyl benz(a)anthracene-induced mammary hyperplasia in rats (Amin et al. 2005). Aqueous extract of fenugreek seed lowered by eight times the viability of human breast cancer cell lines T-47D, ZR-75-1. Immunostaining revealed multiple cellular defects, cell division defects, and apoptosis like features (Vígh et al. 2016).

Chloroform fraction of the methanol extract which contained di-(2-ethylhexyl) phthalate (DEHP) showed the highest anti-cancer activity on MCF-7 breast cancer cell line (Kawabata et al. 2011).

Germinated fenugreek seeds were found to have higher amount of total alkaloids, flavoinodis, saponins, diosgenin compared to the dry seeds. Extract of the germinated seedlings showed higher anti-cancer activity in MCF-7 and AsPC-1human breast cancer cells. There was more capsase-3 and capsase-6 activities and induction of DNA fragmentation (Abbas et al. 2016).

Devasena and Menon (2003) studied the effect of fenugreek seed powder on the colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) by feeding fenugreek powder (2 g/kg) to rats for 30 weeks. 20 mg/kg DMH was given subcutaneously at weekly intervals for 15 weeks. The percentage of incidence of colonic tumors decreased from 93.3 in DMH-treated groups to 16.6 in DMH + fenugreek powder treated animals. The activities of carcinogenic marker enzymes mucinase (in fecal and colon contents) and β glucuronidase (in colon, intestine, liver, and colon contents) were elevated drastically in DMH-treated groups. Feeding with DMH + fenugreek seed powder brought the levels of these enzymes to near normalcy. This inhibition by fenugreek was attributed to its high fiber contents and other anti-cancer phytochemicals like sapogenins and flavonoids present in it. It was also suggested that the polysaccharides may also act as a substrate to mucinase there by sparing mucin that protects the gastric lining from the attack of the carcinogen.

Fenugreek seed powder reduced colon tumor incidence and LPO in DMH-treated rats.

A diet containing fenugreek seed powder reduced colon tumor incidence and LPO in DMH-treated rats and also increased activities of GPx, GST, SOD, and catalase in liver (Devasena and Venugopal Menon 2007).

Methanol extract of fenugreek seed was found to inhibit the production of phorbol-12-myristate-13-acetate-induced inflammatory cytokines such as tumor necrosis factor (TNF)-α in cultured THP-1 cells (Kawabata et al. 2011) and murine melanoma B16F1 cells.

Methanol extract of the fenugreek seeds inhibited DMBA and TPA induced skin tumors in mice (Chatterjee et al. 2012).

Fenugreek extract (FE) had a dose-dependent cytotoxic effect on three of the five pancreatic cancer cell lines tested, with complete elimination of all cells at 10μg/ml. After 48 h of FE treatment, a significant peak was seen in the sub-G1 population of the cell cycle profile of PC-3 and LNCaP cells. FE caused the phosphorylation of many receptor tyrosine kinases that promote cell growth. A surprising finding in this

study was that death of cancer cells occurs simultaneously with growth stimulatory pathways (Shabbeer et al. 2009).

Results of MTT assay demonstrated that hydro-alcoholic extract of fenugreek (HAEF) significantly decreased the viability of human umbilical vein endothelial cells (HUVECs) and 3T3 fibroblast cells. The extract reduced the viability of 3T3 cells and HUVECs with IC50 values of 285.9 μg/mL and 478.8 μg/mL, respectively. While control eggs showed dense vascularized chorioallantoic membrane (CAM), this density was considerably decreased in HAEF treated egg (Iranmanesh et al. 2018).

Fenugreek chloroform extracts significantly decreased HepG2 cell viability in dose-dependent manner from a dose of 100 μg/mL and above. There was shrinkage of cells, increase in capsase-3 activity, and p53 protein (Khalil et al. 2015).

Khalil et al. (2015) analyzed the fenugreek chloroform extract by GC-MS and found fourteen bioactive compounds including flavonoids and terpenoids. Treatment with the extract for 48 h showed a cytotoxic effect and apoptosis induction in a dose-dependent manner that was mediated by upregulation of p53, Bax, PCNA, and caspase-3 activation in HepG2 cells.

Treating the mice with cyclophosphamide and aqueous extract of fenugreek seeds at a dose of 0.4 mg/kg body weight led to an improvement in both histopathological and histochemical parameters of the liver such as cytoplasmic vacuolization of the hepatocytes, congestion of blood vessels, and leucocytic infiltrations (Sakr et al. 2009).

It was found that fenugreek seed extract could phosphorylate a number of proteins, including the insulin receptor, insulin receptor substrate 1, and p85 subunit of PI3-K, in both 3T3-L1 adipocytes and human hepatoma HepG2 cells, leading to the activation of insulin-signaling pathway in adipocytes and liver cells (Vijayakumar et al. 2005). Moreover, fenugreek reduced insulin resistance by increasing the number of insulin receptors in humans (Raghuram et al. 1994), adiponectin levels, and PPARγ protein expression in rats (Mohammadi et al. 2016).

Significant cytotoxic effect of fenugreek seed extract against acute myeloblastic leukemia cell line (KG-1) was noted which resulted in growth inhibition, cell death, and morphological changes. Contrary to many other reports apoptosis induction was not considerable (Alizadeh et al. 2009).

In human chronic myelogenous leukemia (KBM-5) cells, diosgenin was shown to suppress osteoclastogenesis which was induced by a cytokine, RANKL, through activation of NF-$\kappa\beta$. An increasing dose of diosgenin inhibited TNF-α activated transcription factors such as NF-$\kappa\beta$ and Akt (Amin et al. 2005).

Diosgenin inhibited bcl-2 and induction of-caspase-3 protein expression leading to apoptosis in HT-29 human colon cancer cells (Raju et al. 2004).

Li et al. (2010) showed that diosgenin could modulate the STAT3 signaling pathway in hepatocellular carcinoma by suppressing the activation of c-Src, JAK1, and JAK2. Diosgenin also downregulated the expression of various STAT3-regulated genes, inhibited proliferation, and potentiated the apoptotic effects of paclitaxel and doxorubicin in hepatocellular carcinoma cells.

Aggarwal and Shishodia (2006) reported diosgenin to have anti-cancer activity in bone cancer. It suppressed cell proliferation and development of bone cells through inhibition of tumor necrosis factor. Its activity may be due to inhibition of NF-kappaB-regulated gene expression. The diosgenin study indicated that the extract inhibited bcl-2 and induced caspase-3 protein expression, thereby inducing apoptosis and inhibiting cell growth.

Diosgenin was found to have inhibitory effect on the human osteosarcoma cell line, i.e. 1547 cell line by arresting cell cycle at G1 phase and also by inducing apoptosis (Moalic et al. 2001).

Diosgenin prevented telomerase activity by downregulating the human telomerase reverse transcriptase gene (hTERT) expression which is critical for telomerase activity and the survival of A549 lung cancer cells (Rahmati-Yamchi et al. 2014). In case of some human breast cancer cell lines, it was shown that diosgenin inhibited pAkt expression and Akt kinase activity without affecting PI3 kinase levels, resulting in the inhibition of its downstream targets, NF-kappaB, Bcl-2, surviving, and XIAP. The Raf/MEK/ERK pathway, another functional downstream target of Akt, was also inhibited by diosgenin in estrogen receptor featuring (ER+) MCF-7 cells by causing G1 cell cycle arrest, and downregulating cyclin D1, cdk-2, and cdk-4 expression leading to the inhibition of cell proliferation and induction of apoptosis (Srinivasan et al. 2009). Diosgenin inhibited angiogenesis dose dependently by suppressing vascular endothelial growth factor (VEGF) expression (Chen et al. 2015).

Chen et al. (2011) reported that diosgenin inhibits migration and invasion of human prostate cancer PC-3 cells by reducing matrix metalloproteinases expression. Fenugreek seed oil was found to exhibit cytotoxicity in a concentration-dependent manner in HepG2 cells and induced apoptosis via mitochondrial pathway in hepatocellular carcinoma cells. The apoptosis induction was found to be associated with ROS generation and mitochondrial membrane potential. There was upregulation in apoptotic marker genes (p53, caspase-3, caspase-9, and Bax) and downregulation in Bcl-2 gene in the gene expression studies (Al-Oqail et al. 2015).

Protodioscin (PD) strongly inhibited growth of HL-60 cells by inducing DNA fragmentation, but had little effect on KATO III cells in vitro. There was 75.2, 96.3, and 100% apoptosis (hypodiploid phase) of HL-60 cells exposed to 2.5, 5, and 10 μM PD for 3 days (Hibasami et al. 2003).

Thus, fenugreek and diosgenin emerged as very potential agents in the treatment of breast cancer, colon cancer, and liver cancer. Diosgenin being a natural product seems to be much safer and may not pose problems of bio-availability like curcumin. It has emerged as yet another wonder molecule from the armamentarium of natural products. It has to be noted that fenugreek seeds are regularly consumed in various forms in India. They have high degree of safety.

18.3.4 Analgesic and Anti-pyretic Activity of Fenugreek (*Trigonella foenum-graecum)*

The leaf and seed extracts of fenugreek exhibited significant analgesic activity in acetic acid induced writhing and hot plate induced pain models. The extracts exerted their activities both by peripheral and central mechanisms. Anti-pyretic activity was also noted in Brewer's yeast induced pyrexia in mice. In formaldehyde induced pain model, 30 min after the injection of the drugs, 50 ml of 2.5% formaldehyde is injected subcutaneously into the plantar surface of the animals' hind paw. Pain response scoring is determined by recording the pain-like behaviors per unit of time including licking, flinching, raising or shaking of the injected paw for 1 h after formalin injection. The first 10 min, post-formalin, is known as the early phase, and the period between 15 and 60 min as the late phase. *Trigonella foenum-graecum* seed extract exhibited significant analgesic activity in this model (Tjølsen et al. 1992). The first phase of the activity is considered to be of central origin and the second phase is of peripheral origin. In the tail-flick method, the rats show flicking of tail when exposed to radiant heat. When mice are injected acetic acid i.p., they experience severe pain and show writhing and the pain is of peripheral origin. Hot plate induced pain is central in origin where, the animals try to avoid the hot plate by jumping after some latent period. The latent period increases if the test material has analgesic activity.

Ahmadiani et al. (2001) found that the leaf extract of fenugreek (TFG) very strongly inhibited the rat paw inflammation induced by formalin in male NMRI rats both in single dose and chronic application for 7 days (500, 1000, and 2000 mg/kg i.p. and 1000 mg/kg p.o). It was postulated that anti-nociceptive effect of TFG in the second phase was due to its anti-inflammatory effects or at least the anti-inflammatory effect is one reason for anti-nociceptive effects of TFG in this phase of formalin test. The extract also exhibited strong anti-pyretic activity in yeast induced pyrexia in these rats.

Parvizpur et al. (2004) opined that the serotonergic system might be involved in the central anti-nociceptive action of fenugreek leaf extract as they demonstrated that the analgesia induced by *Trigonella foenum-graecum* leaf aqueous extract in the second phase of the formalin test was at least partially dependent on the intact spinal serotonergic system. They also observed that the extract exerted antagonistic effect through P2X receptors and this effect is partially responsible for the analgesia induced by *T. foenum-graecum* in the first and second phases of formalin test and also in tail-flick test, suggesting the involvement of the spinal purinoceptors. *Trigonella foenum-graecum* leaf aqueous extract at 500 mg/kg did not exhibit anti-nociceptive activity in the tail-flick latency. But a dose of 1000 and 2000 mg/kg showed good activity. Similarly at 500 mg/kg, only first phase of nociception in formaldehyde induced pain was inhibited where as higher doses prevented both first and second phases of nociception.

Morani et al. (2012) demonstrated that *T. foenum-graecum* has an anti-nociceptive activity in the neuropathic pain caused by partial sciatic nerve ligation (PSNL) and in the neuropathic pain caused by sciatic nerve crush injury (SNCL).

The animals were evaluated through the hot plate test, motor functional test, and measurement of motor nerve conduction velocity. The treatment was able to improve the evaluated parameters, except for the measurement of motor nerve conduction velocity in the PNSL, which indicates a modulation of sensory pathway or inhibition of cytokine release as one of the probable mechanisms. By employing high performance liquid chromatographic (HPLC) analysis, these authors reported 18%w/w trigonelline HCl in the standardized extract.

Aqueous, hydro-alcoholic, methanol or ethanol extracts of fenugreek leaves and seeds exhibited very promising analgesic activity in one or the other of the models tested.

Methanol extract of fenugreek leaves showed 93% inhibition of acetic acid induced writhing in mice at 400 mg/kg dose. The extract also enhanced the latency time in tail immersion test of the mice in hot water suggesting a central analgesic activity. The extract also exhibited central depression activity as was evident from the open-field test. It also showed cytotoxicity in a brine shrimp lethality test (Akter et al. 2011).

Extracts of fenugreek leaves and seeds prepared with petroleum ether, chloroform, ethyl acetate, and methanol all showed good central analgesic activity at 50 mg/kg i.p. in the hot plate induced pain and peripheral analgesic activity in the acetic acid induced writhing in mice. Of all the extracts, methanol extract of the leaves had the highest activity (Bhalke et al. 2009).

Abbas et al. (2016) observed water extract of the sprouts of fenugreek seeds had significant anti-pyretic activity in Brewer's yeast induced pyrexia in mice. The extract also exhibited significant analgesic activity in acetic acid induced writhing, in hot plate induced pain models and anti-inflammatory activity in carrageen induced paw edema.

Water soluble ethanol extract of fenugreek seeds at 40 mg/kg dose (p.o.) reduced the acetic acid induced writhing in mice by 75% and pain threshold by 45% over control mice. At 20 mg/kg given i.p. to rats, the extract reduced the carrageenan induced hind paw edema by 59% thereby pointing towards a peripheral as well as central mediated analgesic activity of the extract (Vyas et al. 2008).

Water extract of fenugreek seeds at 200 mg/kg exhibited significant analgesic activity in mice in acetic acid induced writhing and Hafner's tail clip method (Satish et al. 2013).

Mandegary et al. (2012) made a detailed study on the total extract of fenugreek seed as well as the alkaline chloroform fraction (AKC), acidified chloroform fraction and water soluble fraction derived from the total extract. Total seed extract at 100 mg/kg and AKC at 5 mg/kg significantly decreased number of painful behaviors at early phase (T10) of formalin test as well as at the late phase of pain in a dose-dependent manner. The total extract showed presence of alkaloids, saponins, and flavonoids while the AKC fraction was found to be devoid of flavonoids and rich in alkaloids suggesting the anti-nociceptive effect of both AKC and total extract might be probably due to the presence of alkaloid. The AKC fraction dose-dependently inhibited both phases of pain in this model. Since the late phase of formalin test is an inflammatory stage mediated by cyclooxygenase, it might be possible that the anti-

nociceptive effect of AKC fraction is mediated via the inhibition of cyclo-oxygenases and/or lipoxygenases (and/or inflammatory mediators).

In carrageenan induced paw edema, aqueous fraction and acidified chloroform fraction (ACC) exhibited optimal inhibition 1 and 4 h after the injection of carrageenan. The anti-inflammatory effect of these fractions was attributed to the flavonoids present in them.

Sindhu et al. (2012) found that treatment with highly polar mucilage fraction of *T. foenum-graecum* seed showed long lasting analgesic activity in adjuvant induced chronic arthritis of rats. It was postulated that the activity might be through the inhibition of leukotriene (LT) synthesis, inducible nitric oxide synthase (iNOS) and the release of inflammatory mediatorsIL-1, IL-6, TNF-α, and TNF-β.

They noted significant decrease in COX-2 level, in the 5-LOX activity, in C-reactive protein, in nitrite levels, in the activity of myeloperoxidase, in the level of erythrocyte sedimentation rate and in total white blood cell count, and an increase in red blood cell count and hemoglobin level, indicating a possible involvement of mediators of inflammatory processes. Strong lipid peroxidation, COX-1 and -2 enzyme inhibitory activities of fenugreek seed extracts were also reported by Liu et al. (2012).

Analgesic and anti-pyretic activity of the various extracts of fenugreek leaves and seeds offer promising avenues in our quest for newer molecules for these disorders. Even though, trigonelline has shown definite analgesic activity, it is not the main constituent in the fenugreek leaves suggesting that other chemical entities possibly flavonoids may also be having good analgesic activity. Trigonelline offers great opportunities to develop newer structural derivatives. The work of Parvizpur et al. (2004) is also very interesting and warrants further in depth studies to isolate the active compound/s from the leaves and unraveling the underlying mechanisms of their analgesic activity.

18.3.5 Immunomodulatory Activity of Fenugreek (*Trigonella foenum-graecum)*

There are a few studies on the effect of fenugreek extracts on the immunomodulatory activity in mice. Since, the immune system is intimately integrated with inflammation and cancer, such studies are very much useful in understanding the various activities of fenugreek. The Aqueous herb extract of fenugreek (100 mg/kg) prevented suppression of lymphoid cellularity and humoral immune functions by a single administration of deltamethrin (15 mg/kg) in mice. There was significant increase in hemagglutination titre (HT), plaque formation, and quantitative hemolysis of SRBC (Rehman et al. 2006).

Hafeez et al. (2003) reported that water extract of fenugreek seeds exhibited significant immunomodulatory activity in the humoral and cellular immunity of mice 100 mg/kg dose showed optimal activity. They did not find any increase in the lymphoid organ cellularity but noted significant increase in bone morrow cellularity, humoral immunity as assessed by plaque forming cell assay and quantitative

hemolysis of SRBC assay. The increase was nearly 100% over the control animals. An increase in haemmaglutinationtitre value, delayed type hypersensitivity response, phagocytic activity and proliferation of lymphocytes as assessed by ^{3}H-thymidine incorporation was also observed in the mice treated with fenugreek seed water extract. These studies suggest that fenugreek increases humoral immunity.

Fenugreek seed powder decreased the immunosuppressive activity of cyclophosphamide by 57–108%. The leucopenia, decrease in weights and cellularity of lymphoid organs, serum g-globulin levels, delayed type of hypersensitivity response and delay in the skin burn healing process were all reversed by the oral treatment of fenugreek seed powder in rats (Gamal Ramadan et al. 2011).

18.3.6 Anti-inflammatory and Anti-arthritic Activity of Fenugreek (*Trigonella foenum-graecum)*

In a number of studies, different extracts of fenugreek exhibited potent anti-inflammatory and anti-arthritic activities in various animal models. The alcohol or hydro-alcohol extracts were found to elicit anti-inflammatory activity by suppressing the production of tumor necrosis factor-α (TNF-α), inhibiting Cyclooxygenase-2 (COX2) and inflammatory cytokines like of IL-1α, IL-1β, IL-2, IL-6.

In an indirect method to assess the anti-arthritic activity of water and n-butanol soluble fractions of fenugreek seeds, Trivedi (2015) noted significant protein denaturation inhibitory and protease inhibitory activity in an in vitro test system. The authors opined that since auto-antigens in arthritis are produced as a result of protein denaturation, this activity of the extracts supports the hypothesis that they have anti-arthritic activities.

The alcohol and aqueous extracts of fenugreek seeds exhibited significant COX2 inhibition to the extent of 57–59%. (Ramadhan 2020).

Methanol extract of fenugreek seeds suppressed the production of TNF-α initiated by phorbol myristate acetate and subsequent production of inflammatory cytokines such as IL-1, IL-6, in human monocytic cell line (THP-1) (Kawabata et al. 2011).

Seventy percent ethanol extract of the seeds at 75 mg/kg and 150 mg/kg p.o significantly reduced the paw edema caused by injection of carrageenan, histamine, and serotonin. The extract, when given for 7 days, significantly inhibited the formation of granulomatous tissue in rats suggesting the inhibition of chronic inflammation. The extract also exhibited significant anti-oxidant activity in DPPS and lipid peroxidation models (Subhashini et al. 2011).

A 400 mg/kg p.o dose of 70% ethanol extract of the seeds administered to Complete Freund's Adjuvant (CFA) induced female rats for 21 days significantly reduced the enhanced levels of IL-1α, IL-1β, IL-2, IL-6, and TNF-α in the synovial fluids by 79.82%, 76.95%, 62.5%, 66.6%, and 74.78%, respectively. There was also a significant reduction in the action of LPO and enhancement of activity in SOD, GSH of cartilage tissue by treatment with 400 mg/kg extract (Suresh et al. 2012).

The effect of oral administration of petroleum ether extract of the seeds was evaluated in rats for its anti-inflammatory and anti-arthritic activities in carrageenan and formaldehyde induced rat paw edema as also in CFA and cotton pellet induced chronic inflammation. The oily extract at 0.5 ml/kg reduced paw edema by 37.3% and 85% in these two models. There was 45% reduction in the cotton pellet granuloma formation in the treated groups. A significant reduction in the elevated serum and liver SGPT and ALP was noted in the cotton pellet induced granuloma model of arthritis. The high content of unsaturated fatty acids are thought to be responsible for the anti-inflammatory and anti-arthritic activities by virtue of their ability to act as powerful anti-oxidants (Pundarikakshudu et al. 2016).

Intra-peritoneal administration of the petroleum ether extract significantly reduced carrageenan induced rat paw edema and also rendered protection against carbon-tetrachloride induced liver injury (Öner et al. 2007).

Purified mucilage from the ethanol extract of the seeds at 75 mg/kg exhibited maximum reduction of inflammation in carrageenan and CFA induced arthritis models. The elevated levels of serum SGOT, SGPT, C-reactive proteins, nitrites, serum and tissue lipid peroxidase, MPO, PBMN, COX-2, and 5-LOX which were increased in the CFA induced arthritis were reduced by daily treatment with fenugreek mucilage from day 5 to day 20. The treatment significantly brought down the elevated levels of ESR, WBC and increased RBC and hemoglobin in these arthritic rats. There was extensive cellular infiltration and edema in the paw tissues of CFA arthritic rats while the paws of rats treated with fenugreek mucilage exhibited mild cellular infiltration and edema. Fenugreek mucilage also prevented the depletion of anti-oxidant enzymes SOD, GSH, and GPx in the CFA treated arthritis rats. These results render strong support for the anti-arthritic activity of fenugreek seeds and the galactomannans present in the mucilage are considered to be mainly responsible for this activity (Sindhu et al. 2012).

Liagre et al. (2004) noted that 40 μM diosgenin treatment dramatically reduced the proliferation of RA fibroblast-like synoviocytes (RA FLS). Cell shrinkage, cytoplasm condensation, and formation of cytoplasmic filaments appeared after 24, 48, and 72 h of diosgenin treatment. Compared to control untreated cells, cells treated with diosgenin exhibited a twofold increase in the caspase-3 activity after 24 h and over-expression of COX-2 and subsequent higher production of PGE2 by four fold after 48 h. It was also noted that IL-1ß, aCOX-2 inducer, significantly increased diosgenin-induced apoptosis of human RA FLS. These authors concluded that the apoptosis caused by diosgenin in RA FLS was due to upregulation of COX-2 and strong production of PGE2. These findings are in contrast to the observations of other researchers who noted an inhibition of COX-2 in vivo experiments with arthritic rats.

Thus these studies on animals clearly suggest that fenugreek has some beneficial effects in arthritis and inflammation.

18.3.7 Anti-oxidant Activity of Fenugreek (*Trigonella foenum-graecum*)

The polyphenols and flavonoids present in fenugreek contribute to various pharmacological activities by virtue of their anti-oxidant activities. They render protection by enhancing the anti-oxidant enzymes superoxide dismutase (SOD), glutathione reductase (GR), catalase, and glutathione peroxidase (GPx) in tissues such as heart, muscle, liver, and brain during diabetes (Baquer et al. 2011).

In diabetic rats, leaf powder of fenugreek significantly lowered lipid peroxidation and increased the anti-oxidant system (Annida et al. 2005).

The flavonoid and polyphenol-rich fraction of aqueous extract of fenugreek significantly increased anti-oxidant enzyme activities in the diabetic kidneys thereby protecting them from functional and morphological injuries (Xue et al. 2011).

Kaviarasan et al. (2004, 2006, 2008) reported that polyphenol-rich fenugreek seed extract significantly reduced H2O2-induced oxidative modifications in normal and diabetic human erythrocytes, prevented ethanol, induced liver toxicity by retaining the activity of enzymes such as alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) and the electron transport component cytochrome c reductase. There was significant reduction in the levels of lipid peroxidation products and protein carbonyl content, an increase in the activities of anti-oxidant enzymes and restoration of the levels of thiol groups was noted.

Administration of fenugreek seed ethanol extract (FPEt) restored the altered levels of liver function enzymes aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin, and gamma-glutamyl transferase and decreased liver glycolgen. The treatment also improved lipid profile and reduced collagen content, aldehyde content and peroxidation (Kaviarasan et al. 2007).

In a goat model of H_2O_2- and CCl_4-induced liver damage, ethanol extract of *Trigonella* leaves showed a significant hepatoprotective effect as evidenced by decreased levels of enzymatic and nonenzymatic anti-oxidant enzymes (Meera et al. 2009).

Dixit et al. (2005) found that diosgenin and trigonelline did not show any anti-oxidant activity. It was demonstrated that the supplementation of fenugreek seed powder in the diet led to a reduction in biomarkers of oxidative damage in alloxan-diabetic rats (Ravikumar and Anuradha 1999).

The phenol rich extracts of fenugreek also showed good anti-oxidant activity in in vitro test systems like DPPH scavenging activity, and ascorbate-Fe* induced lipid peroxidation.

Yacoubi et al. (2011) extracted fenugreek with cold acetone water (70:30). The total phenolic content of the extract was found to be 15.1 mg gallic acid equivalent/g dry powder. The extract showed 74.7% DPPH scavenging activity which is equivalent to the activity of 73.5 mM Trilox. The dried extract was tested for anti-oxidant activity in experimental pulmonary fibrosis induced by bleomycin (BLM) in rats. The animals were treated with either 200 mg/kg extract (p.o) or given feed supplemented with 20% fenugreek seed powder. The treatment was given from

day 3 to day 18 after bleomycin injection. There was a reduction of melondialdehyde (MDA) levels of the treated groups with a corresponding increase in total anti-oxidant status (TAS) as compared to the untreated groups. Immunohistochemistry studies revealed only partial effect of the extract and powder on the bronchiolar and peribronchiolar inflammatory infiltrate. Polyphenols have an effect on inflammation, but no major effect on structural disorganisation resulting from BLM.

The polyphenols have an ability to lower the hepatic lipids in body because of their potential to modify the activities of several enzymes such as enzymes related to glucose and lipid metabolism (Madar and Shomer 1990).

The aqueous and hexane extracts of fenugreek seeds showed strong inhibition of COX-2 activity while the ethyl acetate extract showed more inhibition of COX-1 activity. Flavone-8-C-glycoside isolated from the water extract showed more COX-2 enzyme inhibition (Liu et al. 2012).

Aqueous extract of fenugreek protected the urinary bladder of mice from buthionine sulfoximine and cyclophosphamide induced uterotoxicity. The treatment normalized the decreased activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GP), and catalase (CAT) and brought down the enhanced Lipid peroxidase (LPO) activity (Bhatia et al. 2006).

Germinated sprouts of fenugreek seeds showed highest amount of total phenols on the 7th day of germination and this corresponded with highest free radical scavenging activity (Saxena et al. 2017).

Eighty percent methanol extract of the seeds of fenugreek genotype HM 348 from Swami Keshwan and Rajasthan Agricultural University, Rajasthan, India exhibited highest total phenols, DPPH free radical scavenging activity, Iron III reducing activity, Iron(II) chelating activity, and hydroxyl radical scavenging activity (Pathak et al. 2014).

The phenol extract exhibited strong anti-oxidant activity in all the test systems studied like DPPH scavenging activity, ferric reducing anti-oxidant power, ferrous ion chelating activity and inhibition of OH* mediated DNA fragmentation. The values reported for the anti-oxidant activities of phenol extract and alkaloid extract, respectively, are DPPH radical scavenging activity (0.28 and 0.36 mmol Troloxequi/g); metal chelating activity (0.14 and 4.03 mmol EDTA equi/g); FRAP values (0.43 and 1.78 mmol Fe2+equi/g). Both the phenol extract and trigonelline strongly inhibited dipeptidyl peptidase–IV enzyme which is a key enzyme involved in the progress of diabetes. The activity was found to be four times more with the phenol extract over the alkaloid extract (Sreerama and Rachana 2019).

Seventy percent hydro-alcohol extract of fenugreek seeds exhibited a prominent anti-glycation and glycation reversal activity. The extract was rich in flavonoids and phenols and had free radical scavenging activity on DPPH, FRAP, and ABTS (Abeysekera 2018).

Ethanol extract of fenugreek seeds (250 mg/kg) restored the lipid peroxide, SOD, Catalase, GST and reduced glutathione levels in the brain of alloxan-induced diabetic rats (Pradeepkiran et al. 2020).

Mashkor (2014) found highest amount of total phenols and highest anti-oxidant activity in 50% acetone water and 50% methanol extracts of fenugreek seeds.

Feeding 10 g fenugreek seed powder in 100 ml water and 10 g seed powder in 100 g feed for 28 days to rats reversed the damage caused by ethylene glycol to the kidneys and prevented the formation of urinary caliculi. There was less deposition of stone crystals and there was much lesser malondialdehyde production in the kidneys of the treated group (Shekha et al. 2014).

Paul (2018) reported highest phenol and flavonoid concentrations in the water extract of fenugreek leaves as compared to the ethanol extract or extracts of the seeds. This correlated with higher anti-oxidant activity in this extract. Further, this extract showed more glucose up take in insulin-induced and non-insulin-induced 3T3L1 cell lines.

Thus the strong anti-oxidant activity of the polyphenols synergistically acts with other active substances in fenugreek in their anti-diabetic, hypo-lipidemic, anti-cancer, and anti-inflammatory activities. The activity of these flavonoids and phenols on the liver function enzymes is of greater importance as this will have a definite impact on the lipid and glucose metabolism.

18.3.8 Anti-Ulcer Activity of Fenugreek *(Trigonella foenum-graecum)*

Pandian et al. (2002) found in a rodent model that the aqueous extract and gel fraction of the fenugreek seeds rendered protection to the gastric mucosa in ethanol induced ulcer model. The activity was found to be comparable to omeprazole. The activity was thought to be due to the water soluble mucilage and flavonoid/phenolic glycosides present in the extract. The extract was found to enhance anti-oxidant potential of gastric mucosa and prevent gastric lesion formation

Aqueous extract of the seeds was found to offer protection against reflux esophagitis (RE) in rats (Kheirandish et al. 2013).

Sugaheal is a standardized trigonelline and 4-OH isoleucine rich extract prepared from fenugreek seeds which has been proved to be very effective in diabetes. A significant reduction in the ulcer index of indomethacin induced ulceration was noted with treatment of Sugaheal at 100 mg/kg. Uniformity of mucosal border with less vascular congestion and cellular infiltration were observed in the treatment group clearly showing its protective effect in the stomach. Sugaheal at 100 mg/kg restored the altered biomarkers of oxidative stress caused by indomethacin treatment. The enhanced activities of glutathione peroxidase and glutathione reductase in indomethacin induced gastric ulceration were brought to near normal values by 100 mg/kg of sugaheal treatment. It had a protective effect on the activities of Pyruvate dehydrogenase (PDH) and some of the Krebs' cycle enzymes like Isocritrate dehydrogenase (ICDH), α-Ketoglutarate dehydrogenase (KGDH), and Succinate dehydrogenase (SDH), activities of electron transport chain enzymes like NADH cytochrome C oxidoreductase and cytochrome C oxidase, collagen content of rat gastric tissues which were seriously impaired by the treatment with indomethacin. This conclusively proves the gastric protective activity of Sugaheal (Bandyopadhyay et al. 2016).

The water extract of defatted fenugreek seeds exhibited in vitro, in vivo, and in silico anti-ulcer activity in 15% ethanol induced ulcerogenic activity in AGS cell lines. The extract exhibited cytoprotection in a MTT assay. The extract at 1000 mg/kg completely prevented the formation of ulcers induced by ethanol. Key flavonoid constituents reported to be present in the extract like vitexin-7-O-glucoside, vitexin-1, luteolin, and orientin have shown remarkable degree of interaction with H+/K+ ATPase receptor binding site in silico. Thus these studies clearly support the anti-ulcerogenic and gastroprotective effects of fenugreek water extract (Figer et al. 2017).

Thus the phenolic and flavonoid compounds as well as trigonelline and 4-hydroxyisoleucine present in fenugreek offer protection against gastric ulcers.

18.3.9 Anti-microbial and Anti-Fungal Activity of Fenugreek (*Trigonella foenum-graecum*)

Various investigators have shown effectiveness of *Trigonella* extracts against *Helicobacter pylori* (O'Mahony et al. 2005; Randhir et al. 2004; Randhir and Shetty 2007). Different extracts of fenugreek exhibited anti-fungal activity against *Fusarium graminearum*, *Botrytis cinerea*, *Alternaria* sp., *Rhizoctonia solani* and *Pythium aphanidermatum* (Haouala et al. 2008).

Chandra et al. (2011) found the aqueous extract of fenugreek to be effective against *Escherichia coli* and *Malassezia furfur* but to be ineffective against *Pseudomonas putida*. *E. coli* was found to show the highest sensitivity towards acetone extract, while methanol extract showed an elevated response against *Pseudomonas* spp. It is also suggested that sprouted or the germinated seeds had enhanced anti-microbial activity specifically against *Hellicobacter pylori* (Kor et al. 2013).

Sharma et al. (1996b) observed fenugreek leaf methanol and acetone extracts to have significant anti-microbial activity of on *Staphylococcus aureus* and *Escherichia coli*. Seed extracts did not show any activity. Stem extracts had medium activity.

Since fenugreek has shown promising anti-ulcer activity, its activity against *H. Pylori* is of considerable therapeutic importance.

18.3.10 Other Activities of Fenugreek (*Trigonella foenum-graecum*)

Soluble fiber of fenugreek is hydrocolloidal in nature, gives textural appeal, thickening, emulsifying, stabilizing, gelling, and encapsulating properties. Hence, it is used in nutrition and dairy products, cereal bars, yogurts, and nutritional beverages (Kumar and Maliakel 2008).

Sowmya and Rajyalakshmi (1999) reported that microorganisms in the large intestine utilize soluble fiber of fenugreek as substrate for fermentation.

Fenugreek has been used as food stabilizer, food adhesive, food emulsifier and gum and in the preparation of various types of bakery and extruded products (Wani and Kumar 2018).

Hormonal Balancing Activity of Fenugreek (*Trigonella foenum-graecum*)

It is suggested that uterine and lactation-stimulating properties of fenugreek could be due to the presence of steroids such as saponins in the seed, which may be similar to the activity of oxytocin on uterus (Bingel and Farnsworth 1994).

Saponin I and dioscin derived from fenugreek seed showed increased release of rat growth hormone from rat pituitary cells (Shim et al. 2008).

Fenugreek seed extract has been shown to reduce thyroid hormone concentrations and serum glucose (attributed to thyroxine induced hyperglycemia) in rats (Tahiliani and Kar 2003).

Fenugreek (*Trigonella foenum-graecum*) in Cosmetics

When fenugreek paste was applied to a scalp as a treatment for dandruff, it resulted in numbness of head, facial angioedema, and wheezing. It exhibited strong sensitivity to fenugreek in patients. The presence of IgE-mediated allergy to fenugreek seeds was confirmed in these patients by scratch testing, double blind placebo controlled challenges, and immunoblotting. Healthy humans did not show such response with fenugreek extract (Patil et al. 1997).

18.3.11 Clinical Trials on Fenugreek (*Trigonella foenum-graecum*)

There are quite a few clinical studies on fenugreek involving diabetic and hyperlipidemic patients which showed beneficial effects of fenugreek. However, these studies are scientifically not well designed and the sample size was low in many instances. The type of extracts, dose, and duration of treatment is not uniform making the outcome of the studies questionable. There is much to be done in this area to validate the claims on fenugreek. Results of some of the clinical studies are discussed below.

Snehlata and Payal (2012) observed a reduction in the glycosylated hemoglobin levels and an increase in insulin sensitivity in a small group of diabetic mellitus patients treated with fenugreek seed powder.

In a clinical study involving 50 male volunteers aged 35–65 years, Furosap a standardized fenugreek seed extract containing 20% protodioscin, when given at a dose of 500 mg/kg as a single dose for a period of 12 weeks improved free testosterone levels by 46% in 90% of the study population. 85.4% of the study population showed improvements in sperm counts. There were no toxic symptoms as evidenced by normal lipid profiles, hemogram, liver, and kidney histology. The patients experienced elevated mental alertness and mood (Maheshwari et al. 2017).

Chronic administration of 25 g/day of fenugreek seed powder to 60 non-insulin dependent diabetic patients for 24 weeks was found to significantly reduce the levels of cholesterol, triglycerides, LDL cholesterol, and LDL + VLDL levels by 19%,

18%, 16%, and 23%, respectively, while no significant change was observed in HDL cholesterol. The effect was found to be sustained and long lasting (Sharma et al. 1996a, 1996b). No changes were observed in serum creatinine or blood urea nitrogen (BUN) in a series of 60 diabetic patients given fenugreek powder for 12 weeks (Sharma et al. 1996a).

FENFURO contains furostanolic saponins extracted from the seeds of fenugreek, which exert hypoglycaemic effect in type II diabetic patients. It was given to diabetic patients as an adjuvant therapy at the dose of 500 mg twice daily along with the regular anti-diabetic therapy for 12 weeks. After 12 weeks, there was a significant decrease in the mean fasting glucose levels from baseline value of 159.97 mg/dl to 98.76 mg/dl; in the post-prandial glucose levels from baseline value of 254.33 mg/dl to 142.30 mg/dl; and in glycated hemoglobin (HbA1c) from baseline levels of 9.37% to 6.11% in the patients given FENFURO along with regular anti-diabetic medication (Gupta et al. 2018). Bawadi et al. (2009) found in a clinical trial that drinking 5 g fenugreek seed water extract along with chewing the seeds brought about significant decrease in post-prandial glucose levels in type 2 diabetic patients immediately after 2 h of consumption.

Hasan and Rahman (2016) found that 10 g/40 ml fenugreek water extract had significantly reduced blood sugar level within 5 days in a group of 9 patients.

Sfar et al. (2018) carried out a controlled clinical studies on 56 female obese and type 2 diabetic patients with the mean of age is 47 years, on oral anti-diabetic drugs with unbalanced blood glucose and lipid parameters. Patients received a 15 g dose of fenugreek powder daily in the morning for a period of 4 weeks. There was a significant decrease in BMI and waist circumference, an increase in HDL-C and a decrease in LDL-C levels, as well as a decrease in fasting blood glucose, resistin, and insulin levels. The reduction of resist in has a positive sign to the health of diabetic patients as it has detrimental effects on insulin sensitivity and its proinflammatory role.

In a 3-year randomized, controlled, parallel study, involving men and women aged between 30 and 70 years, type 2 diabetic patients were given fenugreek powder 5 g twice a day before meals. At the end of the study, there was a significant reduction in the cumulative incidence rate of diabetes. Also fasting blood glucose and post-prandial blood glucose levels, LDL cholesterol levels significantly decreased in fenugreek treatment group with a corresponding increase in serum insulin levels. The outcome of diabetes in the fenugreek treated group was positively associated with increased serum insulin and decreased insulin resistance (HOMA IR) (Gaddam et al. 2015).

A study by Ikeuchi et al. (2006) showed that fenugreek seeds increased the strength and endurance in healthy humans.

Repeated administration of fenugreek seed extract to healthy overweight subjects decreased the consumption of dietary fats (Chevassus et al. 2010).

Fenugreek given at a dose of 2.5 g twice daily for 3 months had no significant effect on blood sugar or lipid profiles in healthy volunteers. But the same treatment when given to coronary artery disease patients with or without type-2 diabetes

brought about significant improvements in the elevated blood sugar and lipids (Bordia et al. 1997).

In a pilot study, subjects with frequent heartburn, a symptom of indigestion, when given fenugreek fiber product, 30 min before the meals/day for 2 week, showed diminished heartburn severity (DiSilvestro et al. 2011).

Ruby et al. (2005) undertook a clinical trial on the glycogen resynthesis in six cyclists postcycling exercise. They were fed 1.8 g/kg body weight dextrose + fenugreek standard extract that provided 2.0 mg/kg 4-hydroxy-isoleucine. The trial results showed that 63% higher glycogen resynthesis on dextrose + 4-hydroxy-isoleucine administration over administration of dextrose alone following glycogen depleting cycle exercise in trained male cyclists.

In a clinical study involving 114 newly diagnosed Type II diabetes patients, Geberemeskel et al. (2019) administered solution of 25 g fenugreek powder to 57 patients and metformin to the other 57 patients for 30 days and found significant lowering of triglycerides, total cholesterol and low density lipoprotein cholesterol by 23.53%, 13.6%, and 23.4%, respectively, when compared to the lipid profiles of the metformin group. Similarly, high-density lipoprotein cholesterol increased 21.7% in the patients administered fenugreek seed powder over the metformin treatment groups. The soluble fiber content and saponins are considered to be responsible for the activity which acts by indirectly increasing the thyroid hormone or delayed absorption of glucose and fatty acids in the digestive tract.

In a study conducted by Raghuram et al. (1994) involving 10 non-insulin-dependent diabetic patients, food supplemented with 25 g fenugreek seed powder for 15 days enhanced intravenous glucose tolerance as indicated by a reduction (26%) in the area under curve of glucose, its rapid metabolic clearance and short plasma half-life. Fenugreek treatment significantly increased molar insulin-binding sites of erythrocytes. This clearly suggests an improvement in the glucose utilization. Thus fenugreek seeds may act both locally delaying the absorption of glucose from the gastrointestinal tract and also by improving systemic utilization of glucose.

These clinical trials, though having limitations, clearly indicate the therapeutic utility of fenugreek in diabetes and lipid disorders.

18.3.12 Toxicity of Fenugreek (*Trigonella foenum-graecum)*

Khalki et al. (2010) evaluated the potential toxic effects of fenugreek seeds (lyophilized aqueous extract) on pregnant mice and fetal development. The extract was administered to mate female mice during the entire period of pregnancy, at doses of 500 and 1000 mg/kg/day. The mothers showed no obvious symptoms of toxicity, but an increase in the fetal death rate, a decrease in the litter size, and a reduction in the fetal body weight and increase in the incidence of morphological abnormalities were observed in the offspring.

Pregnant females received 0, 500, or 1000 mg/kg/day aqueous extract of fenugreek seed by gavage for the whole period of gestation. These results suggest that prenatal exposure of mice to a high dose of fenugreek seeds caused growth

retardation and altered neurobehavioral performance in the post-weaning period (Khalki et al. 2012).

The extract, containing a minimum of 40% 4-hydroxyisoleucine, has been evaluated using the standard tests such as reverse mutation assay; mouse lymphoma forward mutation assay; mouse micronucleus assay as recommended by US Food and Drug Administration (FDA) for food ingredients. It has not been found to be genotoxic under the tested conditions, thus providing support that fenugreek extract supplementation of foodstuffs for people with diabetes is expected to be safe (Flammang et al. 2004).

Al-Ashban et al. (2010) noted that while ethanol extract of defatted fenugreek seeds did not show any acute toxicity up to 3 g/kg body weight, long term usage for 90 days at 100 mg/kg, had mortality in the animals. Their body weights reduced. There was significant decrease in the weight of testes in the male mice. Morphological abnormalities in the sperms such as swollen acrosomes, amorphous, microcephali, megacephali, rotated head and flat head increased indicating its spermitoxic activity.

Nakhla et al. (1991) found crude saponins of fenugreek to have caused epithelial degeneration of renal tubules. That fenugreek seeds may have anti-fertility activity was also indicated by Kassem et al. (2006).

Debitterized fenugreek powder did not exhibit any signs of toxicity in acute and sub-chronic toxicity studies in mice and rats (Muralidhara et al. 1999).

When fenugreek seeds were given for 90 days, weanling rats did not experience significant hematological, hepatic, or histopathological changes (Rao et al. 1996).

Though fenugreek has been proved to be safe by and large its male anti-fertility activity and toxic effect on pregnant rats, further in-depth studies are required to establish its overall safety.

List of different pharmacological activities of different plant part and constituents of Fenugreek are mentioned in Table 18.2.

18.4 Conclusion

A detailed study and analysis of the data from various research groups indicate that fenugreek has multiple therapeutic activities. Its anti-diabetic, anti-hyperlipidemic, and anti-cancer activities are really impressive. Saponins, soluble fibers, 4-hydroxyisoleucine, trigonelline, diosgenin, and protodioscin hold great promise in future development of anti-diabetic and anti-cancer molecules. Another new dimension added to the plethora of pharmacological activities of fenugreek is the analgesic and anti-pyretic activities as also its anti-arthritic activity. This common domestic spice is really a wonder spice and has immense medicinal value. Further research and process development are expected to bring out some potential and safe medicaments from fenugreek.

Table 18.2 List of Pharmacological activities of Fenugreek

Name of plant part of *Trigonella foenum-graecum* L.	Type of extract/name of constituents	Pharmacological activity
Seed	Seed powder	Ant diabetic activity
		Analgesic and Anti-pyretic activity
		Immunomodulatory activity
		Anti-lithogenic activity
		Anti-Cholesteremic activity
		Anti-cancer
		Anti-ulcer activity
		Anti-oxidant activity
		Hormonal balancing activity
	Aqueous extract of seed powder	Ant diabetic activity
		Analgesic and Anti-pyretic activity
		Immunomodulatory activity
		Anti-cholesteremic activity
		Anti-ulcer activity
		Anti-oxidant activity
		Anti-microbial activity
		Anti-arthritic activity and Anti-inflammatory activity
		Protease inhibitory activity
	Alcoholic extract of seed powder	Ant diabetic activity
		Anti-inflammatory activity
		Anti-cancer
		Anti-oxidant activity
		Anti-cholesteremic activity
	Seed oil	Anti-arthritic activity and Anti-inflammatory activity
		Anti-oxidant
		Anti-cancer
	Hydro-alcoholic extract of seed powder	Anti-diabetic activity
		Anti-cancer
		Anti-oxidant
	n-Hexane extract of Seed powder	Analgesic activity
	Poly phenolic extract of seed powder	Anti-diabetic activity
		Anti-oxidant activity
	Chloroform seed extract of powder	Anti-cancer
	Germinated sprouts of seeds	Anti-oxidant

(continued)

Table 18.2 (continued)

Name of plant part of *Trigonella foenum-graecum* L.	Type of extract/name of constituents	Pharmacological activity
	Defatted seed powder	Anti-diabetic activity
		Anti-ulcer
		Anti-arthritic activity and Anti-inflammatory activity
Whole plant	Alcoholic extract of plant	Anti-cancer
Leaf	Leaf powder	Analgesic activity
		Anti-pyretic activity
		Anti-cholesteremic activity
		Anti-oxidant
	Aqueous extract of leaf powder	Anti-diabetic activity
		Analgesic activity
		Anti-nociceptive activity
		Anti-oxidant
	Alcoholic extract of leaf	Central analgesic activity
		Anti-microbial
Name of phytoconstituents	Diosgenin	Anti-arthritic activity and Anti-inflammatory activity
		Anti-diabetic activity
		Analgesic activity
		Anti-pyretic activity
		Anti-cancer
		Anti-cholesteremic activity
	4-hydroxyisoleucine	Anti-diabetic activity
		Anti-ulcer
		Anti-cholesteremic activity
	Trigonelline	Analgesic activity
		Anti-diabetic activity
		Anti-ulcer
		Anti-cancer
		Anti-cholesteremic activity
	Galactomannan	Anti-arthritic activity
		Anti-cholesteremic activity
		Anti-diabetic activity
	Flavonoids	Anti-oxidants
		Anti-inflammatory
		Anti-cancer
		Anti-ulcer
	Saponins	Anti-cholesteremic activity
		Anti-diabetic activity
		Anti-cancer
		Hormonal balancing activity

(continued)

Table 18.2 (continued)

Name of plant part of *Trigonella foenum-graecum* L.	Type of extract/name of constituents	Pharmacological activity
	Unsaturated fatty acids and purified mucilage	Anti-arthritic activity and Anti-inflammatory activity
		Anti-oxidants
		Anti-ulcer

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Trigonella foenum-graecum and Its Bioactive Compounds Having Potential Antidiabetic Activity

19

Heena Tabassum and Iffat Zareen Ahmad

Abstract

Most drugs are available in the market for use in diabetes management. Their side effects and high costs, however, underline the need for natural herbal medicines. Diabetes is a significant health problem that can cause substantially higher complications. The treatment of diabetes is still extremely unsatisfactory, despite numerous preventive methods and armoires of medication. The management of diabetes still remains grossly unsatisfactory. Diabetes mellitus is a critical disease with constantly increasing death rates. It is characterized by elevated level of blood glucose due to the insufficient production of insulin and insulin resistance, or both, causing abnormalities in carbohydrates, lipids, and proteins metabolism. The immediate need is to identify novel food based bioactive agents or drugs for curing or preventing diabetes, with comparatively fewer side effects. Plant secondary metabolites have long been known to have health benefits against various oxidative stress related diseases including diabetes. *Trigonella foenum-graecum* herb has an enormous potential to prevent or cure diabetes more than other plant species especially due to the presence of unique active constituents such as alkaloids, flavonoids, steroids, and saponins. Furthermore, the use of fenugreek as a hypoglycemic official medication is still to be examined due to a lack of adequate science or clinical studies. The preventive and healing properties of this potent herb against diabetes and its complications are recommended to be carefully examined. This chapter presents information on bioactive compounds of *Trigonella foenum-graecum* and its strong antidiabetic power.

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Keywords

Fenugreek · Alkaloids · Flavonoids · Steroids · Saponins antidiabetic potential · Diabetes

Abbreviations

AGP	α(1)-acid glycoprotein
AICAR	5-Aminoimidazole-4-carboxamide ribonucleotide
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase test
AMPK	Adenosine monophosphate-activated protein kinase
AST	Aspartate aminotransferase
BSO	Buthionine sulfoximine
CAT	Catalase
CENPF	Centromere protein F
CNAs	Copy number alterations
COX	Cyclooxygenase
CRF	Corticotropin-releasing factor
CRHBP	Corticotropin-releasing hormone-binding protein
CY	Cyclophosphamide
ERK	Extracellular kinases signal-regulated
FOXM1	Forkhead box protein M1
GGT	Gamma-glutamyl transferase
GMP	Guanosine monophosphate pathway
GPX1	Glutathione peroxidase 1
GSH	Glutathione
GSHPx	Glutathione peroxides
GSSG	Oxidized glutathione
GST	Glutathione-s-transferase
H2O2	Hydrogen peroxide
HBV	Hepatitis C virus
ICAM-1	Intercellular adhesion molecule-1
IGFR	Insulin-like growth factor receptor
IL	Interleukin
LDH	Lactate dehydrogenase
LKB1	Liver kinase B-1
LL/2	Lewis lung carcinoma
LOX	Lipid oxidation products
LPO	Lipid peroxidation
MDA	Malondialdehyde
MMP	Matrix metalloproteinase
MnSOD	Manganese superoxide dismutase
MTT	3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl

NAC	N-acetylcysteine
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
ncRNAs	Non-coding RNA
NDEA	N-nitrosodiethyl amine
NF-kB	Nuclear factor kappa B
NO	Nitric oxide
APX	Ascorbate peroxidase
AsA	Ascorbic acid
ASO	Ascorbate oxidase
CA	Citric acid
CAT	Catalase
d-ALA-D	Delta-aminolevulinic acid dehydratase
DHAR	Dehydroascorbate reductase
FRAP	Ferric reducing ability of plasma
GPO	Guaiacol peroxidase
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
GST	Glutathione-S-transferase
LOX	Lipoxygenase
MDA	Malondialdehyde
MDHAR	Monodehydroascorbate reductase
NM	Nonmetalliferous
NPSH	Nonprotein thiol
PAL	Phenylalanine ammonia-lyase
PC	Phosphatidylcholine
PCD	Programmed cell death
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PLNH	Paecilomyces lilacinus NH
PM	Plasma membrane
POD	Peroxidase
PPO	Polyphenol oxidase
PS	Phosphatidylserine
PSH	Protein thiol
PSII	Photosystem II
ROS	Reactive oxygen species
Rtn	Rutin

19.1 Introduction

Diabetes or hyperglycemia is a chronic metabolic disorder possessing a major health challenge worldwide and is very common and most prevalent diseases that affect the citizens of both developed and developing countries. Estimation gives the proportion that about 25% of the world population is affected by the disease. In India, there are around 40.9 million diabetic patients and the expectation is still high up to 69.9 million by 2025 (Mohan et al. 2007). Due to the high rate of the diabetic patients, India has been tagged as the diabetic capital of the world (Joshi and Parikh 2007). Lack of an urgent preventive measure could lead it to become the major health issue among the Indian population. Number of deaths due to diabetes estimated by the Indian Diabetes Federation (IDF) is around 3.9 million that represents 6.8% of the total global mortality count (IDF 2006). The carbohydrate metabolism is affected due to the disruption in the optimum level of the insulin in blood which leads to the disease (Maiti et al. 2004). Diabetes is also caused due to the deficiency of the insulin function or due to the insensitivity of the organ to insulin. This anomaly results in continued hyperglycemia which affects other metabolic pathways of the human body as well (Bastaki 2005). When left untreated this could lead to some severe damages of tissue and vascular damages that can lead to serious compilation such as retinopathy (Bearse et al. 2004), neuropathy (Seki et al. 2004), nephropathy (Looker et al. 2003), ulceration, and the cardiovascular complications (Svensson et al. 2004).

Diabetes is the most common endocrine disorder. The pancreatic endocrine hormones, insulin, and glucagon work simultaneously to control the blood glucose level in the body to an adequate level on the basis of the body's requirement. In normal conditions insulin is secreted by the β-cells of the islets of Langerhans when there is high blood sugar level in the blood. This enzyme increases the potential ability of muscles, red blood cells, and fat cells to absorb the excess sugar out of the blood and use it for other metabolic pathways that could help to restore the optimum level of sugar in blood (Gupta and De 2012). Glucagon enzyme which is secreted by the α-cells of the pancreas in response to the low blood sugar level acts in a different manner as compared to insulin. This enzyme helps the liver and other muscle cells to release the stored glucose into the blood to be utilized by the working cells. Retention of high blood glucose level for long duration can ultimately lead to the long term damage to the organs like kidney, eyes, liver, nerves, heart, and blood vessels. This type of complication in such organs may cause the death of the individual (Pari and Saravanan 2004). Diabetes mellitus acts as the cause for several diseases like end stage renal disease, adult blind, non-traumatic lower extremity amputation (Gupta and De 2012).

The two main widely accepted types of the diabetes mellitus are type 1 and type 2 (Zimmet et al. 2004). Type 1 diabetes occurs in the patients with very little or no insulin secretory capacity. Type 1 is also of two types that is type 1a conferring almost 90% of the type 1 and type 1b conferring 10% of it. It is also referred as the juvenile diabetes. Type 1a results from the destruction of the pancreatic cells caused due to immunological damages and is associated with certain diseases like Addison's disease, Grave's disease, and Hashimoto's thyroiditis (Atkinson and

Maclaren 1994). Type 1b is idiopathic without any etiological basis. The patients suffering from this type of diabetes possess the predominant deficiency of insulin and are susceptible to keto-acids but there is no evidence of any autoimmune disease development (McLarty et al. 1990). The most common form of diabetes is type 2 diabetes in which the insulin production and its resistance is affected (DeFronzo et al. 1992). This type is dominating in the elderly people, over 40 years. It may occur in the obese person, person with decreased body activity and this type may also be inherited from parents to off-springs (Zimmet et al. 1990). This type is also associated with individuals suffering from hypertension and dyslipidemia. Dietary supplements, physical activity, and the oral hypoglycemic agents are responsible to enhance the disease (Zimmet et al. 2001; Wang et al. 2018; Al-Attar and Alsalmi 2019).

Fenugreek is commonly used as a spice in cooking and in small quantities is categorized as "Generally Recognized as Safe" by the U.S. Food and Drug Administration (Fetrow and Avila 1999). Fenugreek is a member of the Leguminosae (Fabaceae) family and is commonly cultivated in India, Egypt, the Middle East, and North Africa. The seeds of the plant have been used as a traditional remedy for numerous conditions including gastrointestinal disorders, gout, wound healing and inflammation, hyperlipidemia, and diabetes (Fetrow and Avila 1999). The antihyperglycemic effects of fenugreek seeds and its subfractions are demonstrated in diabetic rats (Khosla et al. 1995a, b, c), dogs (Ribes et al. 1986), and humans. The seeds also show beneficial effects in hypolipidemic subjects (Sharma 1986) and in cancer (Sur et al. 2001). Supplementation of seeds in the diet enhances the antioxidant potential in control and diabetic rats (Anuradha and Ravikumar 2001). It has been reported to have restorative and nutritive properties and to stimulate digestive processes, useful in healing of ulcers in digestive tract (Khosla et al. 1995a, b, c). Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive, and antioxidant.

Bioactive compounds isolated from fenugreek seeds include saponins (i.e., fenugreekine, diosgenin), alkaloids (i.e., trigonelline, gentianine, carpaine), amino acids, flavonoids, some of which act as insulin secretagogues (i.e., 4-hydroxyisoleucine, arginine), coumarins, mucilaginous fibers (galactomannan), nicotinic acid, and other vitamins and minerals in Fig. 19.1 (Marles and Farnsworth 1995; Fetrow and Avila 1999). Flavonoids are the secondary metabolites which besides being a good antioxidant also show an array of biological activities (Okwu and Nnamdi 2011). Flavonoids form a class of benzo-gamma pyrone derivatives that have high pharmacological potency. A great interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activity of these polyphenolic compounds (Priya et al. 2011). Much of the hypoglycemic effect of fenugreek seeds in clinical studies is likely due to the inhibitory effects of mucilaginous fibers on glucose absorption (Priya et al. 2011; Madar et al. 1988). Accumulating evidence indicates that the bioactives compounds of *Trigonella foenum-graecum* provide protection against diabetes and its complications in various cell line and animal models (Table 19.1).

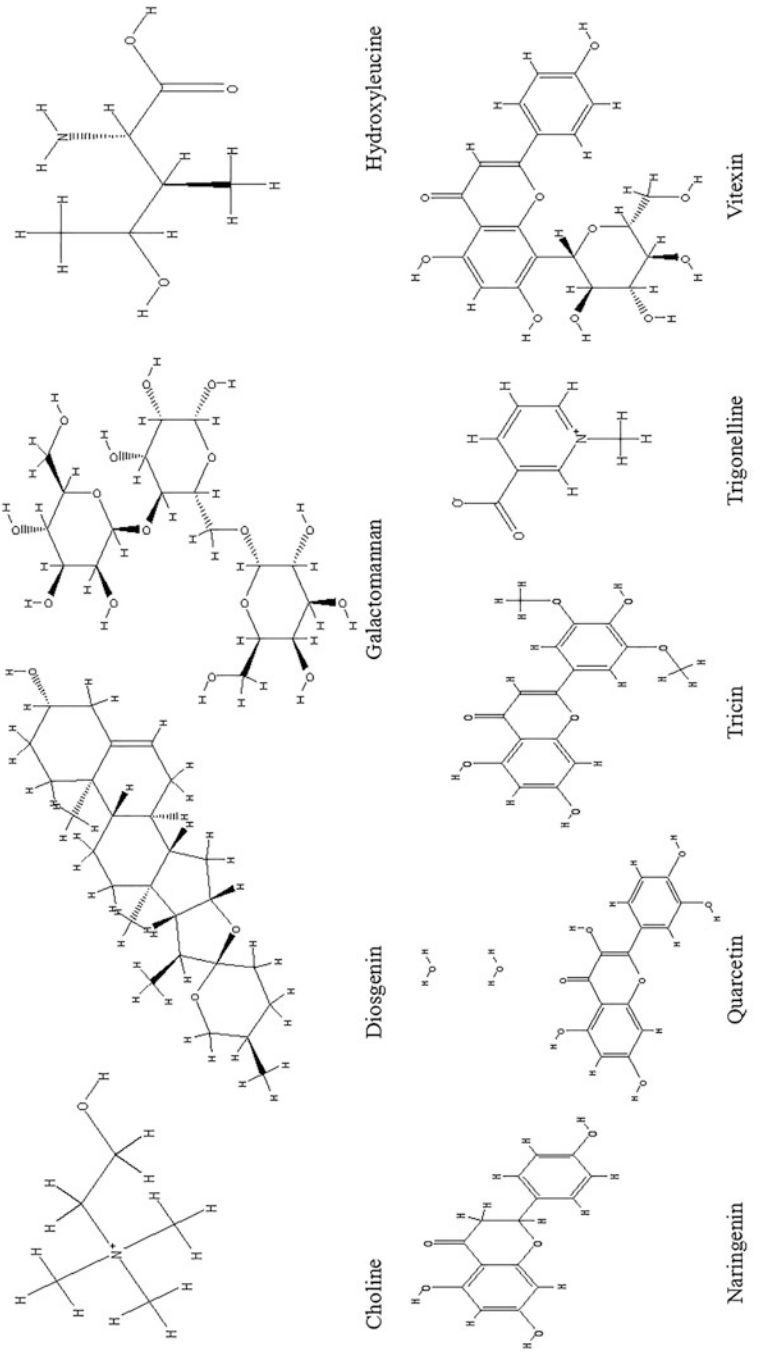

Fig. 19.1 Chemical structures of the bioactive components of *Trigonella foenum-graecum*

Table 19.1 Effects of trigonelline on diabetes and its complications in vivo and in vitro

Compounds	Property	Experiment model	Concentration	Effects	References
Trigonelline	Hypoglycemic effect	Type 2 diabetic seen in male KK-Ay/Ta Jcl and C57BL/6J mice	0.056% of the diet for 28 days	↑ Glucose tolerance in diabetes with obesity on day 22–23 ↑ Liver glucokinase/ glucose-6-phosphatase ratio and decreased serum TNF	Yoshinari and Igarashi (2010)
	Hypoglycemic effect	Streptozocin- and high carbohydrate/high-fat diet-induced type 2 diabetes on Sprague-Dawley rats	(180–220 g) 50 mg/kg (i.g.) for 28 days	↓ Blood glucose	Zhou et al. (2011)
	Hypoglycemic effect	Alloxan-induced diabetes Sabra albino rats	250 or 1000 mg/kg via a stomach tube or in the drinking water	↑hypoglycemic effect	Mishkinsky et al. (1974)
	Inhibition of intestinal glucose uptake	In vitro using rabbit intestinal brush border Membrane vesicles on New Zealand white rabbit	IC50 = 19 mM	⊥ Intestinal sodium-dependent glucose uptake	Al-Habori et al. (2001)
	Increased glucose tolerance	15 overweight men	500 mg/kg (i.g.) 15 min following a 2 h, 75 g OGTT	↓ Blood glucose	Van Dijk et al. (2009)
	Increased glucose tolerance	Non-obese type 2 diabetic rats: male Wistar and GK rats	0.056% of the diet for 43 days	↓ Glucose level between 15 and 60 min of the OGTT compared to controls, ↑ glucose tolerance	Yoshinari et al. (2009)

(continued)

Table 19.1 (continued)

Compounds	Property	Experiment model	Concentration	Effects	References
	Improved insulin resistance and cell regeneration	Non-obese type 2 diabetic rats: male Wistar and GK rats	0.056% of the diet for 43 days	↑ Insulin level after 15 min of the OGTT; ↓ over the next 120 min (unlike the gradual increase observed in the control rats)	Yoshinari and Igarashi (2010)
	Improved insulin resistance and cell regeneration	Type 2 diabetic mice male KK-Ay/Ta Jcl and C57BL/6J mice	0.056% of the diet for 28 days	↓ Fasting serum insulin	Zhou et al. (2011)
	Improved insulin resistance and cell regeneration	15 overweight men	500 mg/kg (i.g.) 15 min following a 2 h, 75 g OGTT	↓ Insulin levels; no effect on insulin area under the curve values during the OGTT	Abe and Kaneda (1975)
	Improved insulin resistance and cell regeneration	Streptozocin- and high carbohydrate/high-fat diet-induced type 2 diabetes Sprague-Dawley rats	50 mg/kg (i.g.) for 28 days	↓ Serum insulin level and increased insulin sensitivity index ↑ Pancreas weight, pancreas-to-body weight ratio, and insulin content in pancreas	Bakuradze et al. (2010)
	Incretin	15 Overweight men	500 mg/kg (i.g.) 15 min following a 2 h, 75 g OGTT	↓ Gastric peptide secretion pattern following an OGTT	Zhou et al. (2011)
	Hypolipidemic effect	Non-obese type 2 diabetic rats: male Wistar and Goto-Kakizaki (GK) rats	0.056% of the diet for 43 days	↓ Serum and liver triglyceride levels ↓ Liver fatty acid synthase activity and increased liver carnitine palmitoyl	Hong et al. (2008)

				transferase and glucokinase activities	
	Hypolipidemic effect	Type 2 diabetic mice male KK-Ay /Ta Jcl and C57BL/6J mice	0.056% of the diet for 28 days	↓ Triglyceride levels in the liver and adipose tissue	Yoshinari and Igarashi (2010)
	Hypolipidemic effect	Streptozocin- and high carbohydrate/high-fat diet-induced type 2 diabetes Sprague-Dawley rats	50 mg/kg (i.g.) for 28 days	↓ Serum total cholesterol and triglyceride level	Zhou et al. (2011)
	Hypolipidemic effect	Rats (strain)	Dose and route of administration not provided	↓ Total and free plasma cholesterol levels	Abe and Kaneda (1975)
	Antioxidant effect	HT-29 cells	30 _x0005_M (24 h incubation)	↓ Cellular reactive oxygen species level	Bakuradze et al. (2010)
	Antioxidant effect	Streptozocin- and high carbohydrate/high-fat diet-induced type 2 diabetes Sprague-Dawley rats	50 mg/kg (i.g.) for 28 days	↓ Malonaldehyde and nitric oxide contents and increased superoxide dismutase, catalase, glutathione, and inducible nitric oxide synthase activities in pancreas	Zhou et al. (2011)
	Treatment of diabetic auditory neuropathy	Diabetic auditory neuropathy induced by streptozocin Male Institute for Cancer Research Mice	10 mg/kg (i.g.) for 9 weeks	Rescue of the hearing threshold shift and delayed latency of the auditory evoked potential induced by streptozocin	Hong et al. (2008)
	Treatment of diabetic auditory neuropathy	Auditory neuropathy induced by increasing doses of pyridoxine	10 mg/kg (i.g.) for 5 weeks	Rescue of the hearing threshold shift, delayed latency of the auditory evoked potential, and	Hong et al. (2009)

(continued)

Table 19.1 (continued)

Compounds	Property	Experiment model	Concentration	Effects	References
		Male Institute for Cancer Research Mice		sensory fiber loss induced by pyridoxine intoxication	
Diosgenin	Ameliorating the inflammatory changes in obese adipose tissues	3T3-L1 adipocytes and RAW 264 macrophages	TNF-a: 0.0770.01, 0.0470.02 ng/mL; MCP-1: 4.3270.23, 0.3670.04 ng/mL; NO: 1.1970.10, 1.9670.03 mM, respectively	↑ MCP-1, ↑ TNF-a, ↑ NF-kB	Hirai et al. (2010)
	Cardiovascular risk, insulin secretion, and beta cells in streptozotocin (STZ)-induced diabetic rats	Albino male rats of Wister strain	Dose of 5 or 10 mg/kg per body weight a period of 30 days	↓ BG, ↓ serum LDL, ↓ serum TC, ↑ serum HDL ↓ Hb A1c, ↓ ALT, ↓ AST, ↓ G6P, ↑ SOD, ↑ catalase, ↑ GSH, ↑ GK	Kalailingam et al. (2014)
	Antioxidant defense and to decrease the lipid peroxidation in aorta	Adult male albino rats of Wistar strain	Doses (10, 20, and 40 mg/kg b.w.) once daily for 4 weeks.	↓ BG, ↓ Hb A1c, ↑ total Hb, ↑ SOD, ↑ catalase, ↑ GSH, ↓ FI	Pari et al. (2012)
	Antioxidative and hypolipidemic effects	Male Sprague-Dawley (SD) rats (6-week-old) were obtained from Korea Experimental Animal Center	Diosgenin on rats fed with a high-cholesterol diet supplemented with either 0.1% or 0.5% diosgenin for 6 weeks	↓ Serum TC, ↑ serum HDL, ↓ TBARS, ↑ SOD, ↑ GPx, ↑ catalase	Son et al. (2007)
	Glucotoxicity, lipotoxicity, endoplasmic reticulum (ER) stress, and oxidative stress	Male Sprague-Dawley rats of age 8–10 weeks and with body weight 150–200 g were supplied by the National Centre for Laboratory Animal Sciences (NCLAS)	T2DM was induced in male Sprague-Dawley rats by feeding high-fat diet (HFD) for first four weeks and then administering streptozotocin (STZ) at a dose of 35 mg/kg b.w	↓ FFA, ↓ TNF-a, ↓ IL-6, ↑ leptin, ↑ PPARg, ↓ endoplasmic reticulum stress	Tharaheswari et al. (2014)

	Attenuating the key enzyme activities of carbohydrate metabolism and glycogen content in streptozotocin-induced diabetic rats	Male Wistar rats with body weight of 150 g to 180 g were obtained from Abdul Hakeem College, Vellore, India	Doses (15, 30, and 60 mg/kg body weight) were administered orally to normal and streptozotocin-diabetic rats for 45 days	↓ BG, ↑ liver glycogen	Saravanan et al. (2014)
	Modulation of the lipid and antioxidant profile	Male Sprague-Dawley rats (150–200 g) of 6–8 weeks old were obtained from Venkateshwara Enterprises, Bengaluru, India	Diosgenin administered orally at two doses (40 and 80 mg/kg body weight) for 14 days	↓ BG, ↓ serum TC, ↓ serum TG, ↓ ROS, ↑ SOD, ↑ GSH, ↑ neutral lipid accumulation	Sangeetha et al. (2013)
	Ameliorate hyperglycemia and diabetes	4-wk-old male KK-Ay/Ta Jcl mice from Nippon CLEA (Tokyo, Japan)	Diosgenin, 1 mM, 5 mM, 10 mM,	↓ Adipocyte size, ↑ adipogenesis, ↓ macrophage infiltration ↓ Adipocyte inflammation	Uemura et al. (2010a, b)
	Diabetic obese KK-Ay mice, HepG2 cells	4-week-old male KK-Ay/Ta Jcl mice from Nippon CLEA (Tokyo, Japan)	Diosgenin, 1 mM, 5 mM, 10 mM,	↓ Serum TG, ↓ SREBP-1c, ↓ FAS, ↓ SCD-1, ↓ ACC, ↓ Hepatic steatosis, ↓ LXRa activation	Turer et al. (2012)
4-Hydroxyisoleucine	Potentiator of insulin secretion	Isolated human and rat pancreas	Dose concentration range of 100 micromol/l to 1 mmol/l	↑ GSIS	Sauvaire et al. (1998)
	Insulinotropic and antidiabetic properties	Male Wistar rats and male mongrel dogs	Dose of 18 (mg/kg)	↑ Oral glucose tolerance, ↑ GSIS	Broca et al. (1999)
	Insulinotropic activity	Male Wistar rats Iffa Ž Credo, Lyon, France	Dose 200 mM	↑ GSIS	Broca et al. (2000)
	Insulinotropic effect	4-week-old male Sprague-Dawley rats (Janvier, Le Genest Saint Isle, France)	10-mg priming dose	↑ Oral glucose tolerance, ↑ IS, ↓ HGP, ↑ PI3K, ↓ FI	Broca et al. (2004a, b)

(continued)

Table 19.1 (continued)

Compounds	Property	Experiment model	Concentration	Effects	References
		7-week-old male Wistar rats (Iffa-Credo)			
	Antidyslipidemic and antihyperglycemic agent	Hamsters	–	↓ Serum TG, ↓ TC, ↓ FFA, ↑ HDL:TC	Narender et al. (2006)
	Insulinotropic and antidiabetic	Male Wistar rats were purchased from the Pharmacological Research Center of Tehran University of Medical Sciences	50 mg/kg per day for 8 weeks	↓ BG, ↑ serum HDL, ↓ ALT, ↓ AST	Haeri et al. (2009a, b)
	Anti-hyperglycemic and antidyslipidemic properties	Black lean C57BL/KsJ-db/þ mice were originally obtained from Denmark through the kind courtesy of M/s Novo Nordisk, Copenhagen (Denmark)	50 mg kg1 dose level	↓ BG, ↓FI, ↓ serum TG, ↓ serum TC, ↓ serum LDL ↑ serum HDL	Singh et al. (2010)
	Antidiabetic activities	Male Wistar rats were purchased from the Pharmacological Research Center of Tehran University of Medical Sciences	50 mg/kg/day for four weeks	↓ BG, ↓ serum TC, ↓ serum LDL, ↓ serum TG, ↑ serum HDL	Haeri et al. (2012)
	Increasing surface GLUT4 level in skeletal muscle cells via phosphatidylinositol-3-kinase-dependent pathway	L6 skeletal muscle cells stably expressing rat GLUT4 with a myc epitope inserted in the first exofacial loop (L6-GLUT4myc)	5 uM and 25 uM dose	↑ Glucose uptake, ↑ pAkt, ↑ PI3K, ↑ GLUT4	Jaiswal et al. (2012)

	Inhibit palmitate-induced, ROS-associated inflammation and restored insulin sensitivity through regulating IRS-1 function	Insulin-resistant skeletal muscle (L6 myocytes)	25 μM	↑ pIRS-1, ↑ PI3K, ↑ pAkt, ↓ ROS, ↓ NF-kB, ↓ JNK1/2 ↓ p38 MAPK	Maurya et al. (2014)
	Improve high glucose-induced IR in 3T3-L1 adipocytes	Insulin-resistant 3T3 L1 adipocytes of mice	5, 10, or 20 micromol/L for 24 h	↓ TNF-a, ↑ glucose uptake	Yu et al. (2013)
Quercetin	Antidiabetic effect	Male Sprague-Dawley rats weighing 200–250 g were purchased from Koatech (Gyeonggi, Korea)	Doses of 30, 10, and 30 +10 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$	↑ Serum blood glucose levels, insulin levels, and dyslipidemia in diabetic rats were significantly improved ↓ Oxidative stress and tissue injury biomarkers ↑ Hyperlactatemia and ketoacidosis	Yang and Kang (2018)
	Antidiabetic and antihyperglycemic	Albino Wister male rats (7–8 weeks old, weighing 150–200 g) were purchased from the GSA Animal Farm, Mayiladuthurai, Tamil Nadu, India	Doses of 50 and 75 mg/kg	↑ Triglycerides, high-density lipoprotein, very-low-density lipoprotein, low-density lipoprotein, and total cholesterol ↓ Blood glucose and urine sugar levels ↑ Plasma insulin and hemoglobin levels	Srinivasan et al. (2018)
	Antidiabetic effects	Adult male albino rats	15 mg/kg bw day	↑ GSHPx, SOD and CAT	Abdelmoaty et al. (2010)
	Stimulation of lipolysis	Adipocytes from epididymal adipose tissue	30–50 uM	⊥ of PDE	Kuppusamy and Das (1992)

(continued)

Table 19.1 (continued)

Compounds	Property	Experiment model	Concentration	Effects	References
		of male Wistar rats (180-230 g)			
	Antidiabetic	Rat adipocytes	100 uM	⊥ of the insulin effect on adipose tissue	Elberg and Shechter (1995)
	Antidiabetic activity	Adipocytes of epididymal tissue of Wistar rat	100 uM	⊥ of methylglucose uptake Inhibition of GLUT4 in adipocytes	Strobel et al. (2005)
	Antidiabetic activity	Caco-2E cells	10–200 uM	⊥ of glucose uptake ⊥ of GLUT2	Kwon et al. (2007)
		Caco-2 cells	31 uM	⊥ of glucose uptake ⊥ GLUT2 and SGLT1	Manzano and Williamson (2010)
	Antidiabetic and antioxidant activity	INS-1-cell line and pancreatic cells of male Wistar rats	20 uM	Potentiation of both glucose and glibenclamide-induced insulin secretion and glucose-induced insulin secretion. Protection of -cells against oxidative damage Protection against oxidative damage induced by H2O	Yoshinari and Igarashi (2010)

	Antidiabetic activity	C2C12 murine skeletal myoblast H4IIE murine hepatocytes	50 uM	↑ t of glucose uptake	Eid et al. (2010)
	Antidiabetic activity	Male Sprague-Dawley STZ-induced diabetes rats (200-220 g)	10 and 15 mg/kg BW/d for 10 days	↓ in the fasting plasma glucose ↑ of glucose tolerance test. Regeneration of the pancreatic islets	Vessal et al. (2003)
	Antidiabetic activity	Male Wistar STZ-induced diabetes rats	(200–250 g) 15 mg/kg BW/d 4 weeks	↓ in serum glucose ↑ in insulin in serum Protective effects against oxidative damage Preservation of pancreatic -cell integrity ↓ in MDA and NO ↑ antioxidant enzymes activity: SOD, GSHPx, CAT	Coskun et al. (2005)
	Antidiabetic activity	Obese Zucker rats	(13 weeks old) 2 and 10 mg/kg BW/d 10 weeks	↓ in hyperinsulinemia	Rivera et al. (2008)
	Antidiabetic activity	Male BALB/c STZ-induced diabetes mice	(7 weeks old) 0.1 and 0.5% diet 2 weeks	↑ of diabetic symptoms and liver injury ↓ in plasma glucose	Kobori et al. (2009)
	Antidiabetic activity	Male C57BL/6J mice	(6 weeks old) 0.8% diet 3 and 8 weeks	↓ of insulin dependent activation of PI-3K	Steward et al. (2009)
		SHR and Wistar Kyoto rats	(24 weeks old) 10 mg/kg BW/d 4 weeks	No effects on serum glucose and insulin levels and insulin resistance	Romero et al. (2009)
	Antidiabetic activity	Male Wistar rats	(280 g) 25mg/kg BW/d 4 weeks	↓ in hyperinsulinemia ↑in adiponectin levels	Wein et al. (2010)

(continued)

Table 19.1 (continued)

Compounds	Property	Experiment model	Concentration	Effects	References
	Antidiabetic activity	Male C57BL/ksJ-db/db mice	(5 weeks old) 0.08% diet 7 weeks	↓ in fasting plasma glucose levels and glycated hemoglobin ↓ the intestinal maltose activity	Kim et al. (2011)
Galactomannan	Antidiabetic and hypolipidemic	Adult male Wistar rats, weighing 179 ± 10 g, obtained from the local Central Pharmacy, Tunisia	(5 weeks old) 0.08% diet 7 weeks	↑in HDL-cholesterol ↓ of AST and ALT and LDH ⊥ diabetes-induced kidney injury through lowering the urea and creatinine content in plasma	Hamden et al. (2010)
	Antidiabetic and antioxidant effects	Male long-Evans male rats were bred at BIRDEM (Dhaka, Bangladesh)	0·5 g/kg	⊥ of carbohydrate digestion and absorption ↑ of peripheral insulin action ↓ Serum glucose ↑ Liver glycogen content ↑ Total antioxidant status	Hannan et al. (2007)

19.2 Alkaloids

19.2.1 Trigonelline

Trigonelline has markedly reduced the blood glucose level, TC, and TG concentration in diabetic rat model. The results showed that trigonelline has positive influence on diabetes by reducing blood sugar and fat levels, enhancing insulin sensitivity index and insulin content, overexpressing antioxidant enzymes, and thereby reducing lipid peroxidation (Zhou et al. 2013). Another study showed a positive effect of trigonelline on main enzymes of carbohydrate metabolism including glucokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, glucose-6-phosphatedehydrogenase in liver tissues of diabetic rats which reached normal levels. Trigonelline improved insulin sensitivity which was obvious from intraperitoneal insulin tolerance test. Thus, trigonelline improved insulin sensitivity and controls carbohydrate metabolism by modifying the key regulatory enzymes in the liver tissues of diabetic animals (Subramanian and Prasath 2014). The positive influence of trigonelline on insulin sensitivity and glucose/lipid homeostasis is facilitated by the augmentation of the insulin signaling and its free radical scavenging property. Moreover, the beneficial effect of VLD on pT308-Akt is an essential component in insulin signaling, and therefore its antidiabetic activity (Aldakinah et al. 2017). A study was carried out to explore the consequence of trigonelline on blood glucose, glycosylated hemoglobin, and plasma insulin levels in high-fat-fed (HFD)/streptozotocin (STZ)-induced type 2 diabetic rats. Diabetes was induced in rats and were administered with trigonelline for a month. The toxicity and biochemical parameters including blood glucose, HbA1C, insulin, insulin resistance (HOMA-IR), and lipid profile were studied. The enzyme activities of AST, ALT, and ALP serum were also evaluated. Trigonelline administration reduced the elevated levels of glucose, glycosylated hemoglobin, AST, ALT, and ALP. The insulin level was enhanced with a beneficial effect in liver and muscle glycogen content in case of insulin-resistant diabetic rats. Trigonelline efficiently regularized the parameters of lipid profile. These data has shown that trigonelline has the capability to reduce sugar level and also showed antidyslipidemic effects in HFD/STZ-induced type 2 diabetic rats (Aldakinah et al. 2017). The islet of pancreas and β-cells impairment were reduced followed by the dosing of trigonelline to diabetic rats. The rise of GLP-1 levels was seen to reduce the blood sugar in enduring diabetic rats and better outcomes were shown in the oral glucose and starch tolerance test. In addition to this, trigonelline regularized important enzyme related to hypertension as ACE and improved the hemoglobin A1c and lipid profiles and reduced the liver toxicity. Consequently, it was shown that trigonelline was fruitful in managing control of sugar level, metabolism, and liver function in diabetic rats. It was finally observed that trigonelline can be a prospective herbal drug lead for the management of type 2 diabetes (Hamden et al. 2013). Trigonelline is an antidiabetic alkaloid which has been complexed with a and b cyclodextrin molecules for sustained release. This complex showed high efficacy of a-Cyclodextrin as compared to b-Cyclodextrin for the sustained release of the drug (Ghosh et al. 2020). Trigonelline

markedly reduced blood glucose, TC, and TG levels in diabetic rat model. Pancreas-to-body weight ratio, insulin level, insulin sensitivity index, insulin content in pancreas, malonaldehyde and nitric oxide contents, and superoxide dismutase, catalase, glutathione, and inducible nitric oxide synthase activities were changed in case of diabetic rats and were almost similar to control levels when treated with trigonelline. These results prove that trigonelline has positive effect on reducing blood glucose and lipid levels, increasing insulin sensitivity index and insulin content, upregulating antioxidant enzyme activity, and decreasing lipid peroxidation (Zhou et al. 2013). In a genetic model of diabetes (KK-Ay obese mice), administration of trigonelline and nicotinic acid lowered blood glucose levels in oral glucose tolerance tests (OGTT) carried out on days 22-23 after feeding, indicating that both trigonelline and nicotinic acid improve glucose tolerance in diabetes with obesity. The fasting serum insulin levels were significantly lower in mice fed trigonelline and showed a lowering tendency in mice fed nicotinic acid, than in the control mice. The liver glucokinase/glucose-6-phosphatase ratios were higher and serum levels of tumor necrosis factor (TNF)—lower in the trigonelline and nicotinic acid-fed mice compared to the control mice, suggesting that the regulation of glucokinase/glucose-6-phosphatase and TNF—by trigonelline and nicotinic acid were closely related to the suppression of diabetes in KK-Ay mice (Yoshinari and Igarashi 2010). Our recent study also showed that in low-dose streptozocin- and high carbohydrate/high-fat diet-induced type 2 diabetes in rats, 4-week treatment with trigonelline decreased the blood glucose levels of the diabetic to near those of the control rats (Zhou et al. 2011). In Sabra albino rats with alloxan-induced diabetes, trigonelline exhibited a mild and transient hypoglycemic effect (Mishkinsky et al. 1974; Mishkinsky et al. 1967). Trigonelline inhibits intestinal sodium-dependent glucose uptake, as shown in vitro using rabbit intestinal brush border membrane vesicles. However, trigonelline (10 mM) did not inhibit glucagon-induced glycogen phosphorylase activity (Al-Habori et al. 2001). Coffee consumption has been associated with a decreased risk of type 2 diabetes. Ingestion of the major coffee components chlorogenic acid (1 g) and trigonelline (500 mg) significantly reduced glucose and insulin concentrations 15 min after a 2-h OGTT compared with placebo (1 g mannitol) in a group of 15 overweight men. Decaffeinated coffee, chlorogenic acid, and trigonelline all failed to affect area under the curve values for glucose or insulin during the OGTT. Therefore, chlorogenic acid and trigonelline reduce early glucose and insulin responses during the OGTT (Van Dijk et al. 2009). In OGTTs in non-obese type 2 diabetic Goto-Kakizaki (GK) rats, a pumpkin paste concentrate-fed group maintained a lower glucose level than the control group between 15 min and 60 min after glucose ingestion. The active compounds in the pumpkin extract were isolated and identified as trigonelline and nicotinic acid. Diets containing trigonelline and nicotinic acid improved and tended to improve glucose tolerance, respectively (Yoshinari et al. 2009). However, in this and another study published by Yoshinari et al. (Van Dijk et al. 2009), no positive control was used. In OGTTs administered to non-obese type 2 diabetic GK rats, insulin levels increased after 15 min in trigonelline-fed GK rats and then gradually decreased over the next 120 min. In contrast, the insulin level gradually increased over 120 min in control

GK rats, suggesting that trigonelline improves insulin sensitivity (Yoshinari et al. 2009). In our studies, trigonelline decreased insulin levels and increased pancreas weights, pancreas-to-body weight ratios, insulin sensitivity indexes, and pancreatic insulin content in type 2 diabetic rats (Yoshinari and Igarashi 2010). Decaffeinated coffee, chlorogenic acid and trigonelline treatment failed to affect the overall levels of glucagon-like peptide-1 or the gastric inhibitory peptide secretion pattern following an OGTT when studied on a group of 15 overweight men. Decaffeinated coffee slightly increased the total glucagon-like peptide-1 concentration 30 min after ingestion (before the OGTT) relative to placebo (2.7 pM), but this change was not accompanied changes in glucose or insulin secretion. These findings do not support the hypothesis that coffee acutely improves glucose tolerance through effects on the secretion of incretin hormones. However, the chronic effects of ingestion of coffee and its major components still need to be investigated (Olthof et al. 2011). Trigonelline reduced the total and free plasma cholesterol levels in rats (Abe and Kaneda 1975). The serum and liver triglyceride levels in the trigonelline- and nicotinic acid-fed GK rats were lower than those in the control GK rats. Trigonelline and nicotinic acid decreased liver fatty acid synthase activity and increased liver carnitine palmitoyl transferase and glucokinase activities in GK rats. These results suggest that the regulation of these enzymes by trigonelline and nicotinic acid is closely related to the suppression of both triglyceride accumulation and the progression of diabetes (Yoshinari et al. 2009). The triglyceride levels in the liver and adipose tissue of mice fed trigonelline and nicotinic acid were lower than those of control mice, indicating that trigonelline and nicotinic acid reduce the changes in lipid levels accompanied with diabetes (Yoshinari and Igarashi 2010). Our study showed that 4-week treatment with trigonelline decreases total serum cholesterol and triglyceride levels in type 2 diabetic rats (Zhou et al. 2011). Possible metabolic pathway of biosynthesis of trigonelline is shown in Fig. 19.2.

19.3 Saponin

19.3.1 Diosgenin

Trigonella foenum-graecum is a rich source of many bioactive compounds, diosgenin is one of them. It is a very important metabolite as it showed estrogenic activity. It is used as raw precursor for the industrial, large scale production of steroidal drugs and hormones such as testosterone, norethisterone, glucocorticoids, and progesterone. Russell Marker and colleagues produced progesterone by adopting the semisynthetic approach from diosgenin at Parke-Davis Pharmaceutical Company, in 1940 (Szabó et al. 2018). Diosgenin is a steroid saponin and it was tested for gestational diabetes mellitus in a study in which the pregnant mice were treated orally with diosgenin. The results were observed and it was seen that it played beneficial role in gestational diabetes in the pregnant mice as was shown by the improvement of glucose, insulin intolerance, and increase hepatic glycogen content. Diosgenin also exhibited antioxidant properties as it can reduce oxidative stress

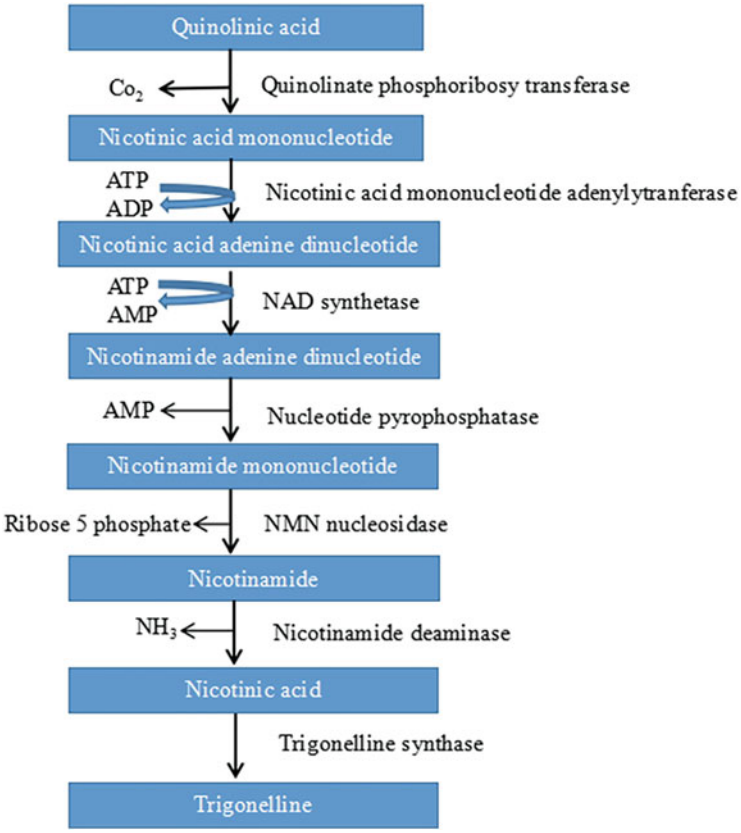

Fig. 19.2 Possible metabolic pathway of biosynthesis of trigonelline

under gestational diabetes condition by reducing TBARS content, increase GSH level, and SOD and CAT antioxidant enzymes activities. Moreover, diosgenin also amended the irregular changes of lipid profiles in pregnant mice through inhibition of sterol regulatory element binding protein-1 which showed the antidiabetic effects of DSG in GD mice (http://www.mskcc.org/mskcc/html/11570.cfm).

Saponins are characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains. Their structural diversity is reflected in their physicochemical and biological properties, which are exploited in a number of traditional and industrial applications (Arivalagan et al. 2013). Fenugreek seeds contain 4.8% saponins in the form of diosgenin, yamogenin, tigogenin, neotigogenin, yuccagenin, lilagenin, gitogenin, neogitogenin, sarsapogenin, and smilagenin. Among them diosgenin (Δ5, 25α-spirostan-3β-ol) represents the principal steroidal saponin (Mullaicharam et al. 2013). Since its discovery, diosgenin a major sapogenin found in fenugreek seed is the single main precursor used in the manufacture of synthetic steroids in the pharmaceutical industry (Raju and Rao 2012). It occurs naturally as a glycosylated compound in fenugreek and can be

liberated by acid hydrolysis (which removes three carbohydrate residues) of the steroidal saponin, dioscin. It is synthesized as part of the melavonate pathway in the biosynthesis of steroids. Steroidal diosgenin is formed by modification of the side chain of cholesterol, in which a spiroketal structure is formed at C-22, yielding a non-polar compound with 6 carbon rings (Mehrafarin et al. 2010). The diosgenyl saponins that are steroidal glycosides and bear diosgenin as aglycone are often found as the major components in the traditional oriental medicines as an antihypercholesterolemic, antihypertriacylglycerolemic, antidiabetic, and antihyperglycemic agent (Manivannan et al. 2013). Additionally, there is considerable commercial interest in growing fenugreek for its high sapogenin content. Saponins are reported to display hypocholesterolemic as well as antidiabetic activity (Wani et al. 2012). These diosgenyl saponins that are steroidal glycosides and bear diosgenin as aglycone are often found as the major components in the traditional oriental medicines as an antihypercholesterolemic, antihypertriacylglycerolemic, anti-diabetic, and antihyperglycemic agent (Manivannan et al. 2013). Depending upon biogeographic origins, genotypes, and environmental factors, reported diosgenin contents in fenugreek seeds vary between 0.1% and 0.9% (Snehlata and Payal 2012). This naturally occurring steroidal saponin, present in fenugreek, has been shown to have favorable effects on glucose lowering, antioxidant activity, lipid metabolism, and myocardial infarction (Al-Matubsi et al. 1998). This compound has shown to reduce diabetes-induced oxidative stress and dyslipidemia in type 2 diabetic rats which is crucial in cardio-metabolic risks by modulating the PPARs (Sangeetha et al. 2013). Fenugreek has been reported to ameliorate diabetes in type 2 diabetic obese KK-Ay mice, by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues, and effects were reported to be mediated by diosgenin (Uemura et al. 2010a, b). Thus, the best documented medical use of fenugreek seeds is to control blood sugar in both type 1 and type 2 diabetes. It is well known that diosgenin lowers plasma cholesterol by increasing fecal cholesterol excretion. Therefore, the hypocholesterolemic effect of dietary diosgenin by increasing fecal cholesterol excretion is primarily attributable to its impact on hepatic cholesterol metabolism rather than intestinal cholesterol absorption (Al-Matubsi et al. 1998). Diosgenin has also been found to exert anticarcinogenic properties, such as inhibiting proliferation and inducing apoptosis in a variety of tumor cells. In a recent study, it has been reported to inhibit migration and invasion of PC-3 cells by reducing MMPs expression, inhibition of ERK, JNK, and PI3K/Akt signaling pathways as well as NF-κB activity and thus suggests a new therapeutic potential for diosgenin in anti-metastatic therapy (Chen et al. 2011). Steroidal saponin, present in fenugreek, has been shown to have favorable effects on glucose lowering, antioxidant activity, lipid metabolism, and myocardial infarction (Al-Matubsi et al. 1998). This compound has been found to mitigate diabetes-induced oxidative stress and dyslipidemia in type 2 diabetic rats which is crucial in cardio-metabolic risks by modulating the PPARs (Sangeetha et al. 2013). Recently, fenugreek has been reported to ameliorated diabetes in type 2 diabetic obese KK-Ay mice, by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues, and effects were seen to be facilitated by diosgenin (Uemura et al. 2010a, b). Thus, the best

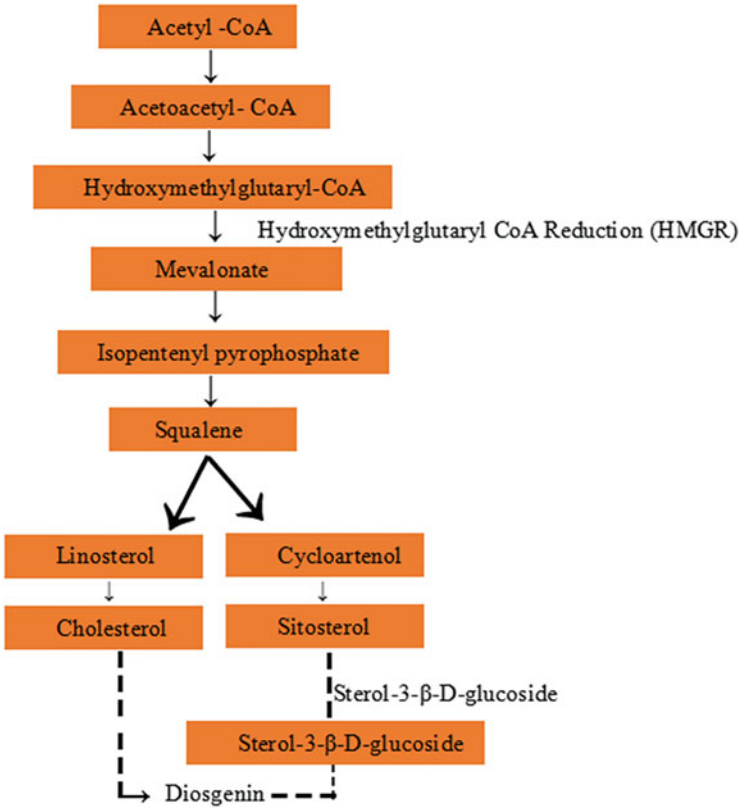

Fig. 19.3 Possible metabolic pathway of biosynthesis of diosgenin from squalene

documented medical use of fenugreek seeds is to control blood sugar in both type 1 and type 2 diabetes. It is well known that diosgenin lowers plasma cholesterol by increasing fecal cholesterol excretion. Therefore, the hypocholesterolemic effect of dietary diosgenin by increasing fecal cholesterol excretion is primarily attributable to its impact on hepatic cholesterol metabolism rather than intestinal cholesterol absorption (Uemura et al. 2010a, b). Diosgenin has also been found to exert anticarcinogenic properties, such as inhibiting proliferation and inducing apoptosis in a variety of tumor cells. In a recent study, it has been reported to inhibit migration and invasion of PC-3 cells by reducing MMPs expression, inhibition of ERK, JNK, and PI3K/Akt signaling pathways as well as NF-κB activity and thus suggests a new therapeutic potential for diosgenin in anti-metastatic therapy (Chen et al. 2011). Recently, diosgenin from fenugreek has been reported to ameliorate diabetes in type 2 diabetic obese by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues (Uemura et al. 2010a, b). Possible metabolic pathway of biosynthesis of diosgenin from squalene has been shown in Fig. 19.3.

19.3.2 Choline

The studies have revealed that elevated intakes of choline and betaine may have beneficial effect on insulin resistance in the common population. In case of type 2 diabetes, the choline uptake was seen to increase inflammation in atherosclerotic plaques in mice. PET tracer 18F-FMCH is a potential tool to study vascular inflammation associated with diabetes (Hellberg et al. 2016).

19.4 Amino Acids

19.4.1 Hydroxyisoleucine (OHIle)

In an in vitro research, it was shown that GLY-L-4-OHIL exhibited better antidiabetic property as compared to other dipeptides. Further, in vivo study also showed extraordinary antidiabetic activity when compared with standard drug and the parent compound 4-OH Isoleucine (Devi and Raju 2020). In another study, 4-HI and L-isoleucine were identified using an electrospray ionization (ESI) ion source, using multiple reaction monitoring (MRM) in positive ion mode. Further, in this study the two-compartment model was statistically fitted based on AIC and SBC values for assessment of the pharmacokinetic parameters of 4-HI. Pharmacodynamic studies were also done by assessing the levels of triglyceride and total cholesterol levels and showed that the pharmacokinetic and pharmacodynamic data of 4-HI correlated with each other (Wadhwa et al. 2020). The antidiabetic effect of 4HO-Ile in animal model of type I diabetes, levels of insulin was seen to decrease to a great extent in streptozotocin-treated rats as compared to control group. Treatment of diabetic rats with daily doses of 4HO-Ile for a month decreased glucose level in blood in the diabetic group. Besides this, the elevated levels of lipids (cholesterol, HDL, LDL and triglycerides) and uric acid in the diabetic rats, was also seen to come to normal levels similar to control group following the treatment of 4HO-Ile. These data showed that 4HO-Ile has substantial antidiabetic properties that are independent of insulin and recommend the importance of 4HO-Ile as a supplement to diabetes treatment and for type 1 as well as type 2 diabetes (Haeri et al. 2012).

4-OHIle is a branched-chain amino acid only present in plants. It is particularly abundant in fenugreek seeds (0.015–0.4%) (Hajimehdipoor et al. 2008; Narender et al. 2006; Mehrafarin et al. 2010). It was synthesized from isoleucine and is responsible for the antidiabetic effects in animals because of its ability to regulate pancreatic insulin secretion (Sauvaire et al. 1998; Fowden et al. 1973; Skaltsa 2002; Broca et al. 2004a, b), hence it has significant potential for the treatment of IR and diabetes (Acharya et al. 2008). The antidiabetic properties of 4-OHIle are related to its ability to stimulate insulin secretion, as observed in human pancreatic islet cells, in isolated perfused rat pancreas (Sauvaire et al. 1984) and in in vivo studies (Broca et al. 1999). An improvement in glucose and insulin tolerance, insulin secretion, and reduced hyperglycemia was observed in diabetic rats and dogs. 4-OHIle functioned as an insulin secretagogue, but only in the presence of elevated blood glucose

concentrations, in a range of 8.3–16.7 mM (Sauvaire et al. 1984; Broca et al. 1999; Broca et al. 2000).

4-OHIle shows in vitro insulinotropic activity related to the glucose concentration of the medium in isolated pancreatic beta cells (Broca et al. 1999; Broca et al. 2000). The secretagogue potential of 4-OHIle is of special interest for various degrees of insulin resistance (Baquer et al. 2009). In streptozotocin-treated rats, an improvement of the diabetic state was associated with the stimulating effect of 4-OHIle on beta cell function, and in normal rats and dogs, 4-OHIle is able to stimulate insulin secretion and improve glucose tolerance, suggesting its potential in the treatment of IR and type II diabetes (Amin et al. 1987; Hajimehdipoor et al. 2008).

Reversing defective insulin secretion is highly desirable in a diabetic state, but enhancing insulin sensitivity in hepatic and peripheral tissues is also important. Insulin sensitivity studies on 4-OHIle demonstrated its efficacy in two rat models (Broca et al. 2004a, b). An improvement in insulin sensitivity was observed in sucrose plus lipid-fed rats, in which peripheral glucose uptake was increased, and also in Zucker fa/fa rats, in which hepatic glucose output was decreased. Injection of 4-OHIle resulted in the activation of insulin receptor substrate-1 (IRS-1) and phosphatidylinositide 3-kinase (PI3K) in insulin-sensitive tissues. In in vitro assays using human and rat pancreatic cells, 4-OHIle showed increased glucose-induced insulin release, and levels of somatostatin and glucagon were not altered (Sauvaire et al. 1998). Cellular glucose uptake in muscle and adipose tissues is dependent on insulin stimulation, which causes the translocation of glucose transporter-4 (GLUT-4) to the plasma membrane. In experimental diabetes tests it has been observed that *Trigonella* molecules are able to reverse the effects on the GLUT-4 transporter to normal levels.

Shukla and Rangari (Shukla and Rangari 2015) studied the antidiabetic activity of 4-OHIle in combination with the natural bioavailability enhancers piperine and ginger oleoresin. Alloxan-induced diabetic rats were treated with fenugreek seed powder containing 28% 4-HOIle, alone or combined with the bio-enhancers, producing significant blood glucose level and body weight improvement when compared to the diabetic control. Narender et al. (2006) studied the effect of 4-OHIle on dyslipidemic hamsters. 4-OHIle resulted in decreased plasma triglycerides, total cholesterol (TC), and FFAs, and a simultaneous increase by 39% of the HDL-C: TC ratio. Haeri and his collaborators (Haeri et al. 2009a, b) determined the effect of 4-OHIle on streptozotocin-induced diabetic and fructose-fed rats. Liver function markers and glycemia improved after an eight-week treatment at a dose of 50 mg/kg. In fructose-fed rats, blood glucose and markers of liver aminotransferases were restored to levels near those observed in control animals. Effects of 4-OHIle were also observed in leptin receptor-deficient db/db mice, with improvement in levels of blood glucose, insulin, and lipids (Singh et al. 2010). Streptozotocin-diabetic rats showed decreased blood glucose and restored blood lipid and uric acid levels after four weeks of treatment with 4-OHIle (Haeri et al. 2012).

The molecular mechanism of action of 4-OHIle has been studied using cell culture models. In rat muscle cells, it has been observed that glucose uptake and GLUT-4 translocation to the plasma membrane were increased after 16 h exposure to

4-OHIle (Jaiswal et al. 2012). The basal phosphorylation of Akt (Ser-473) increased after treatment with 4-OHIle, but mRNA expression of total Akt, IRS-1, GLUT-4, and glycogen synthase kinase-3β (GSK-3β) was unchanged. Treatment of L6 myotubes with 4-OHIle decreased IR induced by FFAs (Maurya et al. 2014). In fact, 4-OHIle restored glucose uptake and GLUT-4 translocation to the plasma membrane after palmitate treatment via insulin induction of IRS-1 phosphorylation. 4-OHIle also inhibited both the production of reactive oxygen species induced by palmitate and the associated inflammation, and it reduced activation of the JNK1/2 pathway, including the extracellular signal-regulated kinase isoforms 1 and 2 (ERK1/2), p38 MAPK, and NF-κB. The effects of 4-OHIle on 3T3-L1 adipocytes included increased glucose uptake in insulin-resistant adipocytes in a dose-dependent manner along with a reduction of TNF-α mRNA expression and secretion, suggesting its anti-inflammatory potential (Yu et al. 2013). Gao et al. (2008) established an IR HepG2 cell line and determined the molecular mechanisms for 4-OHIle in IR. Two potential mechanisms were described: a negative regulation of TNF-α production with an improvement in insulin sensitivity and increased expression of p-IRS-1 and GLUT4 in the insulin-signaling pathway (Zafar et al. 2015). 4-OHIle has been reported as a glucose-dependent insulinotropic compound through its direct effect on pancreatic islets and its insulin sensitivity on muscle, adipose, and liver tissue. These effects, in combination with the absence of acute toxicity or genotoxicity, suggest that this amino acid has a potential role as a natural product for the treatment of obesity and IR (Gong et al. 2016; Olaiya and Soetan 2014). *Trigonella* resulted in a substantial reduction in blood glucose both in the normal and diabetic rats and the hypoglycemic effect was dose-related (Khosla et al. 1995a, b, c).

19.5 Polyphenolic Compounds

Till date more than 8000 polyphenolic compounds, including phenolic acids, flavonoids, stilbenes, lignans, and polymeric lignans have been identified from whole plant foods. These compounds are actually secondary metabolites of the plants that act as a defense against ultraviolet radiation, oxidants, and pathogens (Bahadoran et al. 2013). Phenolic compounds possess antioxidative attributes, which may prevent some forms of chronic diseases (Huang et al. 2009). Recent reports indicate that fenugreek seeds contains five different types of flavonoids, namely vitexin, tricin, naringenin, quercetin, and tricin-7-O-β-D-glucopyranoside (Nanjundan et al. 2009). Among them quercetin and kaempferol are flavonols; luteolin is a flavone; naringenin is a flavanone while vitexin occurs as a glycosylated flavone. Isoflavonoid phytoalexins are also reported to occur in fenugreek in the form of the pterocarpans, medicarpin, and maackiaian (Quintans-Junior et al. 2014). A recent report by Patil and Jain (2014) shows the common phenolic compounds isolated from fenugreek to be scopoletin, coumarin, chlorogenic, caffeic p-coumaric acids, and quercetin (Fig.19.4).

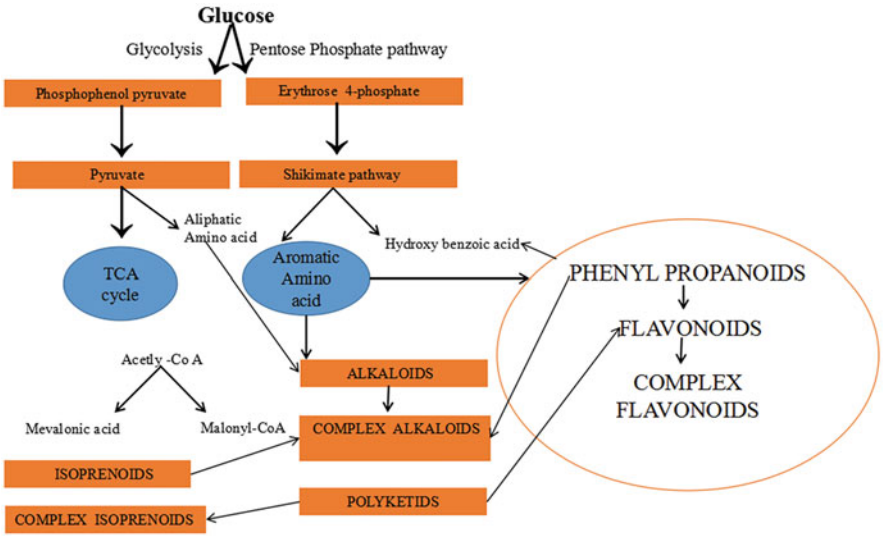

Fig. 19.4 Metabolic pathway of biosynthesis of polyphenolic compounds

19.5.1 Quercetin

Recently, quercetin has been reported to possess beneficial antidiabetic effects under in vitro as well in vivo conditions (Abdelmoaty et al. 2010). The antidiabetic mechanism of quercetin has been reported to involve the reduction of intestinal glucose absorption at the level of glucose transporters (GLUT), blockage of tyrosine kinase activity of β-subunit of insulin receptor, increase insulin secretion from pancreatic β-cells, inhibit11-β hydroxysteriod dehydrogenase type 1 enzyme, increase glucokinase activity, prevent degeneration of β-cells, increase α-glucosidase inhibition, decrease insulin resistance, and increase adiponectin expression (Aguirre et al. 2011). Recent studies indicate that quercetin effectively ameliorates postprandial hyperglycemia in STZ-induced diabetic rats and these effects were mediated through α-glucosidase inhibition with an IC50 of 0.48–0.71 mM (Hussain et al. 2012; Jo et al. 2009). Further, it has also been reported to improve hyperglycemia, hypertriglyceridemia, and antioxidant status of STZ-induced diabetic rats (Jeong et al. 2012). The ethanol extract showed the presence of higher flavonoid content when compared with other solvent extracts. The ethanol extract was subjected to fractionalization by column chromatography. The eluted fractions were run in TLC mobile phase with different solvent ratios. The fractions showed Rf value equal to standard quercetin in TLC was combined and crystallized. The characterization techniques confirmed that the isolated compound was found to be quercetin. The free radical scavenging activity suggests that the isolated compound quercetin could act as a potent source of antioxidants (Sambandam et al. 2016).

19.6 Polysaccharide

19.6.1 Galactomannan

Galactomannan represents the major polysaccharide found in fenugreek seeds and accounts for approximately 17–50% of the dry seed weight (Rathore et al. 2013). It is an integral component of the cell wall which is found concentrated around the seed coat. Galactomannan polysaccharides are structurally composed of a 1,4-β-D-mannosyl backbone substituted by a single galactose unit α-linked at the C-6 oxygen. It is simply mucilage with antidiabetic potential present in plants, and due to high viscosity and neutral ionic properties it is finding wider applications in the food, pharmaceutical, cosmetics, paint, and paper industries also (Nandhini 2010). Fenugreek galactomannans contain a galactose to mannose ratio of 1:1. This high degree of galactose substitution renders the molecule relatively more soluble compared to galactomannans from guar or locust bean, which has a galactose to mannose ratio of 1:2 and 1:4, respectively (Quintans-Junior et al. 2014; Dionisio and Grenha 2012). Presence of galactomannan in fenugreek seed is recognized as the principal source of soluble dietary fiber (SDF) in the plant. The soluble nature of galactomannan fiber from fenugreek has been linked to numerous human health benefits, mainly in the reduction of plasma glucose levels and thus possess an antidiabetic effect (Gupta 2014). It is also known to be hepatoprotective and have the potential to reduce risk of cardiovascular disease and to protect against some cancers through the reduction of low-density lipoprotein (LDL), total cholesterol, and considerably decrease aspartate and alanine transaminases (AST and ALT) and lactate dehydrogenase (LDH) contents in the serum of diabetic rats (Hamden et al. 2010). A study conducted by Hannan et al. (2007) demonstrated that the soluble dietary fiber (SDF) portion of fenugreek can significantly improve glucose homeostasis in type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption. They have also suggested that the SDF fraction may enhance insulin action in type 2 diabetes as indicated by the improvement of oral glucose tolerance in these test subjects.

19.7 Conclusion

Trigonella foenum-graecum has beneficial effects in several human diseases, notably in diabetes disease. Its properties which include hypoglycemic, hypolipidemic activities play a role in improving diabetes. The mechanisms underlying these effects are related to modulation of cell regeneration, insulin secretion, activity of glucose metabolism related enzymes, reactive oxygen species generation and scavenging, axonal extension, and neuron excitability. Referring to the huge amount of data in the scientific literature showed that the *Trigonella foenum-graecum* and its main constituents have effective roles against diabetes. It can be used as a therapeutic drug for curing many types of diseases as well as its extracted compounds can be used individually in drug designing and discovery. Polyphenolic compounds have

antioxidative properties as compared to other extracts. Fenugreek contains 4-hydroxy isoleucine amino acid in free form that has antidiabetic as well as insulinotropic effects. This article also reviews the fundamental role of saponin as an antidiabetic agent. Saponins from various plants and marine animals have been reported to have a hypoglycemic activity. These activities of saponin regulate blood glucose level and prevent diabetic complications due to their antioxidant activity. Dyslipidemia which is attributed to saponin will help to decrease the risk of atherosclerosis and cardiovascular disease in diabetic patients. More research is needed to evaluate the role of saponins and understand their pharmacology in the treatment of diabetes. It has various health benefits which can be enhanced by isolating each and every compound and can be used as a potential candidate for many types of diseases. The consumption of fenugreek has proved safe and secure for humans and may be simply implemented for health benefit as a dietary component, through its rich full fiber packaged and other bioactive components. Fenugreek seeds not only reduce the low-density cholesterol and triacylglycerol but also reduce blood sugar level because of its useful phytochemicals. The various scientific researchers showed that fenugreek which is well known for its immunomodulatory, inflammatory, antioxidative, nutraceutical effects, and induction of labor and lactation during child birth may be a great promising medicinal herbaceous plant. Future research on this plant could lead to the development of drugs which could find potential applications in medicine and pharmaceutical industries due to herbal nature and lower side effects.

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Evaluating the Chemopreventive Properties of Aqueous Seed Extract of *Trigonella foenum graecum* Against p-Dimethylaminoazobenzene (p-DAB) Induced Carcinogenesis in Mice

20

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Abstract

Aqueous seed extract of *Trigonella foenum graecum* broadly used in traditional system of medicine against liver ailments has been tested for its chemopreventive and antigenotoxic effects against p-dimethylaminoazobenzene (p-DAB) through multiple end points, viz. enzymological, cytogenetical, haematological histological and few immunological parameters. Mice (*Mus musculus*) were divided into the following sets. Group I: normal control, Group II: phenobarbital (PB), Group III: only p-DAB, Group IV: p-DAB + PB, Group V: p-DAB + PB + TG 1 (300 mg/kg b.wt.), Group VI: p-DAB + PB + TG 2 (400 mg/kg b.wt.), Group VII: p-DAB + PB + H_2O. They were sacrificed at 90- and 120-day fixation intervals. Chronic feeding of p-DAB + PB resulted in elevated levels of AST, ALT, LDH, GGT along with an increase in chromosomal aberration, micronucleus and decrease in SOD and catalase activities at both fixation intervals ($p < 0.05$ to $p < 0.001$) when compared to normal control, phenobarbital (PB), only p-DAB, p-DAB + PB + TG1 (300 mg/kg b.wt.), p-DAB + PB + TG2 (400 mg/kg b.wt. fed mice). A similar trend was also noticed with regard to proinflammatory cytokines. On histopathological analysis of liver notable modulatory changes were visible between treated and control sets. Both the doses

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offered protection; however, the dose of 300 mg/kg dose offered better protection than the 400 mg/kg dose, thereby making it a good candidate to be used as supporting palliating measure. We want others to confirm and refute our findings.

Keywords

Hepatocarcinogenesis · Fenugreek · Cytokine · GC-MS

20.1 Introduction

Wild biodiversity has been exploited by humans as a resource for thousands of years but the greater part of it still remains to be described and assessed. Herbal medicinal products have been used as a source of putative contender drugs in many diseases. Various experimental and epidemiological studies proposed that food rich in micronutrients tends to reduce the incidence of cancer (Ma et al. 2018; Mokbel et al. 2019; Cena and Calder 2020; Simon et al. 2020). Deficiency of micronutrient leads to DNA damage and it has been recommended as one of the important causes of cancer and therefore studies involving supplementation of nutrients are abundant. It has been recommended by several workers that diet can play pivotal role in the initiation, promotion and progression stages of several types of cancer (Piyathilake 2016; Mokbel et al. 2019). Many cancer chemopreventive agents obtained from natural resources possess antioxidant potential and are non-toxic (Franceschi 1997; Kaegi 1998; Kaya 2003; Fankhauser et al. 2018). Repeated application of carcinogenic azo dyes such as p-dimethylaminoazobenzene (p-DAB) produces neoplastic alterations and induces liver damage. Dietary phenobarbital (PB) also acts as a promoter of carcinogenic effect only when fed conjointly with p-DAB in both mice and rats. The azo dye, p-dimethylaminoazobenzene (p-DAB), is broadly used as a colouring agent of polishes and different dry foods and which has been listed as a Group 2B carcinogen by IARC (1987) and a potent carcinogen of liver (initiator) by several researchers (Daoust and Molnar 1964). Conversely, phenobarbital (PB) is used as an anti-epileptic drug, generally used as a promoter and has an immense potential to promote liver cancer if used in combination with the azo dye (Kitagawa and Sugano 1977; Biswas et al. 2008). Chronic feeding of both p-DAB and PB conjointly has been reported to induce cytogenetical impairment revealed as chromosomal aberrations (CA), mitotic indices including development of micronuclei (MN), and sperm head anomaly (SHA) (Biswas et al. 2004, 2008; Biswas and Khuda-Bukhsh 2005). The success rate of orthodox mode of treatment for liver cancer is extremely poor. Often the allopathic medicines used in treatment of hepatocarcinogenesis have its own side effects and are quite expensive and could not be procured by common masses. In such a set-up there is a need for alternative agents in treating hepatocarcinogenesis which is easily available, has minimum side effects and less expensive and in this regard *Trigonella foenum graecum* Linn. has received considerable attention. So far, the seed extract has not been tested for possible modulatory activity against p-DAB induced carcinogenesis in mice

model. *Trigonella foenum graecum* (TG) commonly known as 'Methi' in Hindi and 'Fenugreek' in English is widely used as a spice in almost whole of Indian subcontinent. It is used against various pathophysiological disorders since time immemorial in Indian traditional medicine such as hypo-cholesterolemic, antidiabetic, nematicidal, immunomodulatory, antifungal, antibacterial and allelopathic activities of fenugreek extract has also been demonstrated by other workers (Yadav and Baquer 2014; Bahmani et al. 2016; Nagulapalli Venkata et al. 2017; Gias et al. 2020). Few other investigators also reported the chemopreventive properties of TG and reported the modulatory properties of the aqueous seed extract against sodium arsenite induced toxicity (Amin et al. 2005; Gupta et al. 2010; Biswas et al. 2019). But to our knowledge crude aqueous seed extract of TG had not been scientifically confirmed so far for its possible anti-carcinogenic effect in any mammalian model in vivo. Hence, the present study was undertaken on the mammalian model mice (*Mus musculus*) to find out if TG can offer protection in induced hepatocarcinogenesis.

20.2 Materials and Methods

Inbred lines of Swiss albino mice were reared and sustained in the animal house of the Department of Zoology (with clearance from the University Ethical Committee and under the supervision of the Animal Welfare Committee, (1973/GO/Re/S/17/CPCSEA date 19/7/2017, Ministry of Environment and Forest, Government of India) Sidho-Kanho-Birsha University, for the investigation. Mice were provided food and water ad libitum and kept in hygienic condition. For the development of hepatic nodules and subsequent hepatocarcinoma, the chronic dietary feeding method used by several workers earlier (Doust and Molnar 1964; Watanabe et al. 2001; Biswas and Khuda-Bukhsh 2002, 2004, 2005; Biswas et al. 2004) was adopted. The general diet comprises of wheat, gram and powdered milk without any animal protein supplementation. All the experimental mice were randomized by fixed random allocation. Groups of 70 healthy mice weighing between 20 and 25 g were used for each treatment series, namely (1) normal, (2) phenobarbital (PB), (3) p-dimethylaminoazobenzene (p-DAB), (4) p-DAB + PB, (5) p-DAB + PB + TG 1 (300 mg/kg b.wt.), (6) p-DAB + PB + TG 2 and (7) p-DAB + PB + H_2O series for each of two fixation intervals, namely at 90 and 120 days.

20.3 Biochemical Assay

For the estimation of AST and ALT, the method of Bergmeyer and Brent (1974a, b) was followed with some minor modifications for estimation of AST and ALT activities. For AST, 100 μL of tissue homogenate was made to react with 500 μL of the substrate solution L-aspartate and alpha keto glutaric acid which was then incubated for 60 min at 37 °C followed by addition of 500 μL of dinitrophenol hydrazine (DNPH) and followed by 5 mL 0.4 N NaOH. The absorbance was

measured at 510 nm. For the analysis of ALT, 100 μL of tissue homogenate was made to react with 500 μL of the substrate solution (L-alanine and alpha keto glutaric acid) incubated for 30 min at 37 °C and the rest of the technique was similar to that of AST and the absorbance was also measured at 510 nm.

For the assay of LDH activity the method of Lum et al. (1974) was adopted with minor modifications. Briefly 1000 μL of working reagent was mixed with 50 μL of homogenate. The mixture was thoroughly mixed and the first absorbance was taken exactly 1 min at 340 nm and thereafter at 30, 60, 90 s. The mean change of absorbance per minute was calculated.

For analysis of the gamma glutamyl transferase (GGT) the technique of Szasz (1976) was followed. In brief 1000 μL of working reagent consisting of gamma glutamyl-p-nitroanilide and glycylglycine was mixed with 100 μL of serum and the rate of increase in absorbance was noted at 405 nm against a suitable blank.

Superoxide dismutase (SOD) activities were measured using the pyrogallol assay by the method of Marklund and Marklund (1974), based on the competition between pyrogallol oxidation by superoxide radicals and superoxide dismutation by SOD. Assays were monitored by spectrophotometry at 420 nm. One unit of SOD activity is defined as the amount of the enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%. SOD activities were expressed as international units per mg of soluble proteins.

Catalase activity was determined by catalytic reduction of hydrogen peroxide by the method of Aebi (1984). In brief, hydrogen peroxide was added to the sample and the mixture was incubated at 37 °C. Catalase activity was measured by recording the decrease in absorbance at 240 nm periodically after addition of sample in a cuvette of the UV-VIS spectrophotometer (UV-1800). The average difference in absorbance in 30 s was calculated. A unit of catalase is defined as the amount of protein that results in a decrease in absorbance of 0.05 in 30 s. The quantitative estimation of total protein was conducted before carrying out the enzymatic estimations by the method of Lowry et al. (1951).

20.4 Cytogenetical Assay

For chromosome preparation, the conventional flame drying colchicine citrate method was followed as described earlier (Biswas et al. 2004, 2008; Biswas and Khuda-Bukhsh 2005) followed by Giemsa staining for scoring bone marrow chromosome aberrations. Chromosome aberrations of various nature have been pooled into two categories: the ‘major’ type comprising aberrations like breaks, fragments, terminal association, etc. and the ‘other’ types comprising less significant aberrations like gaps, erosion, precocious centromeric separation, pycnosis, stretching, etc.

For the assay of mitotic index, a drop of suspension of bone marrow cells in 1% sodium citrate was smeared on clean grease free slides, briefly fixed in ethanol, followed by staining with May-Grunwald and then subsequently by Giemsa as

previously described elsewhere (Biswas et al. 2004, 2008; Biswas and Khuda-Bukhsh 2005).

20.5 Quantification of Haematological Variables

About 30 μL of whole blood in citrate was subjected to AutoAnalyzer (SB22 Plus VET, India), for analysis of WBC, RBC, haemoglobin, HCT, PLT, MCV, MCH and MCHC.

20.6 Determination of Pro-Inflammatory Cytokine Tumour Necrosis Factor-Alpha and Interleukin-6

The tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in serum were determined by using the ELISA kit (R and D Systems, Minneapolis, USA) according to the procedure recommended by the manufacturer.

20.7 Transmission Electron Microscopy

For histological studies by TEM, 1–2 mm liver sections were isolated instantly after sacrifice of mice and fixed in Karnovsky fixative at 4 °C for 5 h, secondary fixation was followed in 1% v/v OsO_4 (E Merck) for about 1 h at 4 °C in dark. Dehydration and embedding were performed according to routine procedures. Serial semi-thin sections (60–90 nm) were cut with the help of an ultramicrotome (Reichert Jung-Ultracut E, United Kingdom) fitted with glass knives. Then ultra-thin sections were stained with uranyl acetate followed by lead citrate and observed under a Philips Microscope (TEM CM-10).

20.8 Phytochemical Screening

The phytochemical screening for flavonoids, alkaloids, tannins, carbohydrates, reducing sugars, glycosides and steroids was determined by the procedure described elsewhere (Cocan et al. 2018).

Gas chromatography-mass spectrometry (GC-MS) investigation of TG seeds was done by a gas chromatograph (Model No. Agilent 190,915–433 CA, USA) which was coupled to a mass spectrophotometer and furnished with a fused capillary column, (Model No. Agilent 190,915–433 CA, USA (HP-5MS, 0.25 mm × 30 m × 0.25 μm). The carrier gas was helium (99%) with a constant flow rate of 1 mL/min. The volume of the sample injected was 5 μL in GC-grade ethanol with an average velocity of 37 cm/s. The initial temperature of the column was 50 °C for 5 min and then, it was programmed to 280 °C. The mass spectra which

were obtained by EI at 69.9 eV over the scan range m/z 50–500 amu. The total GC running time was 28 min.

20.9 Statistical Analysis

Statistical comparisons were made between the positive control groups to that of TG-fed group. The significance of difference between data of the different groups was calculated by Student's t-test. ANOVA (SPSS 10.0 software, (SPSS Inc., Chicago, IL, USA)) was used to compare multiple groups and differences within the groups, and these were also tested for multiple comparisons by Tukey's significant difference test. We mainly focused to analyse more critically the results obtained in only p-DAB + PB + H_2O and p-DAB + PB + TG 1 (300 mg/kg b. wt.), p-DAB + PB + TG 2 (400 mg/kg b.wt.) and inclined to highlight these results more than that of the other doses used and included data leaving aside those of the other doses in the tables.

20.10 Results

There was a substantial increase in activities of AST and ALT in the liver tissue in the p-DAB + PB + H_2O fed group when compared to other groups such as normal, phenobarbital (PB), p-DAB, p-DAB + PB, p-DAB + PB + TG 1(300 mg/kg b.wt) and p-DAB + PB + TG 2(400 mg/kg b.wt) at both the fixation intervals of 90 and 120 days. However, when the data of AST and ALT of p-DAB + PB + TG 1(300 mg/kg b.wt.) and p-DAB + PB + TG 2 was compared it revealed that mice fed with TG had a significant modulatory effect where the activity was suppressed significantly ($p < 0.05$ through $p < 0.001$, Table 20.1). A similar trend was also noted when the LDH activity was compared (Table 20.1). But when p-DAB + PB + TG 1 and p-DAB + PB + TG 2 groups were compared it revealed that the dose of TG1 offered better protection than TG2 which was significant when compared to p-DAB + PB + H_2O fed group ($p < 0.05$ through $p < 0.001$, Table 20.1).

The enzyme GGT catalyses the degradation of the extracellular glutathione in a well identified cycle known as the γ-glutamyl cycle and when the activity of GGT was compared between various groups it revealed that there was an increase in activities in p-DAB, p-DAB + PB, p-DAB + PB + H_2O fed group when compared to normal control, only PB treated and mice treated with TG1 and TG2; however, it was highest in p-DAB + PB + H_2O fed group at both the fixation intervals ($p < 0.05$, Table 20.2).

When the activity of SOD was compared between various groups it revealed that activity was highest in the normal mice but it drastically reduced in p-DAB + PB + H_2O and p-DAB + PB treated mice at both fixation intervals ($p < 0.01$, Table 20.2). However, the activity increased in mice fed with p-DAB + PB + TG 1 when compared to the other dose. A similar trend was also

Table 20.1 Mean activities of AST and ALT (mM/min/mg) in liver of control and treated mice at 90-and 120-day fixation intervals

	AST (mM/min/mg)		ALT (mM/min/mg)		LDH (IU/L)	
	90	120	90	120	90	120
Normal	0.08 ± 0.002	0.07 ± 0.002	0.07 ± 0.001	0.06 ± 0.004	0.82 ± 0.02	0.79 ± 0.02
Phenobarbital (PB)	0.31 ± 0.021	0.38 ± 0.014	0.12 ± 0.014	0.26 ± 0.002	0.94 ± 0.23	0.96 ± 0.003
p-DAB	0.64 ± 0.017	0.58 ± 0.011	0.65 ± 0.024	0.77 ± 0.014	2.96 ± 0.15	3.01 ± 0.02
p-DAB + PB	0.70 ± 0.014	0.62 ± 0.022	0.74 ± 0.036	0.72 ± 0.030	3.02 ± 0.06	3.34 ± 0.05
p-DAB + PB + H_2O	0.65 ± 0.110	0.77 ± 0.014	0.88 ± 0.04	0.85 ± 0.022	3.44 ± 0.11	3.42 ± 0.14
p-DAB + PB + TG 1 (300 mg/kg b.wt.)	0.53 ± 0.002**	0.61 ± 0.012***	0.61 ± 0.021*	0.63 ± 0.008*	2.86 ± 0.03*	2.76 ± 0.02**
p-DAB + PB + TG 2 (400 mg/kg b.wt.)	0.60 ± 0.001	0.69 ± 0.014	0.71 ± 0.019	0.68 ± 0.015	2.93 ± 0.02	2.95 ± 0.25

Table 20.2 Mean activities of GGT, SOD and catalase in liver of control and treated mice at 90- and 120-day fixation intervals

	GGT (IU/L)		SOD (unit/min/mg protein)		CAT (μmole of H_2O_2 consumed/min/mg protein)	
	90	120	90	120	90	120
Normal	13.96 ± 1.02	14.02 ± 0.25	54.33 ± 1.22	53.47 ± 0.12	33.26 ± 1.02	37.51 ± 0.32
Phenobarbital (PB)	17.96 ± 0.589	18.56 ± 1.12	45.02 ± 2.02	47.08 ± 0.45	28.44 ± 0.45	26.02 ± 0.25
p-DAB	22.36 ± 0.145	22.01 ± 0.56	30.04 ± 2.22	32.06 ± 1.05	20.02 ± 0.31	21.88 ± 0.47
p-DAB + PB	27.98 ± 2.11	26.54 ± 0.44	28.12 ± 1.05	26.07 ± 0.22	19.45 ± 2.02	19.52 ± 1.11
p-DAB + PB + H_2O	32.14 ± 0.06	31.56 ± 0.78	27.11 ± 0.58	22.02 ± 0.48	18.77 ± 1.01	18.46 ± 0.58
p-DAB + PB + TG 1 (300 mg/kg b.wt.)	23.66 ± 1.02*	24.99 ± 3.55*	40.02 ± 0.42**	43.02 ± 0.35***	24.66 ± 0.48***	23.87 ± 1.02**
p-DAB + PB + TG 2 (400 mg/kg b.wt.)	26.21 ± 0.56	28.78 ± 0.45	39.46 ± 0.37	39.88 ± 0.89	20.85 ± 0.65	20.73 ± 0.36

n = non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

noticed when the activity of catalase was analysed at both the fixation intervals ($p < 0.001$, Table 20.2).

Cytogenetics analysis of the bone marrow chromosome aberrations revealed that in p-DAB + PB + H_2O, p-DAB, p-DAB + PB the percentage aberrations were more when compared to normal control, only PB fed and groups fed with TG extracts. Though both the TG extracts showed reduction in bone marrow aberrations in the mice fed with TG, the dose of 300 mg/kg b.wt offered better protection when compared to the other dose of 400 mg/kg b.wt ($p < 0.05$ through 0.001, Table 20.3, Fig. 20.1a–d). Similarly, it was found that the percentages of mitotic indices were high at 90 and 120 days in the p-DAB + PB + H_2O p-DAB, p-DAB + PB fed mice when compared to normal control, only PB fed and groups fed with TG extracts. The percentages of mitotic indices were decreased when they were treated with TG extracts. However, it was found that p-DAB + PB + TG1 drastically reduced the percentages of mitotic indices when compared to p-DAB + PB + TG2 ($p < 0.05$ through 0.001, Table 20.3).

On careful examination of the haematological profiles it was found that WBC, neutrophil, lymphocyte, monocytes, eosinophil, basophil and monocytes count was highest in p-DAB + PB + H_2O fed group followed by p-DAB, p-DAB + PB when compared to only PB, normal and TG fed mice ($p < 0.05$) at both the fixation intervals, i.e. 90 and 120 days. Though it was high in both the p-DAB +PB + TG 1 and p-DAB + PB + TG 2 fed mice but it was quite low when compared to p-DAB + PB + H_2O fed group. But the haemoglobin amount reduced appreciably in the mice that was administered p-DAB + PB + H_2O and p-DAB + PB at both the fixation intervals when compared to normal control, PB and p-DAB + PB + TG1 and p-DAB + PB + TG2 which was statistically significant ($p < 0.001$, Tables 20.4 and 20.5). The percentage HCT increased appreciably at both the fixation intervals in mice that was administered p-DAB + PB + H_2O and p-DAB + PB when compared to p-DAB + PB + TG1, p-DAB + PB + TG2 and normal controls and this increase was statistically more ($p < 0.05$, Tables 20.4 and 20.5). A similar trend was also noticed with MCV and MCH values ($p < 0.05$, Tables 20.4 and 20.5). Conversely, it was found that MCHC and PLT decreased quite significantly in p-DAB + PB + H_2O and p-DAB + PB and only p-DAB fed series when compared to normal control and TG fed series ($p < 0.05$ through $p < 0.001$); however, p-DAB + PB + TG1 showed more improvement when compared to p-DAB + PB + TG2.

The different levels of cytokine in various normal and different groups were represented in Table 20.6, where we found that IL6 was 55.77 pg/mL and 58.26 pg/mL and TNFα was 35.65 pg/mL, 32.66 pg/mL in normal mice at 90 and 120 days, respectively. It increased significantly in various groups of mice treated with only PB, only p-DAB, p-DAB + PB and p-DAB + PB + H_2O ($p < 0.05$ through $p < 0.001$) at the two fixation intervals. However, it reduced considerably in groups of mice administered with various doses of TG and this reduction was statistically significant when compared to p-DAB + PB + H_2O group. The dose of TG1 offered better reduction in the expression of cytokine when compared to TG2.

At both fixation intervals we encountered few notable changes in liver through transmission electron microscopy studies. Few changes which were noted in

Table 20.3 The table shows percentage chromosome aberration and mitotic indices in different groups of mice at the two fixation intervals

	Percentage BMCA		Percentage MI	
	90	120	90	120
Normal	0.18 ± 0.02	0.20 ± 0.03	1.11 ± 0.02	0.98 ± 0.47
Phenobarbital (PB)	0.33 ± 0.13	0.37 ± 0.05	2.08 ± 0.14	2.11 ± 0.07
p-dimethylaminoazobenzene (p-DAB)	0.78 ± 0.04	0.85 ± 0.14	4.31 ± 0.33	4.42 ± 0.31
p-DAB + PB	0.87 ± 0.32	0.87 ± 0.11	4.82 ± 0.21	4.96 ± 0.27
p-DAB + PB + H_2O	0.95 ± 0.11	0.93 ± 0.23	4.88 ± 0.64	5.02 ± 0.45
p-DAB + PB + TG 1(300 mg/kg b.wt.)	0.65 ± 0.24**	0.61 ± 0.19*	3.92 ± 0.05**	4.01 ± 0.02*
p-DAB + PB + TG 2(400 mg/kg b.wt.)	0.74 ± 0.07	0.75 ± 0.14	4.04 ± 0.11	4.14 ± 0.12

n = non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

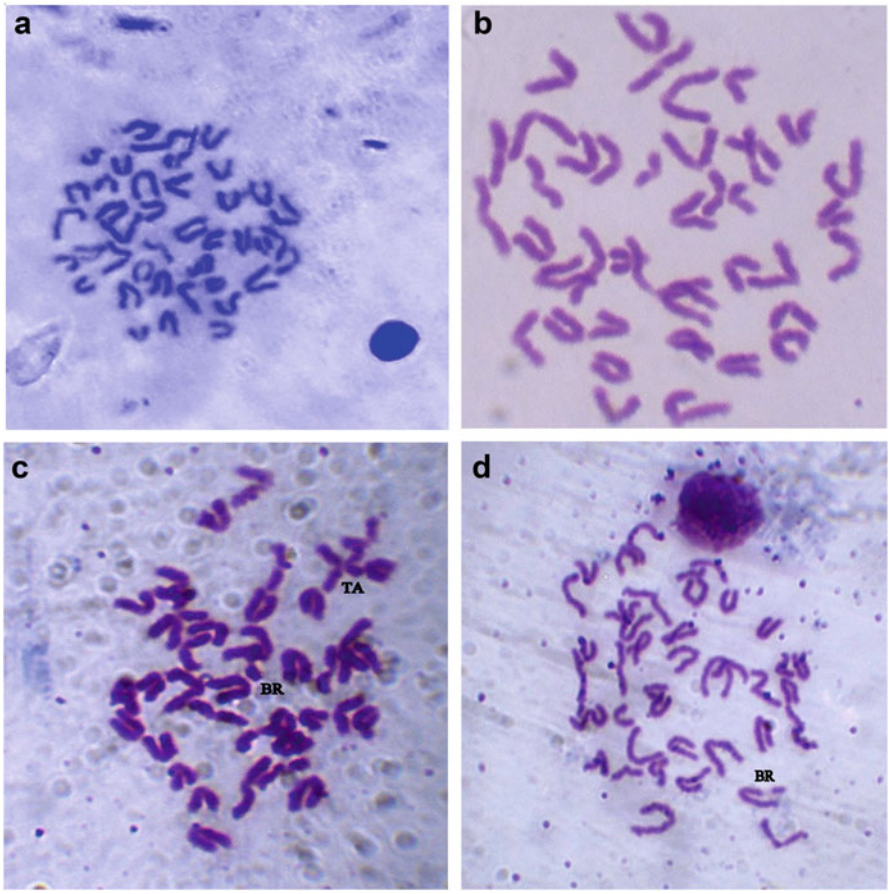

Fig. 20.1 (**a**) Normal metaphase complement of mice. (**b**) Normal metaphase plates from mouse bone marrow. (**c**) Few major aberrations were found in the p-DAB + PB + H_2O such as terminal association (TA) and break (Br). (**d**) Chromosome plate where break (Br) is prominent in p-DAB + PB + H_2O

p-DAB + PB + H_2O group were dispersed nucleoplasm, ER were broken and discontinuous, numerous mitochondria were evident which were more or less rounded in shape, there were numerous lipid droplets and Kupffer cells which were activated (Fig. 20.2a, b). Conversely, when TG 1 fed group was carefully examined, we found that though there was dispersed cytoplasm, ER was more or less continuous. There were lesser number of mitochondria which had distinct orientation of cristae, though lipid droplets were existing they were comparatively lesser in number. The Kupffer cells were present but they were fewer in number (Fig. 20.2c, d) though they were activated.

Table 20.7 represents the various phytochemicals which were estimated qualitatively and the intensity of their existence was represented by + sign. The aqueous

Table 20.4 The mean haematological variables of different treated and control series at 90-day fixation interval

	90 days						
	Normal	PB	p-DAB	p-DAB + PB	p-DAB + PB + H_2O	p-DAB + PB + TG 1	p-DAB+ PB + TG 2
WBC 10^9/L	1.44 ± 0.01	1.59 ± 0.01	2.59 ± 0.002	4.28 ± 0.02	4.11 ± 0.003	3.78 ± 0.02	3.88 ± 0.001
Neu 10^9/L	0.32 ± 0.01	0.54 ± 0.001	0.61 ± 0.002	0.68 ± 0.007	0.60 ± 0.006	0.48 ± 0.001**	0.44 ± 0.012
Lym 10^9/L	0.30 ± 0.022	0.31 ± 0.02	0.55 ± 0.003	0.62 ± 0.014	0.65 ± 0.012	0.45 ± 0.011	0.48 ± 0.030
Mono10^9/L	0.48 ± 0.015	0.68 ± 0.001	0.76 ± 0.012	0.82 ± 0.021	0.85 ± 0.017	0.49 ± 0.018*	0.52 ± 0.007
EOS 10^9/L	0.011 ± 0.011	0.02 ± 0.001	0.03 ± 0.001	0.32 ± 0.002	0.36 ± 0.002	0.30 ± 0.003n	0.40 ± 0.001
Baso 10^9/L	0.012 ± 0.004	0.04 ± 0.003	0.055 ± 0.018	0.052 ± 0.016	0.051 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
Neu %	22.12 ± 1.32	34.0 ± 2.22	77.15 ± 0.36	90.02 ± 0.55	93.06 ± 0.14	67.48 ± 0.15	69.06 ± 2.03
Lym %	15.20 ± 0.78	19.2 ± 1.69	56.02 ± 2.55	63.02 ± 3.21	66.06 ± 2.06	51.01 ± 0.87**	56.02 ± 1.02
Mon %	30.02 ± 1.02	42.9 ± 1.11	45.06 ± 2.56	66.02 ± 3.87	61.11 ± 3.66	50.02 ± 2.51***	62.03 ± 0.78
HGB g/L	13.42 ± 0.55	14.26 ± 0.42	11.58 ± 0.14	10.53 ± 0.11	10.01 ± 0.23	11.02 ± 0.44*	11.36 ± 0.03**
HCT %	14.44 ± 1.22	28.7 ± 1.66	33.33 ± 1.25	35.36 ± 2.31	35.03 ± 2.03	32.02 ± 1.11n	33.01 ± 0.06*
MCV fL	42.08 ± 1.33	72.5 ± 4.03	88.36 ± 2.07	88.01 ± 1.08	87.08 ± 1.98	80.01 ± 0.87	82.11 ± 2.03
MCH pg	14.02 ± 0.47	21.12 ± 4.02	35.41 ± 2.63	34.18 ± 0.46	34.01 ± 0.88	27.02 ± 0.01**	27.88 ± 2.58
MCHC g/L	175.02 ± 2.86	162.75 ± 4.05	105.06 ± 1.09	108.33 ± 4.09	98.06 ± 3.07	123.66 ± 5.01**	121.06 ± 2.03
PLT 10^9/L	42.45 ± 0.24	33.3 ± 0.18	27.33 ± 0.14	26.25 ± 0.06	26.22 ± 0.47	30.16 ± 0.12*	30.09 ± 0.11

n = non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 20.5 The mean haematological variables of different treated and control series at 120-day fixation interval

	120 days						
	Normal	PB	p-DAB	p-DAB + PB	p-DAB + PB + H_2O	p-DAB + PB + TG 1	p-DAB+ PB + TG 2
WBC 10^9/L	1.44 ± 0.03	1.79 ± 0.004	3.21 ± 0.22	4.69 ± 0.002	4.81 ± 0.21	4.12 ± 0.02	4.33 ± 0.33
Neu 10^9/L	0.32 ± 0.12	0.52 ± 0.002	0.58 ± 0.087	0.60 ± 0.02	0.66 ± 0.18	0.61 ± 0.004	0.78 ± 0.07
Lym 10^9/L	0.30 ± 0.007	0.35 ± 0.014	0.55 ± 0.12	0.62 ± 0.018	0.65 ± 0.06	0.45 ± 0.01	0.48 ± 0.02
Mono10^9/L	0.50 ± 0.003	0.61 ± 0.001	0.76 ± 0.006	0.82 ± 0.021	0.85 ± 0.003	0.49 ± 0.012	0.52 ± 0.02
EOS 10^9/L	0.011 ± 0.02	0.014 ± 0.02	0.035 ± 0.001	0.038 ± 0.001	0.036 ± 0.001	0.029 ± 0.003	0.031 ± 0.003
Baso 10^9/L	0.018 ± 0.007	0.038 ± 0.002	0.065 ± 0.021	0.060 ± 0.003	0.058 ± 0.002	0.042 ± 0.02	0.044 ± 0.001
Neu %	22.08 ± 2.06	30.81 ± 1.49	75.06 ± 3.55	88.24 ± 2.02	91.04 ± 3.98	70.01 ± 3.45***	78.04 ± 0.78
Lym %	15.20 ± 0.58	17.86 ± 0.44	56.02 ± 2.01	63.02 ± 3.14	66.06 ± 0.036	51.01 ± 0.58**	56.02 ± 1.02
Mon %	31.01 ± 1.22	42.33 ± 0.61	44.28 ± 1.87	60.24 ± 3.09	61.11 ± 0.78	53.78 ± 3.88*	57.02 ± 2.78
HGB g/L	13.11 ± 1.02	14.22 ± 0.78	11.20 ± 0.56	9.86 ± 1.88	9.72 ± 0.33	11.04 ± 0.60*	10.88 ± 0.38
HCT %	14.44 ± 0.78	20.02 ± 0.86	32.04 ± 1.02	33.66 ± 2.01	35.55 ± 1.02	30.02 ± 3.01**	31.77 ± 4.02
MCV fL	43.66 ± 0.47	68.77 ± 1.54	88.08 ± 0.65	90.27 ± 1.22	90.55 ± 0.88	81.75 ± 3.54**	83.04 ± 1.78
MCH pg	14.77 ± 1.02	21.07 ± 2.03	36.22 ± 0.47	35.88 ± 0.11	35.00 ± 1.22	29.09 ± 1.09	30.06 ± 2.11
MCHC g/L	175.02 ± 3.78	155.27 ± 4.88	108.78 ± 6.03	108.01 ± 2.41	107.26 ± 2.58	128.45 ± 4.98**	118.44 ± 5.02
PLT 10^9/L	44.02 ± 0.47	32.02 ± 0.33	26.08 ± 0.17	27.88 ± 0.66	25.88 ± 0.58	31.01 ± 1.02	29.78 ± 3.26

n = non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 20.6 Variation of the cytokine levels (pg/mL) in different treated and control sets at both the fixation intervals of 90 and 120 days

90 days							
Unit (pg/mL)	Normal	PB	p-DAB	p-DAB + PB	p-DAB + PB + H_2O	p-DAB + PB + TG 1	p-DAB+ PB + TG 2
IL6	55.77 ± 1.02	69.54 ± 0.44	176.66 ± 0.05	183.69 ± 2.09	182.77 ± 1.08	161.28 ± 0.78*	166.88 ± 2.05
TNFα	35.65 ± 0.21	62.16 ± 1.66	216.55 ± 5.02	216.87 ± 0.08	218.77 ± 3.07	196.34 ± 0.44**	202.77 ± 2.55
120 days							
IL6	58.26 ± 0.58	68.26 ± 0.98	173.26 ± 0.88	175.66 ± 6.05	183.56 ± 2.07	152.55 ± 0.14**	157.89 ± 5.66
TNFα	32.66 ± 1.22	86.04 ± 2.03	232.87 ± 0.45	255.26 ± 4.02	269.01 ± 3.33	234.22 ± 4.02***	240.02 ± 0.89

n = non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

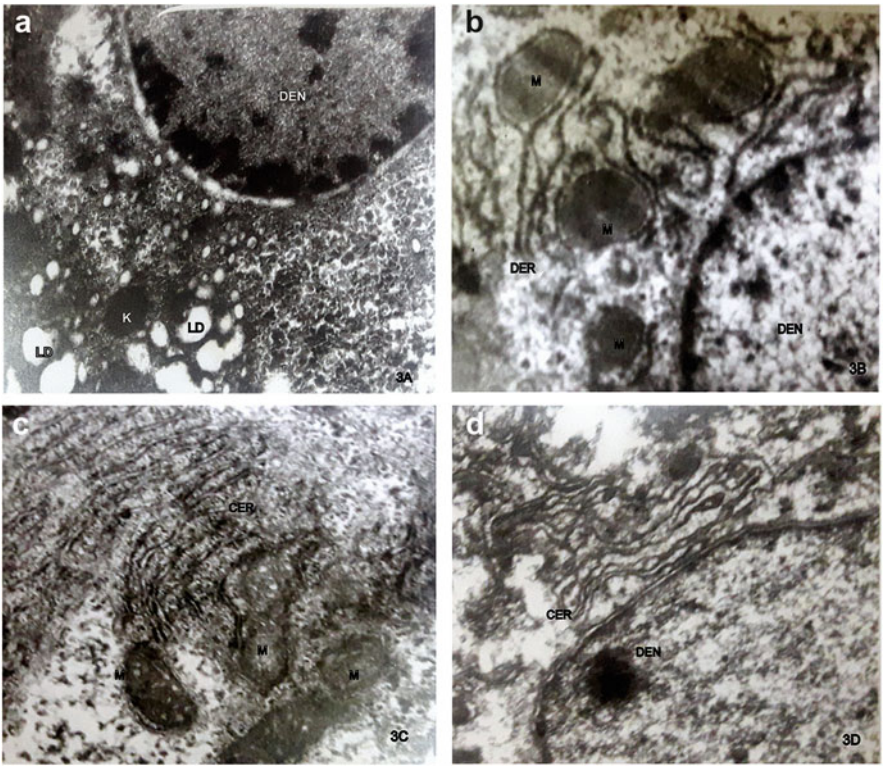

Fig. 20.2 (**a**, **b**) TEM micrograph of liver showing various changes in p-DAB + PB + H2O fed mice at 120-day fixation intervals *M* mitochondria, *DEN* dispersed nucleoplasm, *CER* continuous endoplasmic reticulum, *LD* lipid droplets, *DER* discontinuous endoplasmic reticulum. (**c, d** Few major changes which were noted in mice fed with p-DAB + PB + TG1 (300 mg/kg), *M* mitochondria, *DEN* dispersed nucleoplasm, *CER* continuous endoplasmic reticulum, *LD* lipid droplets, *DER* discontinuous endoplasmic reticulum, *K* Kupffer cells

Table 20.7 Preliminary phytochemical screening of the aqueous seed extract of TG (+ sign indicates intensity)

Chemical compounds	Seed extracts of TG
Alkaloids	+++
Glycosides	+++
Flavonoids	++
Carbohydrate	++
Tannin	++
Steroids	++
Reducing sugars	++

extract of TG seed revealed the presence of various compounds such as flavonoids, alkaloids, tannin, carbohydrate, reduced sugars, glycosides and steroids.

GC-MS study revealed the presence of several phytochemicals as listed in Table 20.8. We have found several useful compounds such as

Table 20.8 GC-MS screening of the aqueous seed extract of *Trigonella foenum graecum*, RT = retention time, Area Pct = area percentage

PK	RT	Area Pct	Library/ID-Wiley 7n.1 Database
1.	6.9986	0.1062	Benzoic acid, 2-amino-, phenylmethyl ester
2.	7.2217	0.1883	Calyculaglycoside A
3.	7.3133	0.2303	2-methylbutanoic acid
4.	8.1029	0.1037	Pentanoic acid
5.	8.2116	0.2503	1,2-Ethanediol
6.	9.5506	0.0982	Isopentyl hexanoate
7.	10.7293	0.101	Allylethylamine
8.	12.1312	0.062	1-Isopropyl-2,2-dimethylpropylideneamine
9.	13.4701	0.6156	Imidazole, 4-fluoro-1-methyl-5-carbhydrazino
10.	15.1467	0.0962	Tetradecane
11.	15.7532	0.2061	5,5-Dimethyl-3-pyrazolidinone
12.	16.1709	0.1054	N-formylproline methyl ester
13.	16.2968	0.1927	Isoamyl butyrate
14.	16.5485	0.1815	Tributylamine
15.	17.1493	5.5985	2,4-Dimethylpyrrole
16.	18.0305	0.4996	Megastigmatrienone 2
17.	18.2651	0.2059	Propanedioic acid, diethyl ester
18.	19.152	2.0299	Silacyclopentane-1,1-D2
19.	19.461	4.2996	Ethyl alpha-d-glucopyranoside
20.	19.5068	1.1743	Thiophene
21.	19.6499	1.2799	n-Butyric acid
22.	19.7014	1.2545	Ribopyranoside
23.	19.7357	1.4731	Heptanoic acid
24.	19.8101	2.2676	Beta-D-glucopyranoside
25.	20.0447	8.3194	Ethyl alpha-d-glucopyranoside
26.	20.491	0.8103	Mome inositol
27.	20.6741	0.8297	1,2-Benzenedicarboxylic acid
28.	20.7599	1.1847	9,11-Octadecadiynoic acid
29.	20.8457	1.7756	1,8-Dioxacyclohexane-2
30.	21.1318	0.4944	Hexadecanoic acid
31.	21.5209	0.1747	Propane, 1,2,2,3,3-pentachloro-1,1-difluoro
32.	21.6583	1.3041	n-Hexadecanoic acid
33.	21.7441	0.2311	1-Nonadecene
34.	21.9959	0.863	2-chloro-1,8-dihydroxy-5-methoxy-6-methyl-9H-xanthen-9-one
35.	22.1618	0.1803	2-Methoxy-1-oxaspiro [4.4] nonane
36.	22.6825	0.0441	Phosphonic acid
37.	22.7626	1.1647	9,12-Octadecadienoic acid, methyl ester
38.	22.8313	1.1189	Ethyl linoleate
39.	22.9343	0.256	Phytol
40.	23.0201	0.203	Stearic acid methyl ester
41.	23.2032	1.0975	Lauramide

(continued)

Table 20.8 (continued)

PK	RT	Area Pct	Library/ID-Wiley 7n.1 Database
42.	23.3863	9.5035	Linoleic acid ethyl ester
43.	23.4607	4.9127	Linolenic acid
44.	24.4163	0.2454	Dodecane, 6,6-Dideutero
45.	24.5421	0.1127	D-Glucitol
46.	24.6623	0.0483	Dibenzazepine-2-carboxaldehyde
47.	24.7195	2.393	3-Heptadecenal
48.	24.7539	0.8071	9-(Isopropoxycarbonyl) phenanthrene
49.	24.7939	2.0648	1-Benzoylamino-5-piperidinyl-1-(4-bromophenyl)-pentane
50.	24.9484	1.524	Dodecanamide, N-(2-hydroxyethyl)
51.	25.1716	0.2688	Nordextromethorphan
52.	26.2816	0.1278	Ethanone, 1-(3-ethylpyrazinyl)
53.	26.3503	1.8714	1,1′,1″-Trimethyl-2,2′:5′,2″-tetrapyrrole 2,2′:5′,2′‘-Ter-1H-pyrrole, 1,1’,1″-trimethyl-(CAS)
54.	26.5391	0.5583	Hexahydroindan)
55.	26.5906	0.1248	Di-n-octyl phthalate
56.	27.3173	0.2255	Campesterol
57.	27.6492	0.1337	6.Beta. -Acetoxy-6,7-dihydro-2,3,9,10-tetramethoxy-7. Alpha.-vinyl-5H-dibenzo[a,c]cycloheptene-5-one
58.	27.7579	4.2918	Linolein, 1-mono
59.	27.8151	1.7494	Linolenic acid

calyculaglycoside A, allylethylamine, ethyl linoleate, phytol, stearic acid methyl ester, lauramide, linoleic acid ethyl ester, ethyl. Alpha-d-glucopyranoside, dodecane, 6,6-dideutero-D-glucitol,dibenzazepine-2-carboxaldehyde,3-heptadecenal, isopropoxy carbonyl phenanthrene, dodecanamide, nordextromethorphan, ethanone, 1-(3-ethylpyrazinyl)-1,1′,1″-Trimethyl-2,2′:5′,2″-tetrapyrrole 2,2′:5′,2′‘-Ter-1H-pyrrole, 1,1’,1″-trimethyl-Hexahydroindan), campesterol, linolenic acid some of which has narrow to wide peak area.

20.11 Discussion

Medicinal plants constitute a basis of raw materials for both traditional systems of medicine and modern orthodox medicine. They represent a significant proportion of the global drug market. Most rural populations, especially in the developing world, still depend on medicinal herbs as their main source of primary health care because they come from low socioeconomic status, reside in remote areas where there is no health care facility. The search for medicines and dietary supplements from ethnomedicinal sources has accelerated in recent times; therefore, the ethno-pharmacologists and chemists are exploring the putative compounds and their sources of therapeutic potential. It has been reported that chronic feeding of both

p-DAB (initiator) and PB (promoter) has been successfully used to develop liver tumours (and subsequently cancer) by some earlier authors (Kitagawa and Sugano 1977; Nesn et al. 1987; Aydinlik et al. 2001; IARC 2001, 2003; Biswas and Khuda-Bukhsh 2004, 2005; Pathak and Khuda-Bukhsh 2007). However, while the chronic feeding of only p-DAB for a very long time 45–61 weeks has been reported to induce liver tumours, but chronic feeding of PB alone has not been reported to produce such tumours (Ueda 1967; Akamatsu and Ikegami 1968). Figure 20.3 showed some of the representative photographs of normal (a), p-DAB + PB + H_2O (b) and p-DAB + PB + TG1 (c) treated mice at day 120. The toxicity of p-DAB is attributed to its degradation products, i.e. p-DAB is metabolized to mono-aminoazobenzene (MAB) by N-demethylation, and MAB is later metabolized to amino azo-benzene (AAB) through demethylation or to N-hydroxy-N-methyl-4-aminoazobenzene (NOH-AAB) Ohnishi et al. (2001). The covalent binding of these metabolites to DNA is not only a chief carcinogenic factor but it can also be supposed to cause the numerous chromosomal aberrations through their interaction with chromosomal DNA in p-DAB-fed mice and pDAB + PB + H_2O fed series as it is evident in the present investigation when compared to normal control (Fig. 20.1a–d). Amino-azo dyes have an exocyclic amino group that undergoes biochemical N-oxidation and further conversion to reactive electrophiles (Aydinlik et al. 2001). It is well established that free radicals which evolved may subsequently form reactive oxygen species (ROS), which are involved in hepatotoxicity and carcinogenesis by these aromatic amines. Further, free radicals are electrophilic species that can react with cellular components. Data obtained in the current work revealed substantial increment in the mean values of WBC neutrophil, lymphocytes, monocytes, eosinophil, basophil count in the p-DAB, p-DAB + PB and p- DAB + PB + H_2O when compared to normal control, only PB fed and TG treated groups at both the fixation intervals. Conversely, there was a considerable decrement in the haemoglobin content in the p-DAB, p-DAB + PB and p-DAB + PB + H_2O which might be attributed to loss of blood internally when compared to p-DAB + PB + TG1 and p-DAB + PB + TG2, in the TG treatment mice the haemoglobin content remarkably improved. It is known that p-DAB + PB enhances ROS production which leads to upregulation of proinflammatory cytokines as was evident in the present investigation. These cytokines result in activation of leucocytes which was also evident while comparing the different positive and negative control groups in the study. The study of haematological variables was important in the sense because they have the highest cell turnover and is quite sensitive to subtle changes. Therefore, investigating the modulatory effects of TG on p-DAB + PB induced in haematological variables could yield more information about its effectiveness as a possible candidate against hepatocarcinogenesis. On GC-MS analysis we encountered numerous phytoconstituents such as carvacrol, calyculaglycoside A, iso-cubenol, phytol, inositol, etc. Carvacrol is a monoterpene phenolic constituent which was present in the extract and is a potent anti-inflammatory substance. It has been reported that carvacrol and iso-cubenol inhibit hypernociception by inhibiting migration of neutrophils and cells involved in the production of pro-inflammatory cytokine such as TNF-α and a decrease in prostaglandins. Treatment with TG extracts resulted

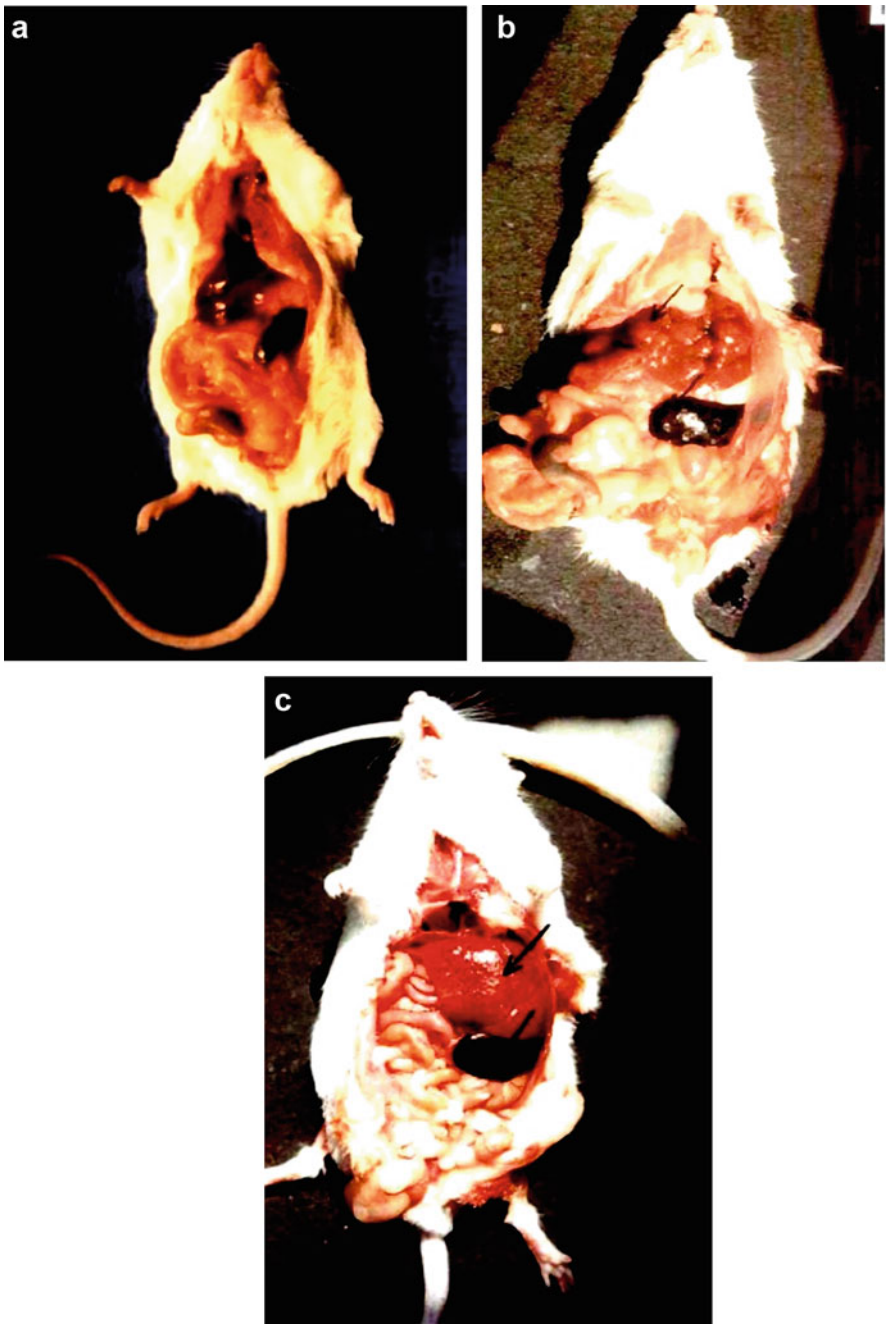

Fig. 20.3 Few representative photographs of mice of treated and control sets at 120-day fixation intervals. (**a**) Normal live and spleen of mice. (**b**) Mice treated with p-DAB+PB+H2O the liver with tumour nodules and enlarged spleen. (**c**) Mice treated with p-DAB+PB+TG1 though the liver has cysts their intensity was low but the spleen was enlarged

in decrease of cytokines in the present investigation. To the best of our knowledge this is the first experiment that demonstrated the modulating potentials of aqueous TG seed extract against p-DAB + PB induced carcinogenesis. Another phytoconstituent calyculaglycoside A inhibits the synthesis of prostaglandin PGE2 and leukotriene, which are inhibitor 5-lipoxygenase and cyclooxygenase; therefore, it might decrease the inflammation of the liver in the TG treated group. In the present investigation there was an increase in SOD and catalase activities with a concomitant decrease in AST, ALT, LDH and GGT activities at both fixation intervals. The outcomes of the present study are encouraging because treatment with aqueous extracts of TG seeds somehow attenuated the p-DAB induced carcinogenesis. During the investigation we encountered flavonoids and phenolic compounds and the reduction in the toxicity induced by p-DAB + PB may be due to a number of phenolic hydroxyl groups attached to the ring structures of the flavonoids. Chopra et al. (2006) reported that alkaloids, flavonoids and reducing sugars are the key sources for the plant to achieve its activity and the sustainability in reducing liver damage. It has been reported by other workers that the main constituents of the fenugreek extracts are polysaccharides, saponins, flavonoids, trigonelline and choline. Polysaccharides act as modulators of carcinogenesis and stimulate macrophages (Choi et al. 2005; Kim et al. 2007). Thakur and Ahirwar (2019) reported the antitumour properties and antimetastatic effect of the steroidal compound ethyl iso-allocholate where they demonstrated that the steroidal derivative induces caspase dependent apoptosis and lessens tumour growth. Allaoui et al. (2019) demonstrated the anticancer efficacy of two protein hydrolysates obtained from fenugreek against colorectal cancer, it triggers membrane permeabilization of mitochondria resulting in cytochrome c release and activation of caspase 3. There are few reports on the antioxidant and anticancer activities of TG in HepG2 cancer cell lines (Khalil et al. 2015; Al-Dabbagh et al. 2018). In the present investigation the two doses of TG were selected based on the earlier studies by the same group (Biswas et al. 2019) where it was found that high doses of TG seed extracts were non-toxic and in this particular study we found that the dose of 300 mg/kg offered better protection than the mice fed with 400 mg/kg of TG extracts. Diosgenin a compound obtained from TG seeds is a steroidal saponin which is biosynthesized from cholesterol has been reported earlier to have numerous pharmacological properties, viz. anti-inflammatory, anti-proliferative, hypoglycaemic activity and a potent antioxidant (Jesus et al. 2016; Sethi et al. 2018). Similarly, others reported methanolic extract of fenugreek seeds retards rate of proliferation and induces p53 expression in experimental skin carcinogenesis (Ali et al. 2014). Though the specific mechanism of the modulatory potentials of aqueous TG seed extract is not yet clear, it can be safe to conclude that the collective actions of all the ingredients contribute mutually to their protective effects against p-DAB induced carcinogenesis. Thus, this investigation creates an avenue for further investigation on this plant for isolation of the phytoconstituents and to determine pharmacological effectiveness.

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Therapeutic Uses and Applications of Fenugreek

21

Ayah Rebhi Hilles and Syed Mahmood

Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is used as traditional medicine as it possesses various therapeutic activities including anti-inflammatory, anticarcinogenic, antioxidant, antianorexic, antiatherogenic, antidiabetic and antihyperlipidemic immunomodulatory antinociceptive, anti-ulcer antimicrobial, anthelmintic, anti-obesity and hepatoprotective effects. Besides, fenugreek has a cleansing action which helps purify the blood, lymphatic system as well as detoxify the body. It is also considered as a galactagogue for promoting lactation, and it may serve as an excellent animal food supplement. The pharmacological uses of fenugreek can be attributed to its bioactive chemical constituents. These chemicals make it a powerful candidate to cure diseases. It holds a promising future in the field of natural products to cure diseases. Fenugreek has been widely studied in in vitro, in vivo and clinical studies which showed significant evidence that fenugreek possesses therapeutic properties applicable to treat many diseases. In this chapter, we summarised the pharmacological aspects of fenugreek.

Keywords

Anti-inflammatory · Anticarcinogenic · Antifertility · In vitro · In vivo · Clinical studies

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21.1 Introduction

Fenugreek is one of the herbs that has been used for a long time in medicine as a laxative and appetiser (Khalki et al. 2013). Many researchers have studied the different properties of this herb and its effect on fertility. Fenugreek seeds have oestrogenic activity (Sreeja et al. 2010). The seeds were also found to cause an increase in serum prolactin level (Kheder et al. 2012). It was reported that fenugreek possesses hypoglycaemic, hypercholesterolaemia, immunomodulatory and antioxidative activities (Rebhi Hilles and Mahmood 2016). Another report summarised the medical application of fenugreek seeds in Chinese traditional medicine as a hypolipidemic, anti-obesity, anti-inflammatory, anticancer, antidiabetic, antibacterial, antioxidant and antifungal agent (Yao et al. 2020). Furthermore, it showed a positive influence on arthritic pain management, high blood pressure, mouth ulcer treatment, diuretic and gastric irritation (Rashid et al. 2018). Moreover, it can be considered as dietary proteins for vegetarians which are not available in animals and fish protein diet (Talib et al. 2014).

21.2 Pharmacological Activities

21.2.1 Anticancer Activity

The fenugreek seeds had shown proliferative inhibition on MCF-7 breast cancer cell line at a concentration of 400 μg/mL after 72 h of incubation, which showed that the herb could be a potent anticancer agent (Al-Timimi 2019). It was also reported that fenugreek ethanol extract (FEE) showed 100% inhibition of blood vessel outgrowth from primary tissue explants in the rat aortic ring assay at 100 μg/mL concentration. In vivo chicken embryo chorioallantoic membrane (CAM) assay was also conducted to evaluate the antiangiogenic properties of FEE which showed significant inhibition of formation of new blood vessels at 400 μg/mL against MCF7 cell lines. These findings confirmed the effect of FEE in inhibiting the vascularisation significantly (Habib-Martin et al. 2017). It was investigated that fenugreek has anticancer properties against MCF-7 human immortalised breast cells due to induction of apoptosis and increasing the expression of pro-apoptotic genes (Khoja et al. 2011). In another study, the growth inhibitory effect of fenugreek extract against MCF7 human breast and pancreatic (AsPC-1) cells showed a significant effect on cell viability by elevating caspase-3, caspase-6 and LDH activity (Abas and Naguib 2019). Treatment with 10–15 μg/mL of fenugreek extract for 72 h revealed inhibition of the growth in breast and pancreatic cancer cell lines through induction of cell death, downregulation of mutant p53 and upregulation of p21 (Shabbeer et al. 2009).

21.2.2 Antioxidant Activity

Setti et al. (2018) revealed that fermented fenugreek has significant antioxidant properties. Different types of fenugreek extract have been examined for their antioxidant effects. The various studies showed that all fenugreek seed extracts possess antioxidant activity (Bukhari et al. 2008). Further, it was reported that the antioxidant properties of fenugreek seeds are directly linked to the presence of polyphenols in it (Naidu et al. 2011). A novel polysaccharide extracted from fenugreek seeds exhibited a significant wound healing potential due to its antioxidant activities. The study of Ktari et al. (2017) revealed that the novel polysaccharide from fenugreek seeds has strong antioxidant properties. In another study, it was incorporated in a novel electrospun scaffold made up of a silk fibroin nanofiber formulated by electrospinning method. The antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. The scaffold's DPPH activity increases with the concentration of fenugreek and the wound healing activity of silk fibroin–fenugreek nanofibres also showed an increment (Selvaraj and Fathima 2017).

Moreover, it was found that fenugreek enhanced the hydroxyl radical scavenging capacity, reflecting high antioxidant activity (Guardiola et al. 2018). It was demonstrated that dietary supplementation of fenugreek seeds improves resistance against hypoxia and starvation in *Oreochromis niloticus* (Basha et al. 2018). Another study has illustrated that fenugreek exhibited a significant increase in phenolic antioxidants (Randhir et al. 2004). Furthermore, it was reported that the antioxidant effect of fenugreek and garlic combination increased the glutathione level in the serum and the liver and the ascorbic acid in the heart and the liver (Mukthamba and Srinivasan 2016).

21.2.3 Anti-Inflammatory Activity

A dose of 200 mg/kg of fenugreek seeds showed significant anti-inflammatory activity (Malviya et al. 2010). The ethanol extract of fenugreek seeds showed anti-inflammatory and antioxidant activities (Suresh et al. 2012). Alkaloids, flavonoids and glycosides are the key components in the fenugreek extract. The anti-inflammatory properties of flavonoid and alkaloid of fenugreek seeds might be due to their ability to inhibit pro-inflammatory enzymes, namely cyclooxygenases (COX) and lipooxygenases (LOX) (Mandegary et al. 2012). Moreover, saponins showed anti-inflammatory activity by inhibiting inflammatory cytokines such as interleukin TNF-α and IL-1β (Kawabata et al. 2011). Furthermore, the aqueous extract of fenugreek leaves has been tested for its anti-inflammatory activity in the formalin-induced rat paw oedema model. The extract was given daily to the rats in two different oral concentrations, i.e., 1000 mg/kg and 2000 mg/kg for 7 days which showed a significant anti-inflammatory effect (Ahmadiani et al. 2001). Another study on the carrageenan-induced rat paw oedema model showed highly significant ($p < 0.001$) anti-inflammatory effect of fenugreek seed extract (10 and 20 mg/kg intraperitoneally) (Vyas et al. 2008). This anti-inflammatory activity could also be

attributed to the presence of linolenic and linoleic acids in the fenugreek extract (Pundarikakshudu et al. 2016).

21.2.4 Antidiabetic Activity

Many researchers have studied the hypoglycaemic effect of fenugreek seeds as well as their aqueous and ethanol extracts in normal and in diabetic models of animals. They found that fenugreek seeds and their both extracts have a hypoglycaemic effect as they cause an increase in insulin secretion (Petit et al. 1993; Zia et al. 2001; Devi et al. 2003; McAnuff et al. 2005; Singh and Garg 2006; Eidi et al. 2007). It has a broad spectrum of hypoglycaemic effects (Murthy et al. 2010). It was reported that intraperitoneal administration of fenugreek seed extracts to alloxan-diabetic mice with 15 mg/kg for 5 and 10 days exhibited insulin-like properties and showed significant enhancement on hepatic glucokinase and hexokinase enzymes (Vijayakumar and Bhat 2008). Puri et al. (2011) mentioned three mechanisms, explaining the hypoglycaemic effect of fenugreek seeds, increasing the insulin secretion, enhancing the utilisation of glucose by the cells and increasing the activity of enzymes that stimulate the glycolysis suggesting that fenugreek could be used as a potent hypoglycaemic agent (Hamden et al. 2017). Gong et al. (2016) demonstrated that fenugreek also has total cholesterol-lowering efficacy besides its hypoglycaemic nature. Diosgenin and 4-hydroxyisoleucine are the most abundant bioactive compounds in the fenugreek. These compounds are known for their beneficial effects on inflammation, glucose tolerance, insulin action, blood lipids, liver function and cardiovascular health. Besides, various reports have been published showing the therapeutic values of fenugreek in type 2 diabetes and metabolic diseases (Fuller and Stephens 2015). A subsequent study also confirmed that fenugreek reduced the blood glucose level by 58%; moreover, it restored liver glycogen content, decreased kidney glycogen and reduced liver glucose-6-phosphatase activity in streptozotocin (STZ)-diabetic rats (Gad et al. 2006). Fenugreek consists of (2S, 3R, 4S) 4-hydroxyisoleucine (4HO-Ile) amino acid, which exhibited antidiabetic activity in STZ-diabetic rats by enhancing insulin secretion under hyperglycaemic conditions and increasing insulin sensitivity (Haeri et al. 2012). Fenugreek exhibited a high capacity in reducing the glucose level and elevating the insulin level in STZ-diabetic rat model which may be attributed to the presence of a significant quantity of bioactive compounds such as saponins, alkaloids and polyphenol (Marzouk et al. 2013). Administration of fenugreek extract was reported to be beneficial for treating 166 patients with type 2 diabetes (T2D). It appeared to have significant hypoglycaemic activity in T2D patients (Bawadi et al. 2009). A dose of 150 mg/kg of fenugreek extract in STZ-induced diabetic rats revealed a significant hypoglycaemic activity by reducing fasting plasma triglyceride level in T2D rats by 22%, 24.6% and 29% at 10, 20 and 30 days of treatment, respectively, without exhibiting any side effects (Swaroop et al. 2014). Soaked fenugreek in hot water was given to 24 patients with type 2 diabetes that showed 25% reduction of fasting blood sugar, 30% reduction of triglyceride (TG) and 30.6% reduction of very low-density lipoprotein (VLDL). Furthermore, the fenugreek seeds can be used as an adjuvant in

controlling T2D when soaked in hot water (Kassaian et al. 2009). The STZ-induced diabetic rats experienced improved urine sugar (30%) and fasting blood glucose (26%) when fed with fenugreek seed mucilage (Kumar et al. 2005).

21.2.5 Antifertility Activity

The male rats were treated with a steroidal extract of fenugreek seeds for 2 months to determine the effect of fenugreek on the male reproductive system. A reduction in the quantification and motility of sperms in treated rats was found when compared with the rats in the control group. Moreover, the testicular weight and androgen-dependent parameters (protein, sialic acid and fructose) were lower in the treated group, indicating reduced androgen levels. Histological examination showed the cease of spermatogenesis, degeneration of seminiferous tubules as well as epididymis (Kamal et al. 1993). The effect of fenugreek seeds on the female and male reproductive organs was also tested in the rabbits. In female rabbits, pre-breeding oestrogen and progesterone levels in the treated group were measured and found to be significantly less than their levels in the control group. In contrast, the level of gestational progesterone was increased dramatically in the treated group compared to the control group. The histological examination of ovaries and uterus showed an increase in the count of corpus luteum and morphological changes in endometrial glands of the treated group. However, in the male rabbits which were treated in the same way as their female counterparts, there was a marked decrease in testosterone level, and the testes showed a decrease in testicular weight and sperm count. Histologically, the tests of the treated group showed a decrease in the number of seminiferous tubules and abnormal spermatogenesis (mild hypoplasia) (Kassem et al. 2006). In 2009, Aswar and his group administrated 75 mg/kg of fenugreek seed extract to 18 pregnant rats on days 1–7 of pregnancy. The implantation rate was detected on day 10 of gestation, and the number of implanted embryos was counted. There was no significant difference in the implantation rates or the number of pups born in the treated group as compared to the control group (Aswar et al. 2009). Modaresi et al. (2012) studied the effect of three different doses (50, 100 and 200 mg/kg) of the alcoholic extract of fenugreek seeds on female reproductive hormones in mice. The extract was administrated intraperitoneally. They found a significant decrease in follicle stimulating hormone (FSH), *luteinising hormone* (*LH*) and estradiol levels in all study groups. Still, the progesterone level was elevated significantly in the second study group (200 mg/kg) only. It was also established that oral administration of fenugreek seed extract (FSE) significantly reduced the level of FSH, when compared to commercial combined oral contraceptive pills (COCPs), indicating that fenugreek seeds can be used as a natural alternative contraceptive method (Hilles et al. 2016). A group of female Sprague Dawley rats was orally administered with 750 mg/kg of fenugreek seeds extract, while other group was given 0.05 mg/kg COCPs for 15 days. The endometrial atrophy and decidual-like cells were observed in COCPs group, while in the FSE extract, the uterine tissues were normal suggesting that FSE could be consumed as a safe natural contraceptive agent (Allow et al. 2016).

21.2.6 Antimicrobial

It was reported that a topical gel formulation of fenugreek leaf aqueous extract could be considered as a safe and effective herbal treatment for various cutaneous fungal infections including dandruff (Kulkarni et al. 2019). The silver nanoparticles of fenugreek seeds showed antibacterial properties against Gram-negative bacteria (*Escherichia coli* and *Staphylococcus aureus*). Furthermore, the ultrasound-assisted nanoparticles from fenugreek reported higher stability as well as antibacterial and antioxidant properties compared with the nanoparticles fabricated by magnetic stirring (Deshmukh et al. 2019). A lethal effect of fenugreek against hazardous bacteria, especially *coli* forms, *Pseudomonas spp., Salmonella typhi* and *Shigella dysenteriae* was studied (Shashikumar et al. 2018). Antibacterial properties of fenugreek seed's methanol extract against *Pseudomonas aeruginosa* were recorded higher than Amikacin (commercial antibiotic) (Alwan et al. 2017). It showed significant bactericidal activity against *B. subtilis*, *K. pneumoniae* and *S. aureus* and antifungal activities against *F. equiseti* and *A. alternata* (Alwhibi et al. 2018). Furthermore, the plant has antimicrobial activity against *Escherichia coli, Shigella dysenteriae, Pseudomonas spp*. and *Salmonella typhi* in addition to *Staphylococcus aureus* and *Bacillus subtilis* (Dash 2011; Norziah et al. 2015; Sharma et al. 2016). Another study also illustrated the antimicrobial properties of fenugreek against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* and one mould: *Aspergillus niger* (Sulieman et al. 2008). It also presented a significant antibacterial activity against peptic ulcer-linked *Helicobacter pylori* (Randhir et al. 2004) as well as *Escherichia coli, Staphylococcus aureus, P. aeruginosa, Klebsiella pneumonia* and *Salmonella typhi* (Alwhibi and Soliman 2014). Furthermore, it exhibited antibacterial activity against *E. coli, S. aureus, S. saprophyticus, S. epidermis, Candida albicans, Streptococcus pyogenes, P. aeruginosa, Klebsiella pneumoniae* and *Proteus vulgaris* (Abdalah 2011; Al-Musrati et al. 2015). . Fenugreek showed high antimicrobial activity against pathogenic bacterial strains (*Serratia marcescens* and *Bacillus cereus*) and pathogenic fungal strain (*Trichoderma viride*) with a high zone of inhibition due to significant potential to be used as an antimicrobial agent due to the presence of different phytochemicals (Dharajiya et al. 2016). It was revealed that fenugreek possessed activity against both Gram-positive (*S. aureus*) and Gram-negative *(P. aeruginosa)* bacterial strains (Al-Timimi 2019). The antimicrobial properties of fenugreek could be attributed to the presence of flavonoids, alkaloids, saponins, tannins, terpenoids and steroids, individually or collectively (Khursheed et al. 2012).

21.2.7 Neuroprotective Activity

It was reported that fenugreek seeds have a neuroprotective effect, which was attributed to the presence of polyphenolic flavonoids such as naringenin, kaempferol, vitexin and steroid saponin in fenugreek seeds (Belaïd-Nouira et al. 2012). It was recorded that fenugreek seeds extract inhibitory effect on acetylcholinesterase activity, a key enzyme involved in the pathogenesis of Alzheimer disease (AD) (Satheeshkumar

et al. 2010). Administration of fenugreek seeds has a neuroprotective effect, a possible treatment of neurodegenerative diseases, including AD (Prema et al. 2016). Moreover, treatment with fenugreek seeds powder showed a neuroprotective activity via immunomodulatory and antioxidant actions (Belaïd-Nouira et al. 2013). It was suggested that the saponin, which is found in fenugreek, could inhibit AD via increasing Acetylcholinesterase (AchE) inhibition activity. It could increase the antioxidants and enhance the apoptosis through regulating apoptotic related genes (Bax, Bcl-2 and casapse-3) in the apoptotic pathway, not by the generation of reactive oxygen species (ROS) in the brain cells of the AD-induced rats (Khalil et al. 2016). The diosgenin, a steroidal sapogenin found in fenugreek seeds, modulates the production of pro-inflammatory cytokines, including IL-1 and IL-6 (Jung et al. 2010). Moreover, it also exhibits an antiparkinson effect by attenuating behavioural changes, preventing apoptosis and restoring the level of malondialdehyde in the rats (Mirzaie et al. 2016). It exhibited positive influence on Parkinson's disease and pathological symptoms of Alzheimer's disease, similarly it has a potential role in various neurological disorders and modulatory effect on cognitive functions (Zameer et al. 2018). Another study recorded that permanent bilateral ligation of the two carotid arteries (two-vessel occlusion, 2VO) in the male Sprague Dawley rats after 8 weeks from 2VO surgery could reduce the superoxide dismutase and glutathione levels besides elevating the malondialdehyde and C-reactive protein (CRP) concentrations. An intervention with 100 mg/kg of fenugreek seeds extract was given to the rats where it enhanced the memory impairment, increased the superoxide dismutase and glutathione enzyme activities and reduced the malondialdehyde and CRP. These findings revealed fenugreek has a neuroprotective effect which can be beneficial in cerebrovascular type dementia (Alruhaimi et al. 2018).

21.2.8 Fenugreek Seeds as Lactation Stimulant

In India, fenugreek is traditionally used as a lactation stimulant (Al-Asadi 2014). It is considered as an effective galactagogue to stimulate and increase milk production in lactating mothers (Gabay 2002). Since dopamine inhibits the prolactin secretion, the fenugreek seeds are supposed to be dopamine antagonists (Ben-Jonathan and Hnasko 2001). Another study explained the lactation stimulant activity of fenugreek where ewes in mid-lactation were given fenugreek seeds orally in two different doses (2.5 and 5 g/kg), once per week for 7 weeks. The milk yield was measured daily, and the prolactin levels were investigated weekly. The results showed a significant increase in milk yield as well as in the prolactin level in treated groups compared to the control group (Kheder et al. 2012).

21.2.9 Hypolipidemic Activity

Many studies indicated that administration of fenugreek seeds produces a considerable improvement in all the lipid profiles. Clinical research was conducted to study the progression of T2D using 5 gm of fenugreek powder twice a day before a meal

offered to the subjects. The study lasted from 3 months to 3 years and their results showed that the rate of diabetes was reduced significantly in fenugreek group when compared to the controls. The fenugreek group also observed a significant reduction in fasting plasma glucose, postprandial plasma glucose and low-density lipoprotein cholesterol (LDL-C), whereas serum insulin increased significantly. The observation was made that the control group can develop diabetes 4.2 times higher than the fenugreek group. The outcome of diabetes in the fenugreek group was positively associated with serum insulin and negatively associated with insulin resistance (Gaddam et al. 2015). It has been previously observed that fenugreek powder possesses a hypolipidemic effect in hypercholesterolaemic patients when an oral administration of (25 and 50) gm of fenugreek powder was given to them twice a day (Prasanna 2000). It was also demonstrated that fenugreek supplements significantly reduced the serum insulin level and slightly lowered the cholesterol levels in the diabetic groups (Abdelatif et al. 2012). Fenugreek seeds have hypocholesterolemic effects, which are attributed to lower serum levels of total cholesterol (TC) and triglyceride (TG) (Aher et al. 2016) by regressing preestablished cholesterol gallstones and controlling possible recurrence (Reddy and Srinivasan 2009). In a study about the hypolipidemic effect of fenugreek seed extract in normal rats, a hydro-ethanolic extract of fenugreek seeds reduced TC, VLDL-C and LDL-C levels. However, there was no significant effect of the extract on levels of high-density lipoprotein cholesterol (HDL-C) (Petit et al. 1993). Meanwhile, Xue et al. (2007) and Eidi et al. (2007) have studied the hypolipidemic effect of fenugreek seeds in diabetic rats. In both experiments, a significant reduction in HDL-C levels was reported. A dietary combination of fenugreek seeds and garlic was given to Wistar rats which showed higher cardioprotective influence when consumed together, and this may be strategic to derive maximum nutraceutical benefit from these ingredients. Hepatic cholesterol was lowered by dietary fenugreek and garlic, suggesting these dietary interventions could significantly counter the hypercholesterolemia. However, the effect produced was more pronounced in the synergistic application. LDL-C was countered by 35%, 15% and 50% with fenugreek, garlic, and the synergistic supplementation of fenugreek and garlic, respectively, while HDL-C was restored. Elevated cholesterol-to-phospholipid ratio and atherogenicity index were also reversed by these dietary interventions, the effect being higher in the case of fenugreek with garlic (Mukthamba and Srinivasan 2015a).

Twenty-eight subjects with high TC and TG for 12 weeks [test drug combination: 1000 mg Amla + 500 mg fenugreek/day] at the end of 12 weeks, there was a significant decrease in serum TC (20-26%), LDL (25–34%), TG (15–30%) and VLDL (15–30%) levels, while an increase was observed in the HDL (0–5%) in the test drug group. This combination has shown a similar response on the lipid profile as statins with lesser adverse effects (Joseph et al. 2012). Administration of fenugreek seeds offered a significant hypolipidemic effect by reducing TC and TG concentrations (Murthy et al. 2010). Oral administration of 25 gm of fenugreek powder for hypercholesteremic T2D patients can mediate the reduction of TC, TG and LDL-C. Still, there was not any significant change in HDL-C (Moosa et al. 2006). It was suggested that fenugreek seeds possessed hypolipidemic effect in obese rats due to the increased activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase,

which is a key hepatic regulatory enzyme that leads the excretion of neutral sterols and bile acids in the faeces (Ramulu et al. 2011). Fenugreek seeds (10%) and garlic powder (2%) were administered to the high-fat diet-fed Wistar rats for 8 weeks; the findings revealed that this combination countered the increase in TG and cholesterol, phospholipid ratio in the cardiac tissue and the elevated lipid peroxides in serum, liver and cardiac tissues (Mukthamba and Srinivasan 2016). An in vitro study aimed to evaluate the hypolipidemic effect of fenugreek seeds showed reduction in TC and TG concentrations, while in its in vivo experiment, it showed a decrease in the serum TG and LDL-C (Vijayakumar et al. 2010). A study indicated that administering a combination of fenugreek seeds and garlic to male Wistar rats could exhibit a powerful cardioprotective effect (Mukthamba and Srinivasan 2015b). Furthermore a mixture of milk thistle and fenugreek seeds could be used as a potent agent for ameliorating lipid, TC and TG levels in alloxan-induced diabetic rats (Saadh 2020). Daily intervention with 2 gm of *Nigella sativa* seeds powder with 20 g of fenugreek seeds powder for 8 weeks to 45 males and 35 females with type 2 diabetes mellitus produced a considerable improvement in all the lipid parameters (TC, TG and LDL-C) without any side effects (Shari et al. 2020). It also exhibited a significant hypolipidemic effect in the rabbits (Sharma and Choudhary 2014).

21.3 Clinical Uses and Applications

Many clinical studies were conducted with fenugreek to evaluate their potential therapeutic activities. A study done by Geberemeskel group (Geberemeskel et al. 2019) showed that fenugreek offered a significant improvement in the lipid metabolism of patients with T2D conditions, with no adverse effects. In another similar study, fenugreek was studied for its antidiabetic potential while comparing it with commercial allopathic medicine, Metformin (Najdi et al. 2019). A previous study by Kiss et al. (2018) for glucose tolerance, especially in patients with impaired glucose responses offered the similar understanding. Ghorbani et al. (2019) studied the potential effect of fenugreek seed in lowering the serum lipid in the diabetic patients with uncontrolled dyslipidaemia. In this study, the fenugreek seed powder mixed with a different herbal cocktail was given to the patient with hypolipidemic and hypoglycaemic drugs, both individually and combinedly. The herbal mixture contained black seeds (1.8 gm), aloe vera (300 mg), garlic (300 mg), psyllium seeds (1 g), milk thistle (500 mg) and fenugreek seeds (2.5 g). The effect of fenugreek in transdermal delivery was evaluated by Ansari et al. (2019). They showed that fenugreek seeds in the patch could reduce the pain score and be utilised as a natural pain management therapy (antinociceptive agent). Its potential as an agent for detoxification and body composition benefits was studied by Tinsley et al. (2019). A hormone modulatory activity potential was evaluated on menopausal women by fenugreek seed extract. The study showed that de-husked seed extract of fenugreek might reduce menopausal symptoms in healthy subjects (Steels et al. 2017). A study conducted by Shamshad Begum et al. (2016) reported that 1000 mg/day dose of fenugreek could result in a significant increase in plasma estradiol (120%) and improvement in various postmenopausal discomforts. A study was

done on symptoms of possible androgen deficiency, sexual function and serum androgen concentration in healthy ageing males where fenugreek (600 mg/day for 12 weeks) was given to the subjects. In this study, both total serum testosterone and free testosterone increased compared to placebo and were also reported safe (Rao et al. 2016). In another study the efficacy of a novel fenugreek seed extract in polycystic ovary syndrome (PCOS) was evaluated where it showed an increase in LH and FSH levels. Furocyst caused a significant decrease in both ovarian volume and the number of ovarian cysts (Swaroop et al. 2015). Robert et al. (2016) showed that fenugreek seed powder reduces the glycaemic response and the GI of buns and flatbreads and can further work to reduce postprandial glycaemia. Ethanolic extract of fenugreek also showed a metabolic activity of CYP3A4 and CYP2D6 (Al-Jenoobi et al. 2015). The authors also report some other studies conducted on the diabetic model (Rafraf et al. 2014; Losso et al. 2009; Kassaian et al. 2009; Lu et al. 2008; Gupta et al. 2002; Abdel Barry 2000). Some studies were done on diabetes and they reported a reduction in TC, LDL-C and VLDL-C and TG (Sharma et al. 1990). The studies done on non-insulin-dependent diabetics (NIDDM) showed that fenugreek influences lipid and glucose level after meal tolerance test (Madar et al. 1988), increases the levels of free testosterone and estradiol (Rao et al. 2015) and manages Parkinson's disease (Nathan et al. 2014). Additionally, the galactagogue herbal tea produced no oxidative stress index (Kavurt et al. 2013), and the same tea could enhance breast milk production as well (Turkyılmaz et al. 2011). It showed the effect of dietary fat intake consumption and reduced intake of lunch in healthy overweight subjects (Chevassus et al. 2009; Chevassus et al. 2010; Mathern et al. 2009). Another study was done to evaluate the effect of fenugreek on LDL-C, apolipoprotein A-1, body mass index and waist circumference, the findings showed it was well tolerated, safe and has significant effect (Vajifdar et al. 2000).

21.4 Other Therapeutic Applications of Fenugreek

Some notable habitually involved uses of fenugreek are arthritic pain management, mouth ulcer treatment, diuretic, high blood pressure and gastric irritation (Rashid et al. 2018). It was recorded that fenugreek fibre preserved bread quality during storage by water-holding capacity and prevention of starch retrogradation, enhanced the resistance and elasticity in the bread dough and maintained the dough texture (Huang et al. 2016). Another study claimed that fenugreek seeds are a rich source of fibre, gum, saponin, alkaloid and flavonoids. It was used as a food stabiliser and emulsifying agent to change food texture due to its high content of fibre (Khorshidian et al. 2016). The effect was evaluated on the gilthead seabream (Sparus aurata L.) growth performance and immune status. The diet was fed for 4 weeks with 0% (control), 1%, 5% and 10% of fenugreek seeds. The results recorded enhancement in all the assayed parameters in fish fed fenugreek diets compared to control fish. The expression of several immune-related genes in head-kidney (MHC1, CSF-1R, IL-8 and IgM) and different antioxidant enzyme genes in the liver (GR, CAT and SOD) of seabream specimens was also investigated. Again, the higher fenugreek doses upregulated most of the genes related with immune response and antioxidant enzyme

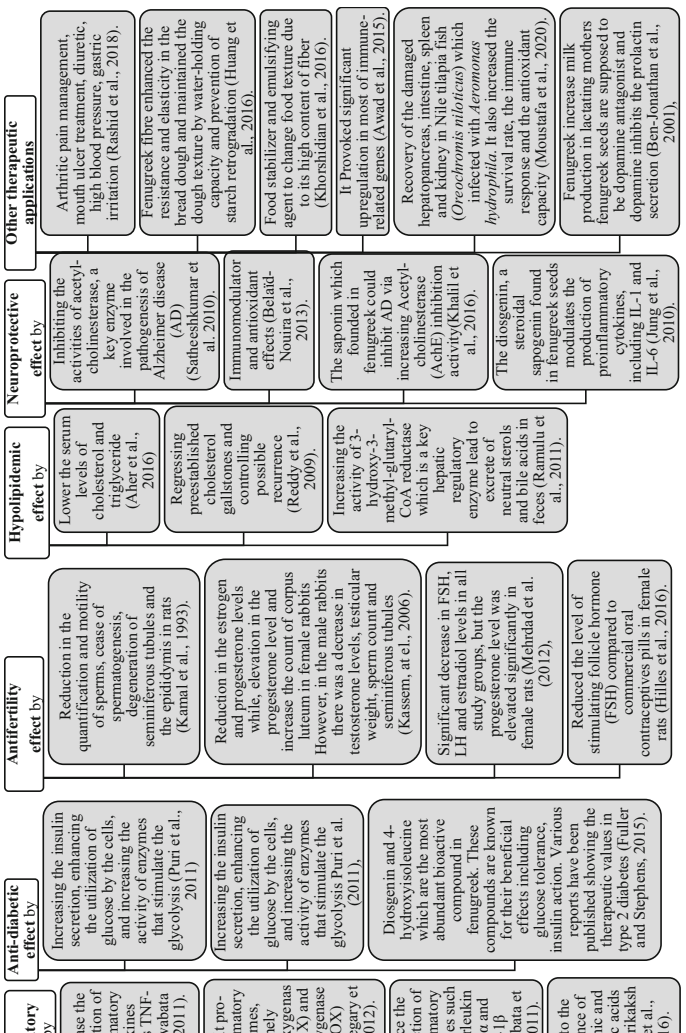
and no adverse effects were observed on intestine and liver morphology on fenugreek-fed fish (Awad et al. 2015). Nile tilapia fish (*Oreochromis niloticus*) infected with *Aeromonas hydrophila* were fed with a diet containing 3% of fenugreek for 8 weeks where fenugreek showed recovery of damaged hepatopancreas, intestine, spleen and kidney. It was also recorded that fenugreek supplemented group showed the highest survival rate, elevated the immune response and the antioxidant capacity which clearly shows that fenugreek can be considered as a natural alternative immunostimulant for Nile tilapia aquaculture (Moustafa et al. 2020).

21.5 Toxicity

A study with 90 days-repeat-dose of fenugreek toxicity was done in mice and rats The study did not report any mortality in animals or any significant body weight changes with 1% and 10% fenugreek diet. As well, there were no significant haematological, biochemical or histopathological changes in the studied organs (brain, heart, kidneys, liver, adrenals, lungs, ovaries, spleen and testes) (Muralidhara et al. 1999). In another study on fenugreek toxicity, fenugreek seeds-fed rats (for 90 days) experienced no changes in the liver function, histopathological or haematological tests (Basch et al. 2003). A methanolic extract of fenugreek seeds was administrated orally to rats where the extract was found to be safe at a dose of 2000 mg/kg with no animal mortality. Therefore, oral dose of 2500 mg/kg was considered as LD_{50} (Pawar and Hugar 2012). Acute oral toxicity was investigated for 24 days at 500, 1000, 2000 and 5000 mg/kg in male and female Sprague Dawley rats and Swiss Albino mice. No adverse pharmacological effects, gross abnormalities or pathological alterations were observed in this study as well. According to these findings, the acute oral median lethal dose (LD_{50}) of the fenugreek extract is more significant than 5000 mg/kg of body weight. In the same study, the sub-chronic toxicity was evaluated for 28 days. All animals survived throughout the study. There were no significant abnormal changes in the body weights and histopathological abnormalities observed for the spleen and ovaries (Swaroop et al. 2014). It was suggested that the low acute toxic dose of fenugreek by oral administration is 2 and 5 g/kg in mice and rats, respectively, whereas by intraperitoneal route, it is 0.65 and 3.5 g/kg in mice and rats, respectively. Accordingly, to avoid overdose in humans by oral administration, 21 g/per adult (60 kg) was suggested as a limit (Ouzir et al. 2016).

21.6 Conclusion

The present chapter covers different therapeutic applications of fenugreek. The major health beneficial properties of fenugreek were reviewed in this chapter, including anti-hyperglycaemic, hypocholesterolemic, anticarcinogenic, anthelmintic, antioxidant and antimicrobial activities since they are the primary medicinal properties of the herb demonstrated in various studies. High fibre content, gummy nature and chemical constituents present in it make fenugreek a natural health-promoting herb. After careful evaluation of these properties, fenugreek can be considered safe and used in daily diet for numerous health benefits.



Flowchart shows the therapeutic properties of fenugreek

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Pharmacological Actions and Therapeutic Potential of *Trigonella foenum-graecum* L.

22

Mohammad Tariq Salman and Fardan Qadeer

Abstract

Trigonella foenum-graecum possesses great medicinal properties. Seeds and their oil have a long history of folklore usage in various systems of medicines. It is popular in traditional systems of medicine like Unani medicine, Ayurveda and Siddha. In Tibb-e-Nabawi (Prophetic Medicine), it is considered a great form of healing medicine and has been recommended for regular use. In Ayurveda, *Trigonella* finds great utility in disorders associated with the gastrointestinal tract as it possesses carminative, digestive and laxative properties. Its pharmacologically active components such as diosgenin and tigogenin possess significant medicinal values. The seed has great therapeutic potential in the treatment of diabetes. It was found to restore the cellular anti-oxidant levels of enzymes playing a vital role in oxidative pathogenesis of diabetes associated complications like nephropathy and neuropathy. It also improves insulin signalling and sensitivity, thereby promoting the cellular actions of insulin. *Trigonella* possesses antihyperlipidaemic effects and studies demonstrated that it lowers serum triglycerides and total cholesterol by inhibiting accumulation of triglycerides and expression of lipogenic genes. Therapeutic effects of *Trigonella* have been extensively studied in clinical studies. An oral preparation of fenugreek seeds improved glucose metabolism by its insulin-sensitizing effect involving mechanisms that included reduction of circulating melanin-concentrating hormone levels. A polyherbal formulation containing fenugreek significantly lowered fasting blood glucose, HBA1c and LDL levels in people with diabetes

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and dyslipidaemia. Fenugreek seed showed beneficial potential in the treatment of diseases associated with female genital tract. A saponin enriched extract of *Trigonella* reduced ovarian volume and number of ovarian cysts while significantly increasing the levels of follicle stimulating hormone and luteinizing hormone in females with PCOD. Another trial demonstrated that de-husked fenugreek seed extract significantly reduced vasomotor, psychological and sexual symptoms associated with menstrual syndrome. Fenugreek extracts significantly increased plasma estradiol levels and improved postmenopausal symptoms such as hot flushes in postmenopausal women. It also produced beneficial effects on male reproductive system like improvement in free testosterone levels and sperm count and morphology.

Keywords

Diosgenin and tigogenin · Diabetes and dyslipidaemia · Clinical uses · Fenugreek

22.1 Clinical Uses in Traditional Medicine

Fenugreek is one of the oldest cultivated medicinal plants in written history. The first recorded use of fenugreek is described on an ancient Egyptian papyrus dated to 1500 B.C. According to writings obtained from ancient civilizations, fenugreek is one of the oldest medicinal plants used in Rome and Egypt to ease childbirth and increase milk flow. Even today, Egyptian women use this plant as Hulba tea to alleviate menstrual pains and for abdominal cramps (Morcos et al. 1981). Fenugreek seeds are used in remedies for diabetes and hypercholesterolaemia in Indian, Arabic and Chinese medicine (Kaviarasan et al. 2006). In the traditional medicine of China also this plant has been used to boost physique, to treat weakness of body and gout. People with very slender physique in East used this plant to have strong and well-developed physique (Yoshikawa). Traditional Chinese herbalists used it for kidney problems and conditions affecting the male reproductive tract. The Chinese also used fenugreek seeds as a pessary to treat cervical cancer (Snehlata and Payal 2011).

22.1.1 Uses in Tibbe Nabawi

In Islamic literature, it is mentioned as a Prophetic Medicine (Tibbe Nabawi) and Prophet Mohammad (SAW) mentioned its therapeutic efficacy and potential of cure. It is stated in books of Hadith that Qasim bin Abdur Rahman narrated that the Prophet Mohammad (SAW) said, 'Mix fenugreek in your medicines and get cure from Methi (Hulba)'. It is also documented that the Prophet (SAW) visited one of his companions, *Sa'ad bin Abi Waqqass*, who had contracted an illness during his stay in Mecca, and then requested that a physician examine him. After a diagnosis was made, the Prophet (SAW) said, 'He will be fine. Give him the soup of a decoction of

dates and fenugreek' (Al-Jauzi 1999). Therefore Hulbah has occupied special place for its medicinal value since centuries in the Middle East and Southeast Asia. It has been traditionally used in the treatment of a number of ailments such as respiratory and gastrointestinal disorders, bladder and kidney diseases, circulatory and immune system problems and for general overall well-being (Khan et al. 2015).

22.2 Traditional Uses of Fenugreek in Unani Medicine

According to opinion of Unani medicine experts, fenugreek has a dry and warm nature. Pharmacologic actions of Fenugreek include: *Munzij* (concoctive), *Jali* (detergent), *Musakkine Alam* (analgesic), *Mudirr-e-Haiz* (emmenagogue), *Mudirr-e-Baul* (diuretic), *Muqavvi-e-Medah* (stomachic), *Mohallil-e-Waram* (anti-inflammatory), *Mulayyin-e-Shikam* (laxative), *Kasire-Riyah* (carminative), *Mosakhhin* (anaesthetic), *Muharrik* (stimulant), antispasmodic, antidiabetic, analgesic activity, nephroprotective, neuroprotective, *Muqavvi-e-Shar* (hair tonic), *Munaffis-e-Balgham* (expectorant), *Mulattif* (demulcent), *Muallid-e-Dam*, *Musaffi-e-Khoon* (blood purifier), *Muqavvi-e-Badan* (general body tonic), *Muqavvi-e-Bah* (aphrodisiac), antibacterial, anti-oxidant, antifungal, anti-hyperlipidaemic and anti-cancerous activity (Khan et al. 2015; Meghwal and Goswami 2012). Its uses in Unani Medicine include diabetes, *Ihtebas-e-Baul wa Haiz* (Amenorrhoea), *Istisqaa* (ascites), *Yerqaan* (jaundice), *Zof-e-Meda* (gastric upset), *Nafakh-e-Shikam* (flatulence), *Qulanj* (intestinal colic), *Bawaseer* (piles,) *Qillatud Dam* (anaemia), *Izm-e-Tehal wa Kabid* (hepatomegaly and splenomegaly), *Warm-e-Rahem* (endometritis), *Inteshar-e-Shar, Wajaul-qutn* (backache), *Falij* (paralysis) *Mirgy* (epilepsy) *Taqteerul Baul* (incontinence), *Nazla* (common cold), *Suaal* (cough), *Dard-e-Chashm* (ophthalgia), skin eruptions and *Fasad-e-Khoon* (*Bahaq, Bars*) (Khan et al. 2015; Ibn-e-Baitar 2003). Also, seeds of the plant have been used as local emollient, a poultice for local inflammation, and as a demulcent to alleviate pain of joints (arthralgia). Infusion of this plant mixed with honey is recommended to treat asthma and internal oedemas (Basch et al. 2003). An infusion of the leaves is used as a gargle for recurrent mouth ulcers, whereas a gargle made from the seeds is used for sore throat. Fresh Fenugreek leaves paste applied over the scalp regularly before bath helps in hair growth, preserves natural colour, keeps hair silky and also cures dandruff. Its leaves have also been used to alleviate cold cough, splenomegaly, hepatitis, backache and bladder cooling reflex. Fenugreek seeds made in gruel if given to nursing mothers increase the flow of milk (Yadav et al. 2011).

Zakariya Al-Razi has used fenugreek to treat diabetes and Sheikh Bu Ali Sina has presented some information about therapeutic properties and benefits of this plant in eliminating mouth odour, undesired odour of body and sweat in his book named *Medicine Law*. Mucilage of fenugreek seeds, especially if mixed with oil of flower treats striae created by cold. The plant is used in the treatment of skin diseases like black spots and bad odour of body, mouth and sweat. Boiled form of fenugreek helps treat the red spot of eye, helps soften throat and chest and provides relief from cough. Using this plant in the form of powder, infusion, decoction and pomade has been

very common in traditional medicine of Iran from ancient times (BuAli Sina 1988). The plant can be used for vaginal washing. It is locally used as an emollient in treatment of pellagra, loss of appetite, gastrointestinal disorders and as a general tonic (Bahmani et al. 2016). In the Middle East and the Balkans, the aerial parts of plant are a folk remedy for abdominal cramps associated with both menstrual pain and diarrhoea or gastroenteritis. They are also used to ease labour pains (Snehlata and Payal 2011).

22.2.1 Use in Ayurveda and Indian Folk Medicine

Carbonized fenugreek seed recovered from Punjab, India indicates its value as far back as 2000–1700. B. C. A wide range of uses were found for fenugreek in ancient times. The plant has been used in traditional medicine of India to treat arthritis, asthma, bronchitis, improve digestion, increase libido and male potency, to cure skin problems (wounds, rashes and abscesses), to treat sore throat, to cure acid reflux, for treatment of reproductive disorders, to induce labour, to treat hormonal disorders, to help with breast enlargement, to reduce menstrual pain and to help in blood sugar regulation (Nathiya et al. 2014).

The nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia. They are also found helpful in lowering fever. The seeds' soothing effect makes them of value in treating gastritis and gastric ulcers. The seeds freshen bad breath and help restore a dulled sense of taste. The oil in the seeds is used as a skin softener and emollient. Fenugreek has a beneficial action on cleansing the blood. As a diaphoretic, it is used to bring on a sweat and to help detox the body. It also has the reputation as a lymphatic cleansing herb. Fenugreek is considered useful for all mucus conditions of the body, particularly the lungs, by helping to clear congestion. It acts as a mucus solvent and throat cleanser, which also eases the urge to cough. Drinking the water that the seeds have been soaked in and rinsed with helps to soften and dissolve accumulated and hardened masses of cellular debris. Fenugreek is recommended for use in head colds, influenza, catarrh, constipation, bronchial complaints, asthma, emphysema, pneumonia, pleurisy, tuberculosis, sore throat, laryngitis, hay fever as well as sinusitis. Fenugreek has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel and is believed to create a protective coating over inflamed areas and absorb toxic material and eliminate it. Fenugreek has a powerful demulcent action, as it is rich in mucilage and it can soothe irritated or inflamed tissue. The seeds are beneficial for digestion. For relief from the symptoms of irritable bowel syndrome, colitis and diverticulitis, the 'soak-and-rinse water' is drunk and the sprouts blended to a liquid. Fenugreek has been known to help reduce fever when taken with lemon and honey. It is often used in many teas and other products that help balance women's hormones and/or enlarge the breasts. It is also used as a remedy to ease childbirth since fenugreek stimulates uterine contractions and can be helpful to induce labour. Used as a lactation aid, the seeds contain hormone precursors that increase milk supply. It has been found that

fenugreek can increase a nursing mother's milk supply within 24–72 h after taking the herb (Snehlata and Payal 2011).

22.3 Pharmacological Activity and Therapeutic Uses

22.3.1 Effect of *Trigonella foenum-graecum* in Diabetes, Dyslipidemia and Obesity

Effect on Diabetes

Diabetes mellitus is the disorder related to impaired metabolism of carbohydrate, proteins and fats and is marked by hyperglycaemia. The long-term outcome of untreated diabetes leads to various microvascular and macrovascular complications. Reactive oxygen species and damaged anti-oxidant machinery has been linked to development of these complications. The complications can only be prevented by keeping a strict glycemic control. However, none of the approved antidiabetic medications reverses the complications of diabetes. Therefore, the explorations of never molecules for the treatment of diabetes has highlighted the role of *Trigonella foenum-graecum* (TG) in the treatment of diabetes.

The antidiabetic effect of TG has been widely studied on various animal models and clinical trials have been conducted in order to establish its therapeutic benefits. Trials have been conducted on various animal models of diabetes mellitus, animal model of streptozotocin induced diabetes showed that TG significantly reduced fasting blood glucose to near normal levels. This effect may be attributed to its anti-oxidant activity and its ability to significantly reduce thiobarbituric acid reactive substances (TBARS). TBARS is a marker of lipid peroxidation and its elevated levels in diabetes signify the damage due to reactive oxygen species (Sankar et al. 2012) The soluble dietary fibre of TG has been shown to significantly reduce postprandial blood glucose levels in rats fed with high carbohydrate diet, the major constituent of soluble dietary fibre of TG is galactomannan which has ability to reduce carbohydrate digestion and its intestinal absorption. It is also noteworthy that correction in postprandial blood glucose has been shown to significantly reduce the development of cardiovascular complications (Ali et al. 1995).

Fenugreek seed polyphenolic extract has been shown to improve insulin signalling and sensitivity and promotes cellular actions of insulin in high fructose fed animals and this effect is comparable to Metformin (Kannappan and Anuradha 2009). These effects on the action of TG have been studied in various animal models. Now it is worthwhile to discuss the antidiabetic effect of *Trigonella foenum-graecum* on clinical trial data to validate its therapeutic activity. A double blinded trial conducted to evaluate the clinical efficacy of TG seed in comparison to placebo showed that TG seeds significantly reduced blood glucose levels, improved HOMA derived insulin resistance and improves insulin sensitivity. This study concluded that TG seeds can be used along with diet and lifestyle modification in early stages of diabetes mellitus (Gupta et al. 2001).

A clinical trial conduced on patients of diabetes to compare the effectiveness of fenugreek seeds (2 g/day) with glibenclamide showed that fenugreek reduced blood glucose levels almost comparable to glibenclamide but significantly improved the levels of fasting insulin. Therefore, fenugreek seeds may be used as an adjuvant to pre-existing antidiabetic medications (Najdi et al. 2019).

Effect on Dyslipidaemia

Changing lifestyle and stress has markedly increased the risk of coronary artery disease. Various risk factors are implicated for its development, dyslipidaemia is one of the major modifiable risk factors for coronary artery disease. Various animal studies and clinical trials have concluded that fenugreek seed and its extract is effective in maintaining a favourable lipid profile. A study conducted by Stark and his colleagues demonstrated that the saponin content of fenugreek seed provides a hypocholesterolaemia effect. The study demonstrated that ethanol extract of *Trigonella* seeds caused significant reduction in the levels of plasma cholesterol and lowered concentrations of liver cholesterol (Stark and Madar 1993).

Dyslipidaemia in diabetes further enhances the risk of coronary artery disease. Fenugreek leaves supplementation in streptozotocin induced diabetic rats for 45 days caused a reduction in blood glucose, total cholesterol, triglycerides and free fatty acids. There was a reduction in the levels of glutathione, superoxide dismutase and catalase in the liver, heart and kidney in such animals (Annida et al. 2004). Monosodium glutamate, a commonly used flavour enhancer in foods is commonly implicated in childhood obesity and dyslipidaemia. A study was done to evaluate the lipid lowering effect of *Trigonella foenum-graecum* on monosodium glutamate (MSG)-induced dyslipidaemia and oxidative stress in Wistar rats. *Trigonella* produced significant reduction in serum total cholesterol, triglycerides, lactate dehydrogenase, aspartate amino transferase, alanine amino transferase, hepatic and cardiac lipid peroxides levels and elevation in serum high density lipoprotein cholesterol (HDL-C) hepatic and cardiac anti-oxidant enzymes [glutathione (GSH) and superoxide dismutase and catalase levels] (Bhandari and Kumar 2013).

The mechanism suggested for the lipid lowering effect of fenugreek is unclear; however, it has been suggested that *Trigonella* seed-derived saponins, diosgenin inhibited the accumulation of TG and the expression of lipogenic genes in a hepatic cell line (HepG2 cells) by inhibiting the transactivation of liver-X-receptor-α and suggested that fenugreek could ameliorate dyslipidaemia by decreasing the hepatic lipid content in diabetic mice via diosgenin (Uemura et al. 2011). It has been suggested that lipid lowering effect of fenugreek seeds can be attributed to its role in modulating the enzymes implicated in glucose and lipid metabolism. The enzymes such as glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), isocitrate dehydrogenase (ICDH) and the activities of lipogenic enzymes, namely ATP-citrate lyase MTP-CL) and fatty acid synthase (FAS) were improved by administration of fenugreek seeds extract (Raju et al. 2001). A placebo-controlled trial concluded that fenugreek given in a dose of 2.5 g twice daily for 3 months to healthy individuals did not affect the blood lipids and blood sugar (fasting and

postprandial). However, administered in the same daily dose for the same duration to CAD patients also with NIDDM, fenugreek decreased significantly the blood lipids (total cholesterol and triglycerides) without affecting the HDL-C (Bordia et al. 1997).

Effect on Obesity

Obesity is defined as the abnormal and excessive deposition of fat in adipose tissue and internal organs. Obesity itself is a major risk factor for development of insulin resistance and subsequent diabetes mellitus. It is also associated with increased risk of development of coronary artery disease, cancer, osteoarthritis and other morbidities. Various drugs for the treatment of obesity were subsequently withdrawn as there was increased incidence of various adverse effects. In the face of this unmet medical need, there is requirement of new potential anti-obesity drugs.

A study done on animals fed with high fat diet revealed that aqueous extract of *Trigonella* seeds significantly reduced body weight, body mass index and white adipose tissue weights. There was reduction in levels of leptin, lipase and apolipoprotein B. In addition, liver and uterine WAT lipogenic enzyme (fatty acid synthetase (FAS) and glucose-6-phosphate dehydrogenase (G6PD)) activities were restored towards normal levels. The finding clearly demonstrated the anti-obesity effect of aqueous extract of *Trigonella* (Kumar et al. 2014). Another study demonstrated that INDUS810, a natural compound extracted from *Trigonella foenum-graecum* L. had a proven anti-obesity effect. It was observed that INDUS810 can reduce high-fat diet-induced weight increase in epididymal white adipose tissue, interscapular brown adipose tissue and liver, as well as serum levels of total cholesterol and low-density lipoprotein cholesterol. It was also noted that the compound has the ability to inhibit lipid accumulation at either differentiating or mature stages and increase lipolysis activity in mature adipocytes. There was an increased activity of adenosine monophosphate-activated protein kinase, which leads to the reduction of lipid contents in adipocytes (Cheng et al. 2018).

4-Hydroxyisoleucine (4-OHIle), a peculiar nonprotein amino acid isolated from fenugreek (*Trigonella foenum-graecum*) seeds, exhibits interesting effects on insulin resistance related to obesity. 4-OHIle increases glucose-induced insulin release. There was a reduction in plasma triglycerides, total cholesterol, free fatty acid levels and the improvement of liver function. The mechanism of action was also established in the study, it was related to increased Akt phosphorylation and reduced activation of Jun N-terminal kinase (JNK)1/2, extracellular signal-regulated kinase (ERK)1/2, p38 mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB. 4-OHIe has demonstrated unique therapeutic potential against insulin resistance and obesity and the harmful effects associated with inadequate glucose uptake, lipotoxicity and liver dysfunction, which ultimately cause diabetes (Avalos-Soriano et al. 2016).

Another study was done to investigate the anti-obesity mechanism of fenugreek seed extract and its compounds on HepG2 cell lines. Cell lines were treated with hydroalcoholic fenugreek seed extracts, diosgenin and 4-OH-lle and orlistat. Results concluded that hydroalcoholic fenugreek seed extracts, diosgenin and 4-OH-Ile

significantly modulate the expression of some important lipid metabolism related genes, which is similar to orlistat (Hajizadeh et al. 2019). The anti-obesity effect of *Trigonella* and its extract is well established in animal models. However clinical trials are needed at this hour in order to establish its true therapeutic efficacy and safety.

Effect on Women Health

Trigonella foenum-graecum has modulatory effect on oestrogen and its receptor, therefore has been found to be beneficial in disorders of female genital tract. Ovarian hyperstimulation syndrome occurs as a result of excessive oestrogen mediated stimulation of ovaries in assisted reproductive procedures. An aqueous extract of fenugreek was studied in this regard. Female Sprague Dawley rats were given human chorionic gonadotropin for the development of hyperstimulation syndrome. The effect of FSA extract was evaluated by measuring the concentration of serum E2 using the enzyme-linked immunosorbent assay. FSA extract reduced serum E2 level significantly in the treated OHSS model (p-value <0.050) compared to the positive control group. Thus fenugreek extract may be used as an adjuvant drugs for safe assisted reproduction technology cycles in terms of OHSS prevention (Ben Hameid et al. 2019).

The study was to examine the effect of *T. foenum-graecum* seed extract in reducing the metabolic and inflammatory alternations associated with menopause. The study was conducted in ovariectomized rats. Metabolic parameters like body weight and blood sugar levels were reduced. The serum level of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) was significantly reduced when compared to sham controlled animals. The results of the study showed that administration of *T. foenum-graecum* corrects metabolic and inflammatory alterations associated with ovariectomy and has a potential for the management of menopause (Abedinzade et al. 2015). A study aimed to evaluate the effect of fenugreek seed extract in Letrozole induced PCOS in female albino rat showed that there was a marked improvement in ovarian structure after fenugreek supplementation. There was disappearance of cysts and number of corpora lutea were seen suggesting ovulation (Magdy Mohamady et al. 2018).

Clinical trial was conducted to establish the efficacy of *Trigonella* in PCOD. A prospective randomized, double-blind, placebo-controlled trial was conducted in Iran. Menstrual disturbance and metabolic parameters (markers of insulin resistance and hormonal parameters) were measured. Insulin resistance based on HOMA-IR (homeostasis model assessment for insulin resistance) fenugreek showed improvement in the condition of ovaries, significantly decreasing the cystic appearance of the ovary at 8 weeks of trial when compared to placebo. The effect was comparable to metformin. Thus it may be used as an adjunct therapy in PCOD (Hassanzadeh Bashtian et al. 2013). Another trial was done to demonstrate the effect of de-husked seed extract of Trigonella in pot menopausal syndrome. A double-blind, randomized, placebo-controlled trial was done in females aged 40–65 years. Menopause-Specific Quality of Life (MENQOL) was used to assess the improvement in these females. There was significant reduction in menopausal symptoms.

There was reduction in vasomotor, sexual symptoms and psychological symptoms when compared to control (Steels et al. 2017).

22.3.2 Anti-Cancer Effect of *Trigonella foenum-graecum*

Anti-cancer chemotherapy has a limited therapeutic efficacy and is associated with serious adverse effects. Adverse effects such as pancytopenia, reticuloendothelial cell toxicity and bone marrow suppression may lead to worsening of the condition and poor compliance. Therefore, there is a constant need to look for more efficacious and less toxic compounds having a preventive and therapeutic effect on different cancers. Fenugreek and its derivatives have been studied to have anti-cancer potential. A study demonstrated that when cancer cell lines were exposed to fenugreek extract at different concentrations and at different time points a selective cytotoxic effect of fenugreek extract was observed in different cell lines including T-cell lymphomas. Thus, fenugreek is a substance which provides significant cytotoxicity to cancer cells which may be useful to prevent cancer in-vitro (Alsemari et al. 2014).

Anti-proliferative effect of *Trigonella* extract was observed on human hepatoma (HepG2) cancer cell lines and was assessed on MTT assay. There was a dose dependent reduction in cell viability in cells treated with ethanolic extract of *Trigonella*. Fifty percent HepG2 cells were eradicated at a concentration of 200 μg/mL. Thus it was observed that *Trigonella* is effective cytotoxic agent against hepatocellular cancer cells (Al-Dabbagh et al. 2018). Studies have shown than fenugreek seeds not only directly cause cytotoxicity on tumour cells but also provide modulatory effects. Wistar rats treated with 1,2-dimethylhydrazine (DMH), a colon carcinogenic compound were used in the study. DHM causes enhanced hepatic lipid peroxidation (LPO), reduced glutathione content (GSH) and reduced enzymes such as glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) activities. Subsequently inclusion of fenugreek seed powder in the diet of DMH treated rats reduced the colon tumour incidence to 16.6%, decreased the LPO and increased the activities of GPx, GST, SOD and CAT in the liver (Devasena 2007).

Protodioscin (PD) is compound purified from fenugreek (*Trigonella foenum-graecum* L.) seeds. The effects of PD on cell viability in human leukaemia HL-60 cells were investigated. PD displayed strong growth inhibitory effect against HL-60 cells. Morphological change showing apoptotic bodies was observed in the HL-60 cells treated with PD. Flow cytometric analysis showed that the hypodiploid nuclei of HL-60 cells were increased. Increased tumour cell apoptosis was observed which was concentration and time dependent. Thus *Trigonella* compound produced cytotoxic effects against human leukaemia cells (Hibasami et al. 2003). Diosgenin, a steroid saponin derived from *Trigonella*, was found to inhibit azoxymethane (AOM)-induced aberrant crypt foci formation, a preneoplastic colonic lesions in animals (Raju et al. 2004). Further, it was observed that same compound diosgenin

could modulate the STAT3 signalling pathway in hepatocellular carcinoma by suppressing the activation of c-Src, JAK1 and JAK2 (Li et al. 2010).

Effect of ethanolic extract of fenugreek on growth of MCF-7 cell lines was studied. MCF-7 cells are lines of oestrogen receptor positive breast cancer. Fenugreek decreased the cell viability and induced early apoptotic changes such as flipping of phosphatidylserine and decrease of mitochondrial membrane potential. Degradation of cellular DNA into fragments comprising multiples of approximately 180–200 base pair was also observed. It also caused cell cycle arrest at G2/M phase (Sebastian and Thampan 2007). There are numerous studies demonstrating the in vitro and in vivo anti-cancer effect of *Trigonella* and its derivatives. Clinical trials are needed to establish its efficacy in humans.

22.3.3 Antimicrobial Activity of *Trigonella foenum-graecum*

Recently, there has been a surge interest in the antibacterial properties of the plants extract. Many investigations were carried out on the therapeutic applications of various plants species on different diseases such as fungal, viral and bacterial infections. A number of studies have revealed antimicrobial activity of fenugreek seeds as well as other plant parts against pathogenic bacteria and fungi.

Spectrum of Activity

Antimicrobial activity of *Trigonella foenum-graecum* (TF) against following pathogens has been reported:

- Gram positive bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae*, *Bacillus subtilis*.
- Gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, *Vibrio parahaemolyticus, Klebsiella pneumonia, Proteus vulgaris.*
- Fungi: *Candida albicans*, *Aspergillus fumigatus, Fusarium solani*, *Colletotrichum musae.*

Antibacterial Activity

Antimicrobial activity of fenugreek leaves, seeds and stem extract (methanol, acetone and aqueous extract) against *E. coli* and *Staphylococcus* was determined by the well diffusion method. Desired bacteria were isolated from spoiled cabbage by serial dilution method. The maximum zone of inhibition was given by methanol, i.e. 20 mm and 19 mm against *E. coli* and *Staphylococcus*, respectively, followed by acetone extract which gives the equal zone of inhibition for both organisms, i.e. 16 mm while the aqueous extract shows nill zone of inhibition (Sharma et al. 2016). Fenugreek seed (FS) extracts were prepared using ethanol (75%), methanol (75%) and water as extraction solvents. Ethanol, methanol, water and hot water extracts were obtained from ground FS, while water extract was obtained from germinated FS. The results revealed that all extracts of the ground FS exhibited

antimicrobial activities. Based on disc diffusion method, water extract obtained from germinated FS exhibited highest antimicrobial activity against all tested bacterial pathogens (*Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*) (Norziah et al. 2015). FS oil also exhibited strong antimicrobial activity in vitro. MIC of the volatile oil ranged from 0.375 to 1.5 mg·mL ~ (−1) (He et al. 2020).

The antibacterial activity of the crude plant seed ethanolic and aqueous extract was studied on six clinically isolated bacteria strains, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, *Escherichia coli* and *Vibrio parahaemolyticus,* using Agar well diffusion technique and zones of inhibition were measured. These strains had been obtained from different clinical specimens (stool, wound infections, urine, skin lesions) of patients admitted to 'Al-Shafaa hospital', Basrah. Results showed that ethanol extract has prominent effect on *S. aureus* and *P. aeruginosa* and moderate activity on the remaining types of the bacteria. The aqueous extract showed low to moderate activities on the bacteria except on *Staphylococcus aureus* and *E. coli*. The concentrations used in MIC for ethanol extract were in the range 50–500 μg/mL. Most of the bacteria were inhibited at MIC of 50 μg/mL (Al-Timimi 2019). TF extracts were tested against bacterial and fungal pathogens responsible for the vaginal infections. Hydroalcoholic and ethanol extracts of TGF showed the highest activity against *A. fumigatus* and good activity against *C. albicans* at 6 mg/mL concentration; the hydroalcoholic extract was found to inhibit bacterial growth to a greater extent than the ethanol extract. Highest antimicrobial activity was seen against *S. aureus*, followed by *E. coli* and *S. agalactiae*. TF extract also showed synergistic antimicrobial activity against above pathogens when combined with *Azadirachta indica* (AI) and *Cichorium intybus* leaf extracts (Yadav et al. 2019).

Ethanolic extracts of fenugreek seeds (FSE) were screened for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* using disc diffusion method. The extract revealed antimicrobial activity against studied bacterial strains. In addition, the effect of FSE combined with chitosan coating on the shelf life of Pacific white shrimp (PWS) (*Litopenaeus vannamei*) during refrigerated storage has been studied. Results indicated that using FSE during the refrigerated storage of PWS led to significantly decreased total bacterial count (Hatab et al. 2018). Blood and oral swab cultures were taken from 40 oral cancer patients undergoing treatment. Prevalent bacterial pathogens isolated were *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Proteus vulgaris*. The fungal pathogens were *Candida albicans* and *Aspergillus fumigatus*. The antimicrobial efficacy of TF leaf extract was evaluated by modified Kirby-Bauer disc diffusion method. MIC and MFC were investigated by serial two fold microbroth dilution method. Pet. ether extract of *T. foenum-graecum* showed 100% activity to all clinical isolates. Pet. ether, benzene and chloroform extracts of *T. foenum-graecum* revealed significant antibacterial activity against *P. vulgaris*. Benzene extract of *T. foenum-graecum* exhibited good antibacterial activity against *P. mirabilis*, *E. coli and K. pneumonia.* The MIC ranged from 31 to 250 μg/mL and similarly the MFC ranged from 31 to 250 μg/mL (Panghal et al. 2011).

Antifungal Activity

Nateqi et al. studied antifungal activities of fenugreek extracts on growth of *Fusarium solani*, an important plant pathogen, soil saprophyte and one of the causal agents of fusariosis in human and animals. The value of calculated MFC was 30 mg/mL for fenugreek aqueous extract (Nateqi and Mirghazanfari 2018). Anthracnose of banana is infected by *Colletotrichum musae*. It is recognized as one the most destructive diseases of mature and immature banana fruits, resulting in huge economic losses all over the world. Fenugreek oil was screened both in vitro and in vivo for antifungal activity against *C. musae*. Fenugreek oil exhibited significant inhibition of mycelial growth, 61% at a concentration of 2 μL/mL. Scanning electron microscopy revealed severely damaged mycelium. Hence, this oil can be considered as potential alternatives to chemical treatments (Rizwana 2018).

A total of 50 *Candida* isolates were isolated and identified from clinical specimens of pus swab, sputum, urine, gastric aspirate and blood samples at Integral University, Lucknow and tested for resistance to various antifungal drugs. Multidrug resistance was observed in all *Candida* isolates tested by 84%, 62%, 60%, 76%, 46, 30% and 22% against fluconazole, clotrimazole, Amphotericin B, itraconazole, ketoconazole, miconazole and nystatin, respectively. The isolates, which were found to be resistant to antifungal drugs were subjected to antifungal testing using agar well diffusion method against ethanolic extract of TF which was found to be effective against all MDR *Candida* isolates. To determine MIC of plant extracts the broth micro-dilution method was performed with some modifications. MIC value was found to be 1.56 mg/mL in MDR candida isolates (Khan et al. 2017).

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