Chapter 12
Role of Biofertilizer in Biological Management of Fungal Diseases of Pigeon Pea [(*Cajanus cajan*) (L.) Millsp.]

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12.1 Introduction

The world population is increasing at a high growth rate and is expected to reach ~9.6 billion in 2050 according to a recent United Nations report (UNPAN 2010). With a projected emphasis on sustainable genetic improvement of major staple crops including rice, wheat and maize, it is also important to lay light on the production of protein-rich foods to reduce global malnutrition and hunger. Proteins are the foremost building block of the human system. It is a known fact that developing countries have only 33% of the normal requirement of protein, hence making it a challenge for various nutritional development programs to fulfil the protein demand.

Leguminous plants (legumes or pulses) are one of the best available protein sources that can contribute a handful amount of proteins in the diet of developing countries as they require minimum care during cultivation and low inputs. Pigeon pea or red gram (*Cajanus cajan* (L.) Millsp.) occupies a chief place in worldwide agriculture among different legume crops (Saxena et al. 2010). It occupies 5.4 million hectares in 22 countries in the continents of Asia and Africa. Out of this India alone has more than 3.9 million hectares, i.e. 72% of the area, of all the pigeon pea-growing countries of the world (FAOSTAT 2018). Uttar Pradesh is the largest producer of pigeon pea in India, but the average yield released by the crop is much less than its other neighbouring states like Bihar and Jharkhand (Ahlawat et al. 2005; Prasad et al. 2017).

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is the most vital legume crop in the world. India is one of the largest producers of pigeon pea commonly known as "arhar" in its northern part followed by the eastern side of Africa and Central

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America. It is roughly cultivated in at least 25 tropical and sub-tropical countries. This crop is greatly influenced by weather conditions; it is well raised in semi-arid tropical areas which are rain-fed. Cropping of pigeon pea is intermixed with maize, sorghum, pearl millet and some other legume crops like groundnut etc. It supplements soil through nitrogen fixation.

The term “biofertilizers” refers to live microbial culture, which when applied to plants, soil or composting pits helps in mobilization of various nutrients by their biological activity. Application of biofertilizers such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) in agricultural field soils is well known. Assessment of native microbial field community is indispensable for developing tracing tools to monitor the introduced biofertilizers. Pigeon pea is affected by almost 60 plant pathogens comprising of bacteria, nematodes, fungi, viruses, etc., but luckily, only a few of them are of economic importance. Out of which, it is withered by numerous fungal diseases, viz. fusarium wilt, Phytophthora blight, Phoma stem canker, Alternaria blight and Macrophomina root rot.

12.2 Some Major Fungal Diseases of Pigeon Pea

Diseases of economic importance in the country are fusarium wilt caused by *Fusarium udum* Butler, Phytophthora blight caused by *Phytophthora drechsleri* Tucker f. sp. *cajani*, Macrophomina root rot caused by *Macrophomina phaseolina* (Tassi) Goid., stem canker caused by *Phoma cajani* (Rangel) and Alternaria blight caused by *Alternaria* sp. Fusarium wilt caused by *Fusarium udum* Butler, a soil and seed borne fungus spreads through wind, water and soil and can survive up to 3 years on infected plant debris and is of great economic importance (Shinde et al. 2014). Symptoms of the disease appear during flowering when the plant is just 1–2 months old. Likewise, *Phytophthora* blight another fungal disease caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* is a common infection of *Cajanus cajan* (L.) Millsp. (Pande et al. 2011). It is a soilborne fungus and thus is fast spreading, surviving as dormant mycelia and chlamydospores in the soil. It is greatly affected by the weather. Rainy season favours the growth of the fungus. The spores of the fungus are spread through air and water. Warm and humid weather after the infection has occurred is a serious concern as it damages the plant and facilitates infection. Phoma stem canker of pigeon pea caused by *Phoma cajani* is one of the emerging diseases of the crop. The symptoms of the disease first appear on the stems as a necrotic spot and later turn into canker, resulting in the wilting of the whole plant. Macrophomina root rot is also among one of the important fungal infections of *Cajanus cajan* (L.) Millsp. caused by *Macrophomina phaseolina* (Tassi) Goid. This disease along with Alternaria blight caused by *Alternaria alternata* is a major problem for late-sown crops. Both these diseases are greatly affected by the weather. They are more prominent in hot and humid season. Under these conditions, root rot spreads to the base of the stem. The lesions further coalesce and cause the branches and then the entire plant to dry up and die.

12.3 Management of Disease

12.3.1 Cultural Management

Cultural practices are the traditional practices used by farmers to overcome diseases caused by pathogens in the crop. The commonly used practices include crop rotation, intercropping, interrow spacing, removal of diseased plant, spraying of nitrogen, etc. Verma and Rai in 2006 reported crop rotation with *Sorghum bicolour* (L.) Moench (sorghum), *Nicotiana tabacum* L. (tobacco) or *Ricinus communis* L. (castor) every 3 years terminates the pathogen from the field. They also stated that growing sorghum or fallow for 1 year on the same field of pigeon pea reduces the incidence of wilt disease up to below 20%. The spray of green manure with *Crotalaria juncea* reduces rot and wilt diseases to a great extent (Upadhyay and Rai 1981). The application of nitrogen as farmyard manure has also been found to be effective. One of the common and effective practices to control the diseases of pigeon pea is intercropping. Growing of other crops like sorghum or black gram as intercrop has proved to be effective (Table 12.1).

12.3.2 Chemical Management

Chemical management involves the treatment of the disease through chemical sprays. Numerous chemicals have been suggested for the management of fungal diseases of pigeon pea for long (Singh 1998). Pigeon pea seeds when treated with

Table 12.1 Cultural practices for disease control against some major fungal diseases

Disease	Common cultural practice
Fusarium wilt	• A field with no previous record (up to 3 years) of *Fusarium* wilt should be selected • Seeds used should be collected from disease-free fields of pigeon pea • The intercropping pattern is preferred • Rotation of 3 years and mixed cereal crops like sorghum, tobacco, etc. is beneficial • Solarization of soil in summer is also encouraged to reduce disease incidence
Phytophthora blight	• Field with no previous disease record is preferred • Sowing of seeds should be avoided in waterlogging areas like the low-lying patch • Good drainage should be ensured through raised seedbeds • Interrow spacing also proves to be helpful
Dry root rot	• Field with no previous disease record is preferred • Late sowing of seeds should be avoided to reduce the risk of high temperature and drought conditions
Phoma stem canker	• Field with no previous disease record is preferred • Infected plants should be removed subsequently to reduce the spread of infection
Alternaria blight	• Seeds used for sowing should be taken from healthy fields • Avoid late sowing of the crop

Table 12.2 Chemical practices for disease control fungal diseases

Disease	Chemical practice
Fusarium wilt	• Seed bacterization with Benlate and thiram in 1:1 (3 g per kg of seed)
Phytophthora blight	• Foliar spray at 15 days interval with Ridomil MZ (2 sprays)
Dry root rot	• Dressing of seeds with tolclofosmethyl or thiram
Alternaria blight	• Foliar spray with Indofil M45

an equal part mixture of benomyl and thiram eradicate the disease (ICRISAT 1987; Reddy et al. 1993). Supplementing soil with boron, manganese or zinc and methyl bromide (CH_3Br) reduces the incidence of fusarium wilt. Ingole et al. (2005) also reported similar findings with a mixture of carbendazim + thiophanate (0.15 + 0.10%) against wilt disease of pigeon pea. Few antibiotics like bulbiformin have also found to be an effective tool against pathogens (Table 12.2).

12.3.3 Biological Management

The application of hazardous fungicides affects the environment in adverse ways, and moreover, chemical fertilizers are not targeted specifically. It not only degrades the ecosystem but also has negative effects on human health. Fungicides affect the food chain as they are toxic to species like earthworms and microorganisms and also to an extent affect genotoxicity of humans (Shuping and Eloff 2017). They cause water and soil pollution too. The solution to this above problem lies in sustainable agriculture. The application of potential microorganisms which are part of the existing ecosystem serves as an effective means against plant protection system. Biological management of diseases has been reported by several workers and serves as an attractive tool for eco-friendly management of soilborne as well as other pathogens degrading the crop. Disease incidence of fusarium wilt has been reduced by the application of antagonistic microorganisms like fungi and bacteria (Passari et al. 2017; Anjaiah et al. 2003; Mandhare and Suryawanshi 2005; Maisuria et al. 2008; Singh et al. 2002). Out of cluster of scientific reports, few of them have notable biological measures that are functional for the management of pigeon pea diseases. Seed inoculation with rhizosphere bacteria like *Bacillus subtilis*, *Pseudomonas fluorescens and Pseudomonas aeruginosa* is very effective against fungal disease of pigeon pea (Mahesh et al. 2010). Integrated management strategies (IDM) which involve a combination of fungicides and biocontrol agents also prove to be beneficial for the management of *Fusarium udum* Butler (Pande et al. 2012). Oil formulations of *Trichoderma* strains like *Trichoderma harzianum* reduce the traces of soilborne pathogens from the diseased plants (Khan and Khan 2002). Siddiqui and Shakeel (2007) suggested that various rhizobacteria are efficient biocontrol agents. Plant extracts like neem and eucalyptus, garlic and henna, ginger and tulsi are also found to have an inhibitory effect against Alternaria blight of pigeon pea (Rathore et al. 2018).

12.4 Biocontrol Agents

The property of microorganisms to fight against phytopathogens is termed as a form of biological control (Duffy and Defago 2009). This approach is eco-friendly, much effective as well as cost-efficient. These PGPRs produce antifungal metabolites, creating competition for nutrients that act as chief modes of biocontrol activity (Duffy and Defago 2009). Rhizobacteria produce some antifungal metabolites like HCN, phenazines, pyoluteorin and tensin which kill the fungal pathogen (Bhattacharyya and Jha 2012). *Bacillus* spp. (Gong et al. 2006) and *Pseudomonas* (Leonardo et al. 2006) are two PGPRs that have been reported being effective biocontrol agents. Among these bacterial species, *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Bacillus cereus* are the most effective ones for controlling plant diseases through various mechanisms (Passari et al. 2016a; Francis et al. 2010). PGPRs like *Bacillus* spp. and *Pseudomonas* spp. have this ability to make endospores which allows them to sustain in a wide range of environmental conditions and hence make them efficient biofertilizers (Perez-Garcia et al. 2011). Application of *T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens* when mixed with neem or karanj cake and compost not only reduces the diseases but also enhances the longevity of biocontrol agents (Narayanan et al. 2015; Shanmugapackiam et al. 2016).

Application of biocontrol agents can be done in three forms:

1. By application of fungi
2. By application of AMF
3. By application of bacteria

12.4.1 By Application of Fungi

Trichoderma sp. secretes secondary metabolites which are antifungal and hence has great potential to act as biocontrol agents. They reduce the fungal pathogen either directly by mycoparasitism or through indirect mechanisms like competition for nutrients and space to survive and modifications of environmental conditions. They help in the promotion of plant growth and also activate the defence mechanism of the plant. Whipps and Lumsden (2001) stated that species of *Trichoderma* have been widely accepted as biocontrol agents against numerous phytopathogens. *Trichoderma* species are useful virulent saprophytes that act as biocontrol agents against phytopathogenic fungi by various mechanisms such as rhizosphere competition, mycoparasitism and antibiotic and enzyme production and induce resistance. Growth promotion activity of *Trichoderma* has also been reported (Cumagun 2012; Harman et al. 2004). Strains of *Trichoderma* (*T. viride*, *T. harzianum*, *T. virens*) were evaluated under field conditions against *Fusarium udum*; out of which *T. viride* was found to be most promising at 15% concentration (Chaudhary et al. 2017). The inoculation of seeds with antagonists helps in externally managing seed and soilborne pathogens. Talc-based formulation of *Trichoderma* sp. has been used to coat seeds.

12.4.2 By Application of AMF

AMF or arbuscular mycorrhizal fungi are the groups of fungi that act as promising biofertilizers. Dumas-Gaudot et al. (2000), Garmendia et al. (2005) and Garcia-Garrido (2009), in their respective studies, reported that AMF-mediated bioprotection is accepted as a key practice for disease control. AMF is currently exploited for its anti-pathogenic properties. Linderman (2000) reported that induced systematic resistance or ISF is the mechanism behind AMF phytoprotection. This mechanism concentrates more on nutritional changes like competition with infection sites, changes in the morphology of root and shoot tissues, abiotic stress reduction and changes in the mycorrhizosphere and chemicals, constituting changes in plant tissues (Hause and Fester 2005). All these properties make AMF a good biofertilizer also in the coming future.

12.4.3 By Application of Bacteria

Plant growth-promoting bacteria are the bacteria present in rhizospheric soil which enhance the growth of the plant directly or indirectly. The awareness of PGPR is increasing steadily in the world. They are applied to several economically important crops to increase the yield of the crop by enhancing the growth of the plant and protecting it from different pathogens. PGPR promotes plant growth by procurement of minerals like phosphorous, nitrogen, etc. directly from the soil (Gyaneshwar et al. 1998) and also indirectly by acting against plant pathogens as a biocontrol agent. Several reports suggest an increment in the quality and the number of different crops worldwide through the application of PGPRs under normal as well as stressed conditions (Passari et al. 2019). The application of PGPR is encouraged because it reduces the dependence on hazardous chemical fertilizers for improving plant growth and helps in reducing plant pathogens, which destabilizes the agriculture system. PGPR exhibits positive effect on the germination of the seeds, the yield of the crop and their tolerance towards stresses like drought and salt (Passari et al. 2019; Brown 1974). PGPR is an effective antagonist against plant pathogens like *Fusarium udum* and *Macrophomina phaseolina*. Soil microbe's interaction with the rhizosphere plays an important role in solubilizing and mobilizing a limited amount of nutrients available and also their uptake by the plant (Bolton et al. 1993; Mantelin and Touraine 2004). PGPR has beneficial effects as a biocontrol agent to important crops like legumes, cereals, fruits, vegetables, etc. According to reports, the exact estimate is unknown, but an average of more than 50% of crop losses in pigeon pea is due to pathogenic microorganisms (Rajash 2005). Thus, the need of the hour is to exploit and enhance the efficacy of soilborne control agents and use their best possible combination against plant pathogens (Mishra et al. 2016; Chang et al. 2005). The encouragement for the use of PGPR as biofertilizers against plant pathogens will serve as a promising alternative to deadly chemical fertilizers and pesticides (Goldstein 1995). Screening of soil for bacterial antagonist against pathogens is a notable biological advancement (Passari et al. 2016a; Karimi et al. 2012; Siddiqui et al. 2005), mostly for PGPR as a biocontrol agent (Siddiqui and Shakeel 2007; Prasad et al. 2002). Inoculation of *Pseudomonas aeruginosa* in the seed is effective against fusarium wilt disease of pigeon pea (Mahesh et al. 2010).

12.4.3.1 Modes of Action of PGPR

The mechanism of action of PGPR is not completely known; however, they are reported to exhibit several beneficial activities for plant growth promotion (Khan et al. 2009; Zaidi et al. 2009). PGPR promotes plant growth in two ways: directly and indirectly (Glick 2012). Pigeon pea is the most staple and proteinaceous food available in many developing countries; hence, it becomes important to protect this crop from damage. Root-nodulating bacteria *Sinorhizobium* inhibited the growth of fusarium wilt of pigeon pea as it possesses chitinase and β-gluconase production (Kumar et al. 2010). Plant growth promotion takes place indirectly when PGPR increases plant growth by decreasing the activity of plant pathogens (Xiang et al. 2017).

12.4.3.1.1 Nitrogen Fixation

Nitrogen is a vital nutrient required for the growth and productivity of the plant. The atmospheric N_2 is converted into plant-utilizable forms by biological N_2 fixation during which nitrogen gets converted into ammonia, and this is done with the help of nitrogen fixation bacteria present in the rhizospheric soil catalysed by nitrogenase enzyme (Kim and Rees 1994). Biological nitrogen fixation, also known as BNF, usually takes place at mild temperatures, by widely spread nitrogen-fixing bacteria (Raymond et al. 2004). This provides an economically beneficial and environmentally friendly alternative to chemical fertilizers (Ladha et al. 1997). Nitrogen-fixing bacteria (symbiotic bacteria) show symbiosis with plants belonging to leguminosae family like rhizobia (Ahemad and Khan 2011; Zahran 2001) However, non-symbiotic nitrogen-fixing bacteria provide only a small amount of the fixed nitrogen that bacterially associated host plant requires (Glick 2012).

12.4.3.1.2 Phosphate Solubilization

After nitrogen, phosphorus is the second most vital nutrient required for plant growth. This is also abundantly available both in an organic and inorganic form in the soil (Khan et al. 2009). The low availability of phosphorous to the plants is due to its presence in the insoluble form which plants are not able to absorb (Bhattacharyya and Jha 2012). The only soluble form of phosphorous available for the use of plants is monobasic and dibasic (Jha and Saraf 2015). To fulfil the phosphorous requirement, phosphatic fertilizers are given as a supplement in the fields. As plants do not absorb the full amount of applied fertilizer, the rest gets converted into insoluble complexes in the soil (McKenzie and Roberts 1990). This practice not only affects the environment but is also not cost-effective. Hence finding a better reliable solution to this problem is necessary. PGPR has coupled with phosphate solubilizing activity which may provide the available phosphorous to the plants in a much eco-friendly way (Khan et al. 2006).

12.4.3.1.3 Siderophore Production

Iron is a prominent nutrient available for all lives possible on earth. It is needed by all living beings.

In properly aerated soils, iron in the form Fe^{3+} (ferric iron), which is easily precipitated as iron oxide, is absorbed by plants (Duffy 1994). This property of microbes to secrete siderophores makes them suitable biocontrol agents as they induce competition for iron availability in the rhizosphere, hence restricting the proliferation of fungal phytopathogens in the vicinity of the crop, because of less availability of iron. CAS or chrome azurol agar media is used to isolate siderophore-producing bacteria. Rajkumar et al. (2008) have reported the growth of the plant through siderophore, because of the siderophore-producing bacteria in the rhizosphere.

12.4.3.1.4 Phytohormone Production

Microbes are known to synthesise phytohormones like auxins or IAA, i.e. indole acetic acid, for a long time. About 80% of the microbes isolated from the rhizosphere, of many crops, secrete secondary metabolites like auxins (Patten and Glick 1996). Indole acetic acid has a prominent function in bacteria-plant interactions (Passari et al. 2016a, b; Spaepen and Vanderleyden 2011). It is also reported that IAA has a plant defence mechanism against plant pathogens, and it produces a signalling effect to reduce the IAA production by the plant pathogen (Spaepen and Vanderleyden 2011).

12.5 Microbial Consortium

Most applications of biocontrol of plant diseases use single biocontrol agents as the antagonist against plant pathogens. The microbial consortium works well as, biopesticides, against a wide spectrum of plant pathogens which is a little difficult to be fulfilled using a single biocontrol agent. Biocontrol agents individually or in consortium attack pathogens through antagonism effect. They act better and more effectively when combined and when belonging to the same ecosystem. Vital and future promising candidates of the microbial consortium are *Trichoderma* sp., *Pseudomonas* sp. and *Bacillus* sp. Seed bacterization with a consortium of *Rhizobium* and *Pseudomonas putida*, *P. fluorescens* and *Bacillus* increased yield and biomass of pigeon pea crop (Tilak et al. 2006). *Trichoderma* sp. in association with AMF has great potential against plant pathogens (Wehner et al. 2010). The consortium of bio-organic (municipal waste) and applied organic (*Rhizobium* sp.) showed prominent improvement in the growth of pigeon pea over control plant (Rizwan and Mahmood 2017). Didwania et al. (2019) have also reported integrated management for Alternaria blight in oil-yielding crops.

12.6 Biotechnological Approaches to Biological Management

The detailed information on biotechnological techniques and genetics is important for developing a mechanism against susceptible varieties. Numerous resistant theories are known against fusarium wilt, and hence a single dominant gene has been established (Owuoche and Silim 2010; Kotresh et al. 2006). Many well-characterized or little-known genes, earlier reported being involved in legume crops, defend against fungal infection in pigeon pea. Resistant varieties available in the market against Phytophthora blight are Hy 4, ICPL 150, ICPL 288, ICPL 304, KPBR 80-1-4 and KPBR 80-2-1 (ICAR database). Out of 80 entries evaluated under sick plot, 18 entries WRP-1, BDN-2004-1, MAHABEJ, BRG-14-2, PT-257, BRG-14-1, MA-13, BWR-133, GRG-160, IPA8F, KA-12-03, ICPL-87119, KPL-44, KPL-43, BSMR571, BSMR-846, BSMR-579 and BSMR-2 have showed moderate resistant reaction with 0.00–10.00 per cent disease incidence. Similarly, Mishra and Dhar (2005) reported the same findings in vitro. Prasanthi et al. (2009) have reported a disease score of zero in treated and untreated pots of genotype ICP 8863, in pot culture screening technique against fusarium wilt-resistant/fusarium wilt-susceptible genotypes. IVT-520, IVT-509 and AVT-603 were found to be resistant against pod bug damage among 29 genotypes screened (Singh et al. 2017).

12.7 Conclusion

With the increasing population of the world, the demand for staple food like legumes, which are rich in protein, would also increase. Hence measures are required to fulfil the demand of the crop.

Decades ago the green revolution happened which increased the agriculture supply globally. This revolution saved the then population from hunger and malnutrition but, in turn, also triggered the use of chemical fertilizer. These chemical fertilizers are very harmful to our environment as they enter the food chain. So it is the need of the hour that we adapt better means to improve the quality as well as quantity of the crop but keeping in mind the environment safety also. Biofertilizers are an excellent solution to this problem of chemical fertilizers. Biofertilizers help in the improvement of plant growth and also act as biocontrol agents. They are eco-friendly and cost-effective means for crop improvement. Their use will serve as an instrument to ensure productivity and stability which will lead us to perfect agricultural practices in the world. A combination of biotechnological approaches with microbial consortium can contribute to go a long way in fighting with fungal diseases of pigeon pea and also to increase the yield.

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