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RESEARCH ARTICLES

Potentiality of selected plants extracts as green fungicides against fennel soil borne diseases

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Abstract

Fennel (*Folium vulgare* L.) is susceptible to various fungal diseases such as damping off and root rot. *Macrophomina phaseolina, Fusarium solani,* and *Pythium aphanidermatum* are the causes of these diseases, which radically lower crop productivity in agricultural industries across the globe. Plant extracts can be used to reduce damping off and root rot, so ensuring a sustainable food supply and safeguarding the environment. Thus, extracts of three medicinal crops of pomegranate peel (*Punica granatum* L.), halfa bar (*Cymbopogn proximus*), and acacia seeds (*Acacia nilotica*) in a variety of organic solvents, such as n-hexane, acetone, ethanol 80% (v/v), and water have been studied for their ability to protect fennel plants against diseases using three different concentrations 150, 200, and 250 ppm. The linear development of the fungus *M. phaseolina, F. solani,* and *P. aphanidermatum* was significantly reduced by all pomgranate peel, halfa bar, and acacia seed extracts in vitro. The most successful against fungal growth were pomegranate peel extracted with acetone and acacia seeds extracted with ethanol. All extracts at varying doses, significantly reduced fennel plant damping-off and root-rot diseases in greenhouse studies. The best treatments for fennel root rot and damping-off were pomegranate peels extracted with acetone and acacia seeds extracted with ethanol at a concentration of 250 ppm. It was also shown that these extracts stimulated the activity of antioxidants as peroxidase, polyphenoloxidase, and chitinase as well as soluble proteins, which are hydrolytic enzymes. Under field conditions, fennel seeds were treated with three plant extracts: acacia seeds, halfa bar and pomegranate peel at 250 ppm concentrations. According to the results, acetone-extracted pomegranate peel was the most successful treatment. Overall, they showed the greatest improvement in plant growth and yield, as well as volatile oil percentage, and its components throughout both seasons Application of plant extracts may have many benefits for sustainable agriculture, due to several advantages, including improved cultivated plant tolerance to biotic stress, higher crop yields and quality, and a decrease in pesticide use. Based on the findings, plant extract may be used as a natural and eco-friendly fungicide by inducing resistance and utilized as a sustainable.

Keywords Fennel · Soil-borne diseases · Plant extracts · Induces systemic resistance

Introduction

One of the most commonly used medicinal crops in Egypt and around the world is fennel (*Foeniculum vulgare* L.) (Khare et al. 2014; Khder Sara et al. 2022, Abd El Mageed et al. 2024). The seed's essential oil was used as a flavoring component in manufacturing (Abdellaoui et al. 2020; Dahmani et al. 2022). Farmers suffer large losses as a result of several fungus that damage fennel. The most prevalent and hazardous fennel diseases in Egypt and globally are *Macrophomina phaseolina, Pythium aphanidermatum, Fusarium solani,* and, which induce damping-off and root rot of seedlings (Khare et al. 2014; Khalequzzaman 2020; Khder Sara et al. 2022; McGovern 2024). Plant secondary metabolites are the subject of much research due to their advantageous and non-toxic properties as anti-bacterial, antioxidant, and biocidal agents for human health and others (Belgacem et al. 2021). Alternative methods that have been suggested include the use of plant extracts, which can be used either independently or in conjunction with other integrated pest management programs (Mari et al. 2016).

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Of these botanicals, pomegranate peel (PPEs) (*Punica granatum* L) extracts show the most promise due to their high potency and application as a green fungicide (Mari et al. 2016; Belgacem et al. 2021). Pomegranates are a natural source of various biological activities and active compounds, such as phenolics, lignans, tannins, organic acids, flavonoids, terpenes, terpenoids, vitamin C, fatty acids, and saccharides (Maphetu et al. 2022). The findings indicated that the most susceptible to PP treatment was the mycelium growth of *Fusarium oxysporum f.sp. lycopersici*, *Penicillium expansum, Aspergillus niger* and *Rhizoctonia solani* (Leontopoulos et al. 2022). Pomegranate peel extract has been demonstrated to include antibacterial and antifungal components to reduce plant and foodborne phytopathogens (Gosset et al. 2021). Aqueous leaf extracts from *P. granatum* exhibit antifungal activity against *Candida albicans, Aspergillus niger, Penicillium chrysogenum,* and *Candida sp*. in both in vitro and in vivo studies (Bassiri-Jahromi et al. 2015, 2018; Bhinge et al. 2021,). The plant pathogenic fungus *Pyricularia oryzae* was shown to be susceptible to the antifungal activity of acetone peel extract at five distinct concentrations (50, 100, 150, 200, and 250 mg/mL), owing to a range of medicinal and pharmacological effects (Jayaprakash and Sangeetha 2016). Punicalagins are responsible for the suppression of *Aspergillus flavus* and *Fusarium proliferatum's* conidial germination and hyphal growth (Sudharsan et al. 2019), as well as the prevention of *Fusarium oxysporum f. sp. lycopersici's* germination and hindrance of its mycelial development. Osman et al. (2021) indicate that pomegranate can be utilized as a natural alternative fungicide against *Sclerotium rolfsii* plant infection since it has the maximum of all growth parameters and yield of root, the percentage of sucrose and the content of soluble solids, as well as the lowest disease severity.

Acacia nilotica is said to have a wide range of active ingredients with various uses as phenolic compounds that exhibit phytotoxic, antibacterial and antifungal qualities (Batiha et al. 2022). *A. nilotica* is safe, and has anti-fungal and microbial qualities, (Hosni et al. 2021). Using ethyl acetate extracts of *A. nilotica* and *A. farnesiana* in benzene, ethyl alcohol, methanol, and ethyl acetate, showed antifungal activity against *Colletotrichum gleoesporioides* in all four organic solvents (Tripathi and Singh 2015) and against both *Sclerotinia sclerotiorum* and *Aspergillus flavus* (Abbassy et al. 2018). *Acacia saligna* has antifungal activity against root rot diseases caused by *Fusarium culmorum* and *R. solani* (Al-Huqail et al. 2019).

The halfa bar (Cymbopogon proximus, Poaceae), belongs to a large genus that comprises roughly 140 species found in tropical and subtropical regions of the world (Elhassan et al. 2016). The main ingredients of the oil were 72.44% peritone, 9.43% elemol, 4.34%, eudesmol, 2.45% limonene, and 1.26% eudesmol (Selim 2011). The presence of terpenoids in the plant extract has also been shown to have antibacterial and antioxidant properties (Falana and Nurudeen 2023).

The objective of this research was to assess the potential of three medicinal crop extracts of pomegranate peel, halfa bar, and acacia seed extracts to effectively develop resistance against fennel plant diseases that result in damping off and root rot.

Biochemical analyses of plant defense systems were conducted in response to a variety of treatments, including soluble proteins, enzyme activities (peroxidase and chitinase), and their effects on plant growth, yield, percentage of volatile oil, and testing frequency.

Materials and methods

Isolation of pathogenic fennel fungi

Soil-borne fungi were isolated from diseased root sections of fennel plants grown in Fayoum Governorate (Abshoi) (DD COORDINATES 29.30995 30.8418.

DMS COORDINATES 29°18′35.82″ N 30°50′30.48″ E) and Qaluobiya (Gezirt el sheir El-Qanater El-Khairya) (Latitude and longitude coordinates are: 30.243404, 31.244295) and identified in National Research Centre, Egypt according to Domsch et al. (1980); Booth (1977). Fungi were maintained on slant media (Potato Dextrose Agar), at 5 °C, and regenerated once a month.

Laboratory experiments

Preparation of plant extracts

Acacia seeds, halfa bar, and pomegranate peel that were bought from the local Egyptian market were subjected to a 15-h extraction process using a variety of organic solvents, such as n-hexane, acetone, ethanol 80% (v/v), and water. Following the extraction and drying of the waste, 1500 ml of distilled water that had been sterilized were added and the combination was then filtered to create an aqueous extract (Parera et al. 2019). Subsequently, the separate extracts were mixed together in a brown container, filtered through 45-m sterile filters, and vacuum-dried at 40 °C using a mini-rotary evaporator (NN series, EYELA) until nearly all of the solvent was removed. All solvent's semi-dried extracts were stored at 20 °C for later use (Rahnemoon et al. 2016; Toklu et al. 2007).

Antifungal effect of plant extracts

The antifungal effects of plant extracts were evaluated at 150, 200, and 250 ppm against the growth of pathogenic fungus. Three duplicates of each treatment were used. The

pathogenic fungus' linear growth (mm) was measured. Reduction was calculated according to the formula created by Pandey et al. (1982): R is equal to (G2- G1/G2) × 100. Here, R:% decrease in hazardous fungal growth G1 represents the average mycelial growth under the treatment; G2 represents the average mycelial change under the control.

Greenhouse test

The local type of fennel seeds (received from Horticulture Research Institute for aromatic and medicine crops) were planted in pots of 25 cm in diameter, filled with sterile clay/sand soil (2:1), and allowed to grow for 16 h at a temperature between 22 °C and 25 °C. Plants were irrigated in greenhouse conditions to maintain a field-capable moisture content. The greenhouse experiment used a random whole block design. A National Research Centre (NRC), Egypt greenhouse hosted a greenhouse experiment. On sterile corn/sand medium, pathogens were cultivated at 25 °C for two weeks. Before seeding, each inoculum was inoculated with three grams per kilogram of soil (clay/sand 2:1) and left to soak for seven days. After sterilizing them with 0.1% sodium hypochlorite, the seeds were subjected to a variety of treatments including varying amounts of plant extracts.

Impact of plant extracts on fennel plant diseases related to damping off and root rot

At 200 and 250 ppm, various plant extracts were employed to make pomegranate peel, half-a-bar, and acacia seeds. Plant extracts were tested against fungi to determine their biofungicidal efficacy.

Analysis of enzymes

To assess the activity of soluble proteins and antioxidative enzymes such as peroxidase, polyphenoloxidase, and chitinase, one gram of fresh fennel plants that had been treated and untreated was collected. The activity of enzyme was determined using the supernatant (Kar and Mishra 1976).

Polyphenoloxidase (PPO) measurement: The absorbance at 420 nm was assayed using spectrophotometer in according to Shi et al. (2002).

Peroxidase (P) measurement: The rise in absorbance at 412 nm at 25 °C was monitored for five min using a spectrophotometer using the Bradford (1976) method.

Chitinase measurement: To prepare colloidal chitin for the chitinase assay, chitin powder was used. The absorbance at 530 nm was measured using a UV spectrophotometer (Vahed et al. 2013).

Soluble protein measurement : Bovine serum albumin is detected by the Bradford assay approximately two times more sensitively than "average" proteins. Most commonly used protein standard is immunoglobulin G (IgG-gamma globulin).

Field trial

Field in trails were conducted during winter seasons during 2020–2021 and 2021–2022 at El Qanater El-Khairia, Qaluobiya governorate, which has a history of high pathogenic soil-borne fungal infestation. The dimensions of the plots were 3 by 3.5 m. The local variety of fennel seeds, obtained from Medici, Hort. Res. Inst., were utilized. There were four plots with 100 identical plants each after 25 seeds were placed on each ridge. A completely randomized plot design was used for each treatment. Fennel seeds were sterilized and then treated with plant extracts at a concentration of 250 parts per million. *Bacillus subtilis*, a commonly utilize commercial biofungicides and the natural product for root rot and damping off, was also used as a comparator at a concentration of 0.5 ml/L at the same time. Untreated served as the reference point. Furthermore, the fennel leaves were sprayed with the same treatments 30 and 60 days after seeding.

Impact of plant extract on fennel soil- borne disease control: Soil- borne diseases were assessed after 15, 30 and 45 days of sowing as pre, post and root rot incidence, respectively.

Growth parameters: At the end of each growing season, the following parameters were assessed for the crop: the weight of 1000 seeds (g), the dry weight of the seeds (g), the height of the plant (cm), and the overall output per plant (g).

Analysis of essential oils content

All samples were tested for percentage of essential oils using 100 g, in accordance with Egyptian Pharmacopoeia (1984). A deep freezer was used to store the refined essential oil until GC–MS studies could be performed.

Frequency

The essential oil samples were subjected to GC–Ms analysis under the following circumstances using apparatus stands for gas chromatography–mass spectrometry located at the NRC, Department of Medici and Aromatic Plants Research, Egypt. The device is a mass spectrometer detector made by THERMO Scientific Corp., a US company that produces the TRACE GC Super Gas Chromatograph. The system was connected to the GC–MS equipment using a 30 m × 0.25 mm TG-WAX MS column with a 0.25 µm film thickness. In the tests conducted in helium gas, electron ionization (EI) at 70 eV was used to produce the mass spectra of the compounds spanning the spectrum range of m/z 40–450. The mass spectra of the original compounds were used to

determine the presence and relative concentrations of the compounds. The Spectral Wiley Library.

Analytical statistics

The means and standard deviations of the experimental data were reported. The data was analyzed using SAS version 9.4 and compared using the Duncan's Multiple Range Test.

Results

Laboratory experiments

Antifungal effect of plant extracts

Three plant extracts i.e. pomegranate peel, half-a-bar, and acacia seeds were tested against the pathogenic fungi *F. solani, M. phaseolina*, and *P. aphanidermatum*, measuring three different concentrations of 150, 200, and 250 ppm. As compared to the control, all extracts considerably lowered the growth of the three tested fungi, according to the results shown in (Fig. 1). The most successful therapies against the growth of the fungi under test were pomegranate peel extracted with acetone and acacia seeds extracted with ethanol. When comparing the acetone extract of pomegranate peel at 250 ppm to the untreated control, all examined fungi showed significant reductions in their growth: *M. phaseolina* at 96.70%, *F. solani* at 95.66%, and *P. aphanidermatum* at 94.30%. Comparing the ethanol-extracted acacia seeds at 250 ppm to control plants, which consisted of acacia seeds and pomegranate peel extracted at 250 ppm with n-hexan, showed a similar pattern in terms of reducing the growth of *M. phaseolina* (94.76% reduction), *F. solani* (94.03% reduction), and P. aphanidermatum (96.60% reduction). *M. phaseolina* (2.00 mm) showed a 96.70% reduction in growth, while *F. solani* (3.66 mm) showed a considerable reduction in growth at 250 ppm of pomegranate peel acetone extract.

Pomegranate peel acetone extract at 250 ppm significantly reduced the growth of all tested fungi*, M. phaseolina* at 96.70% reduction, *F. solani* at 95.66% reduction, and *P. aphanidermatum* at 94.40% reduction when compared to the untreated control. The same result was also observed for Acacia seeds extracted by ethanol at 250 ppm, which reduced the growth of *M. phaseolina* at 94.76% reduction, *F. solani* at 94.03% reduction, and *P. aphanidermatum* growth at 96.60% reduction. Acacia seeds and pomegranate peel extracted by n-hexan at 250 ppm were both effective against the growth of tested fungi.

The n-hexan-extracted acacia seeds showed a 94.04% reduction in *M. phaseolina* growth, an 87.13% reduction in *F. solani* growth, and a 94.03% reduction in P. *aphanidermatum* growth when compared to the untreated control. While pomegranate peel extracted by n-hexan reduced the growth of *M. phaseolina, F. solani,* and *P. aphanidermatum* by 89.53%, 86.60%, and 86.60%, respectively, when compared to the control plants, On the other hand, the pomegranate peel aqueous extract had a moderate effect, reducing the growth of *P. aphanidermatum* by 91.76%, *F. solani* by 91.76%, and M. phaseolina by 869.6%,respectively.

Greenhouse experiments

Impact of plant extracts on fennel plant diseases

The effectiveness of pomegranate peel, half a bar, and acacia seed extracts in inhibition of soil-borne infections brought on *F. solani, M. phaseolina*, and *P. aphanidermatum* was investigated in a greenhouse during the 2019–2020 growth season. Data on Fig. 2 shows that, all treatments, at varying doses, decreased the disease incidence of fennel, which has been experimentally infected with root rot and damping off pathogens. The best treatments for fennel root rot and damping-off were pomegranate peel extracted with acetone and acacia seeds extracted with ethanol at a concentration of 250 ppm. Pomegranate peel extracted with acetone at a concentration of 250 parts per milliliter reduced damping-off caused by *F. solani* from 34.0% in the control treatment to 6.0%, while also reducing root-rot from 20.0% in the control treatment to 2.0% and increasing the percentage of survived plants from 46.0% to 92.0%. Pomegranate peel, had a moderate effect on reducing the incidence of *M. phaseolina* disease. It reduced damping-off from 46.0% in the control treatment to 6.0%, reduced root-rot from 24.0% in the control treatment to 0.4%, and increased the number of plants that survived from 30.0% to 90.0%. It also suppressed damping off caused by fungi such as *P. aphanidermatum* from 22.0% in the control treatment to 2.0%, decreased root-rot from 12.0% in the control treatment to 2.0%, and increased the number of plants that survived from 66.0% in the control treatment.

Impact of plant extracts on fennel plant oxidative enzymes.

Figure 3 shows that in plants infected with three pathogens, the activity of polyphenoloxidase was enhanced by the extraction of pomegranate peel using acetone and the extraction of acacia seeds using ethanol. Acacia seeds and pomegranate peel extracted with acetone both showed an increase in the activity of polyphenoloxidase to 0.31, 0.32, and 0.34 mg−1 min, respectively. Conversely, the least amount of polyphenoloxidase activity was increased in treatments involving *M. phaseolina, F. solani*, and fungi such as *P. aphanidermatum* (0.014, 0.013, and 0.011 mg−1 min), respectively. Uninoculated controls reported 0.017 mg/min

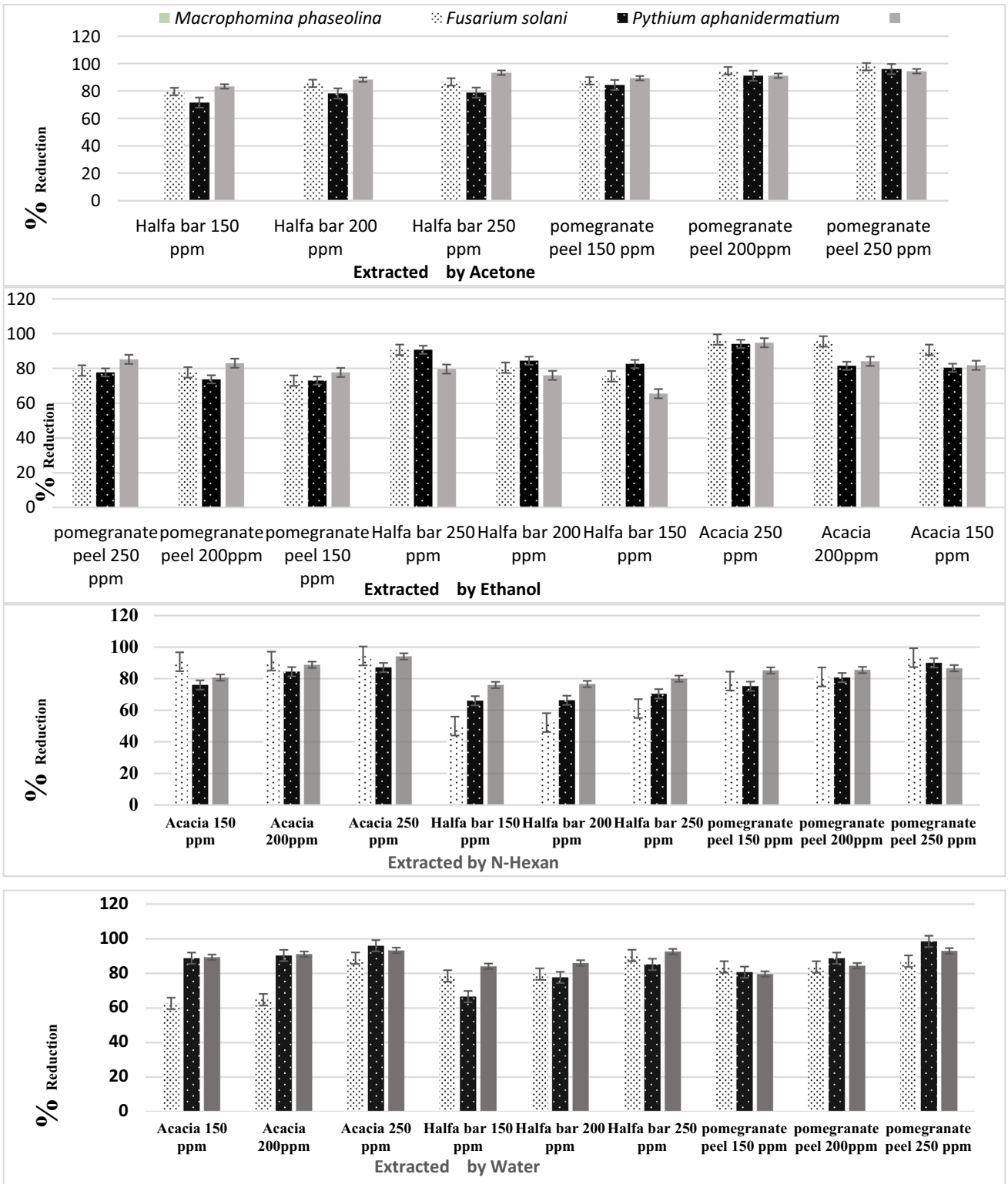

Fig. 1 Assessment of a plant extract's ability to decrease the linear growth of the tested fungi in vitro

in the meantime. The highest rise in peroxidase activity was seen at 0.158, 0.165, and 0.197 mg–1 min, respectively, after those removed by ethanol, which were 0.136, 0.137, and 0.147 mg-1 min, respectively.

Conversely, the lowest peroxidase activity was seen in treatments using *M. phaseolina, F. solani*, and *P. aphanidermatum,* with respective values of 0.193, 0.176, and 0.131 mg–1 min. The untreated control group recorded

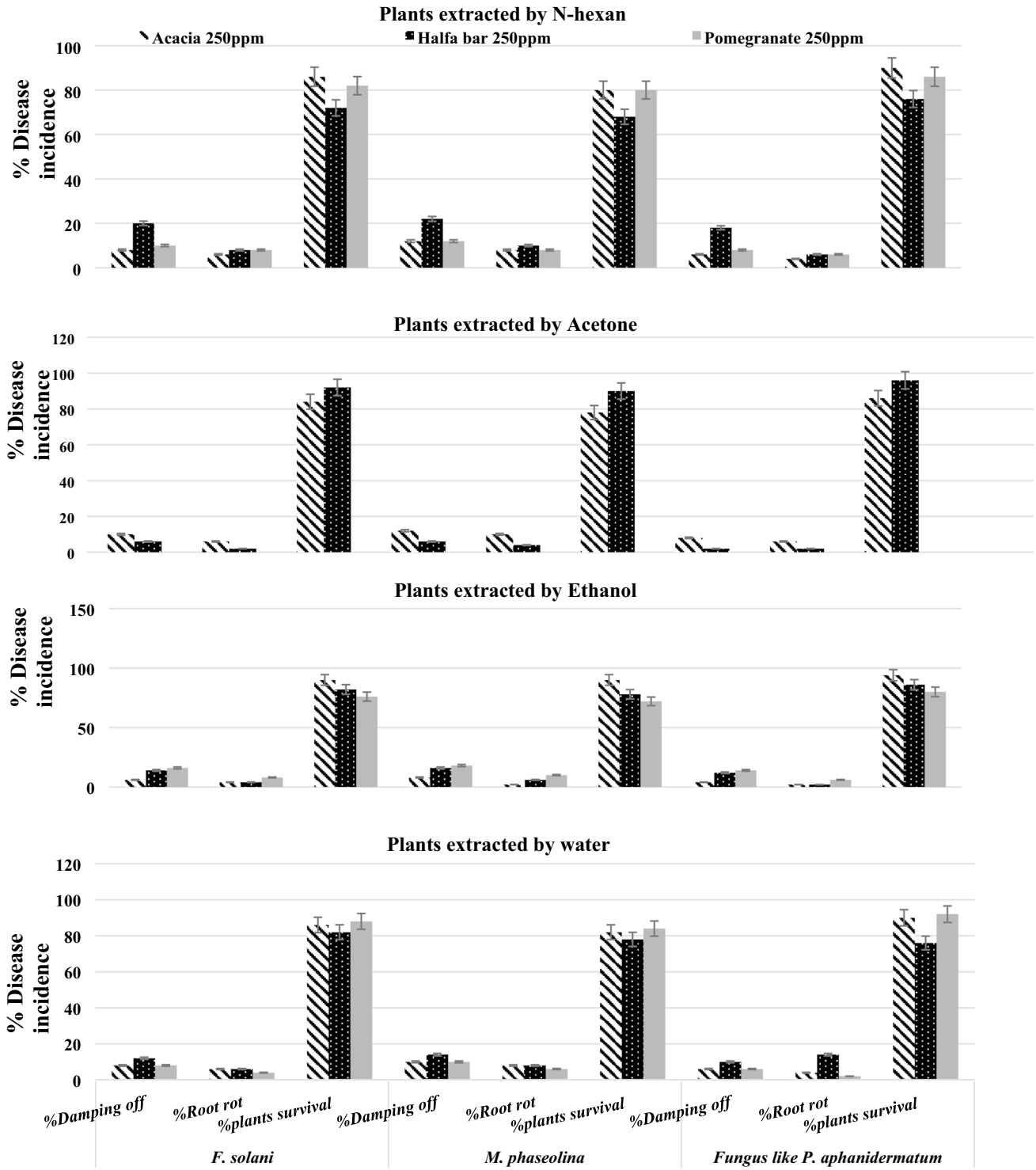

Fig. 2 The effectiveness of various plant extracts in management the three examined fungi in greenhouse

0.0854 mg–1 min at the same time. According to data shown in Fig. 3, pomegranate peel extracted with acetone and acacia seeds extracted with ethanol both enhanced the chitinase activity in fennel plants ioculated with three pathogens. These extracts recorded the highest increases in chitinase activity, coming in at 801.34, 796.42, and 801.34 ppm as glucose, respectively, while the ethanol-extracted seeds showed the lowest increases, coming in at 720.5, 678.36, and 711.636 ppm as glucose). Conversely, the lowest increases in chitinase activity were observed in treatments involving *M.*

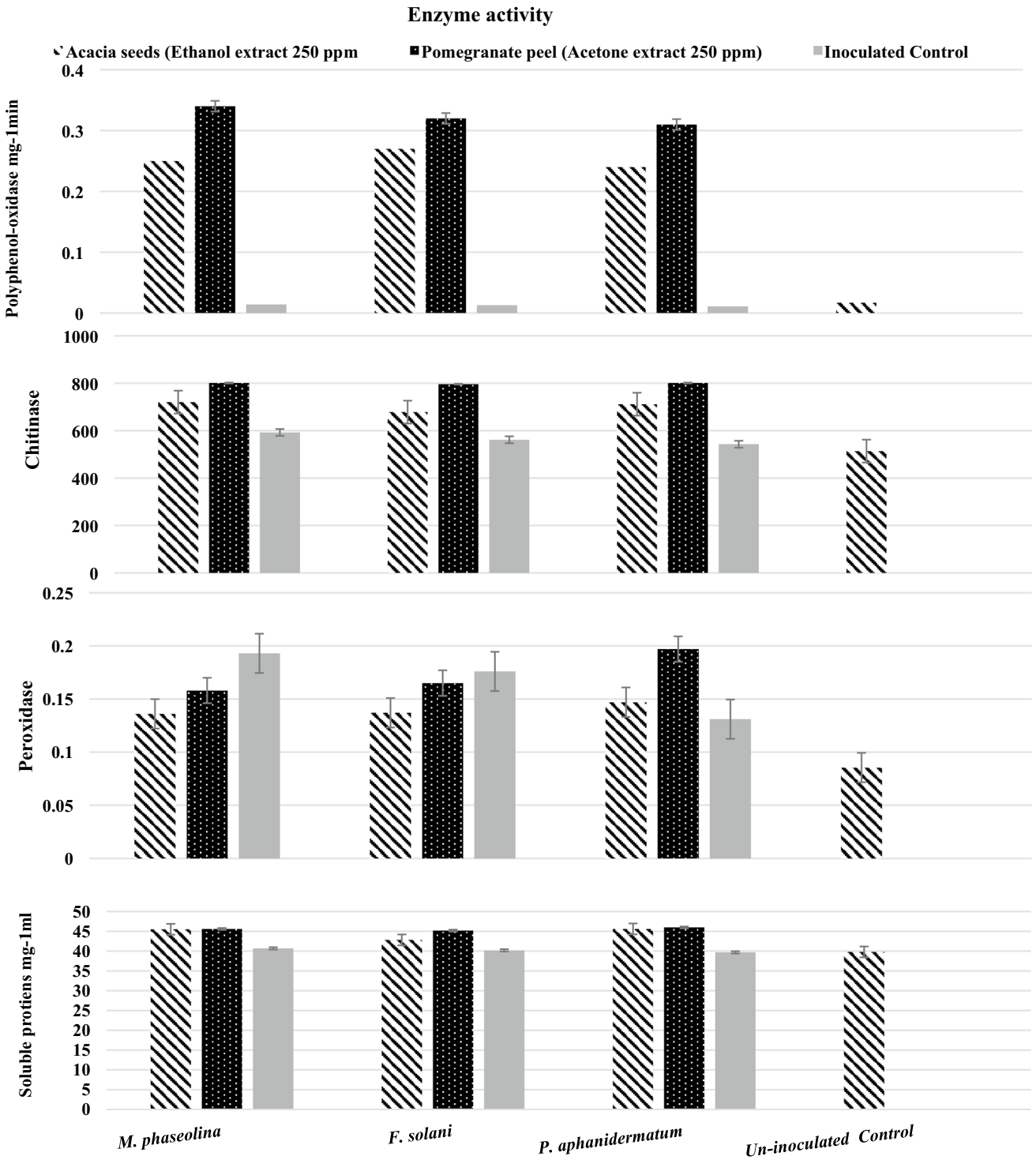

Fig. 3 Impact of plant extracts on the activity of oxidative enzymes in fennel plants grown under artificial inoculation with the tested fungi

phaseolina, F. solani, and *P. aphanidermatum,* which were reported to be (592.5, 561.7, and 542.8 ppm as glucose), respectively. In the meantime, the untreated control group measured 513.8 ppm of glucose. Additionally, pomegranate peel extracted with acetone and acacia seeds extracted with ethanol both enhanced the activity of soluble proteins in infected plants with three pathogens. The untreated control showed the greatest increase in soluble protein activity, recording 45.61, 45.20, and 46.00 mg–1 ml, respectively, followed by ethanol, at 45.62, 42.84, and 45.62 mg–1 ml,

respectively. Meanwhile, the control group that did not receive treatment recorded 39.80 mg/ml.

Field trails

Other treatments were discarded from field trials and only those that demonstrated an effect on disease control in greenhouse settings were selected.

Impact of plant extracts on fennel soil-borne disease control

Based on the data shown in Table 1, pomegranate peel extracted with acetone (250 ppm) was the most successful treatment in lowering the incidence of disease, with rates of 6.0% and 5.0%, respectively, during the two following growth seasons as compared to the control. Furthermore, data indicated that plant survival increased throughout the course of the two seasons, rising from 61.0% and 58.0%, respectively, in comparison to the control group's 94.0 and 95.0%. When comparing the two growing seasons of disease incidence reduction to the control, acacia seeds extracted with ethanol (250 ppm) ranked second in terms of plant survival, rising from 61.0 to 92.0% in the first season and from 58.0 to 93.0% in the second. In addition, pomegranate peel extracted with acetone proved to be more efficient in lowering disease incidence than *B. subtilis*, another commercial biofungicide, according to the data, which unambiguously showed the effectiveness of the commercial biofungicides.

Impact of plant extracts on fennel growth and yield

Over the course of the two following growth seasons, the application of 250 ppm of pomegranate peel extracted by acetone to fennel seeds produced the largest improvements in plant height, dry weight of inflorescences and seeds as well as plant yield.

In comparison to the control, these values were 221.0 cm, 333.0 g, 190.0 g, 32.5 g, and 581.2 g in the 2020/2021 growing season and 228.0 cm, 427.8 g, 237.7 g, 28.7 g, and 351.7 g, respectively, in the 2021/2022 growing season. Furthermore, ethanol-extracted Acacia seeds and proved to be efficient in improvements plant height, dry weight of inflorescences and seeds as well as plant yield, meanwhile

Table 1 Impact of plant extracts on fennel soil-borne disease management under field conditions

Treatments	Extracted by	2020/2021 season				2021/2022 season			
		%Damping off	%Root rot	% Disease incidence	%Plants survival	%Damping off	%Root rot	% Disease incidence	%Plants survival
Acacia	N-hexan	$7.0 \pm 0.21^{b*}$	2.0 ± 0.06^{b}	9.0 ± 0.26^{bc}	91.0 ± 0.26^{ab}	6.0 ± 0.38^{cd}	4.0 ± 0.44^{b}	10.0 ± 0.70^{bcd}	90.0 ± 0.71^{a}
Halfa bar		10.0 ± 0.24^{b}	3.0 ± 0.32^{b}	13.0 ± 0.52^{b}	87.0 ± 0.52^{a}	7.0 ± 0.15^{bcd}	5.0 ± 0.29^{abc}	12.0 ± 0.37^{bcd}	88.0 ± 1.31^{abc}
Pomegranate		5.0 ± 0.44^{b}	5.0 ± 0.47^{b}	10.0 ± 0.91^{bc}	90.0 ± 0.91^{a}	4.0 ± 0.38^{cd}	2.0 ± 0.17^{c}	6.0 ± 0.43^{d}	94.0 ± 0.43^{cde}
Halfa bar	Acetone	8.0 ± 0.30^{b}	4.0 ± 0.33^{b}	12.0 ± 0.68^{bc}	88.0 ± 0.68^{a}	8.0 ± 0.40^{bc}	5.0 ± 0.21^{b}	13.0 ± 0.33^{bc}	87.0 ± 0.33^{ab}
omegranate		3.0 ± 0.12^{b}	3.0 ± 0.21^{b}	6.0 ± 0.27^{bc}	94.0 ± 0.27^{a}	3.0 ± 0.25^{d}	2.0 ± 0.12^{b}	5.0 ± 0.49^{d}	95.0 ± 0.49^{a}
Acacia	Ethanol	6.0 ± 0.31^{b}	2.0 ± 0.10^{b}	8.0 ± 0.23^{bc}	92.0 ± 0.23^{a}	4.0 ± 0.28^{cd}	3.0 ± 0.27^{b}	7.0 ± 0.23^{bcd}	93.0 ± 0.02^{abcd}
Halfa bar		9.0 ± 0.41^{b}	3.0 ± 0.06^{b}	12.0 ± 0.37^{bc}	88.0 ± 0.37^{a}	6.0 ± 0.13^{cd}	5.0 ± 0.13^{abcd}	11.0 ± 0.13^{bcd}	89.0 ± 0.13^{ab}
Pomegranate		6.0 ± 0.15^{b}	5.0 ± 0.14^{b}	11.0 ± 0.28^{bc}	89.0 ± 1.09^{a}	5.0 ± 0.47^{cd}	2.0 ± 0.14^{b}	7.0 ± 0.41^{cd}	93.0 ± 0.41^{a}
Acacia	Water	3.0 ± 0.11^{b}	3.0 ± 0.32^{b}	6.0 ± 0.33^{bc}	94.0 ± 0.33^{abc}	7.0 ± 0.06^{bcd}	3.0 ± 0.25^{bc}	10.0 ± 0.27^{bcd}	90.0 ± 0.27^{ab}
Halfa bar		9.0 ± 0.26^{b}	2.0 ± 0.13^{b}	11.0 ± 0.32^{bc}	89.0 ± 0.32^{a}	11.0 ± 0.32^{b}	3.0 ± 0.41^{bcd}	13.0 ± 0.49^{b}	86.0 ± 0.49^{ab}
Pomegranate		3.0 ± 0.09^{b}	2.0 ± 0.00^{b}	5.0 ± 0.09^{c}	95.0 ± 0.09^{a}	5.0 ± 0.36^{cd}	3.0 ± 0.32^{bc}	8.0 ± 0.67^{bcd}	92.0 ± 0.67^{bcd}
Commercial biofungicide (*Bacillus subtilis*)		6.0 ± 0.50^{b}	5.0 ± 0.25^{b}	11.0 ± 0.62^{bc}	89.0 ± 0.62^{a}	7.0 ± 0.25^{bcd}	5.0 ± 0.25^{ab}	12.0 ± 0.40^{bcd}	88.0 ± 0.40^{a}
Control		29.0 ± 1.88^{a}	10.0 ± 0.64^{a}	39.0 ± 1.25^{a}	61.0 ± 1.25^{b}	30.0 ± 0.64^{a}	12.0 ± 0.91^{a}	42.0 ± 0.86^{a}	58.0 ± 0.86^{b}

*Data with the same letter are not significantly different at $P > 0.0001$, according to Duncan's Multiple Range Test (Duncan 1955)

*The recorded values are the percentage ± standard deviations

Table 2 Impact of plant extracts on growth parameters and yield of fennel plants grown under field conditions

Treatments	Extracted by	2020/2021 season					2021/2022 season				
		Plant height (cm)	Dry weight of inflorescences (g)	Dry weight of seeds (g)	Weight of 1000 seeds (g)	Yield/plant (g)	Plant height (cm)	Dry weight of inflorescences (g)	Dry weight of seeds (g)	Weight of 1000 seeds (g)	Yield/plant(g)
Acacia	N-hexan	204.6 ± 22.9^{a}	205 ± 4.1^{e}	186.6 ± 9.2^{ab}	27.3 ± 2.1^{a}	270.3 ± 3.0^{d}	207.0 ± 21.5^{ab}	257.3 ± 7.2^{bc}	125.9 ± 6.2^{cde}	25.8 ± 2.8^{a}	222.3 ± 1.7^{d}
Halfa bar		180.0 ± 11.5^{a}	257.0 ± 2.1^{cd}	124.0 ± 2.3^{cd}	22.2 ± 1.2^{a}	93.6 ± 4.9^{h}	183.0 ± 4.0^{ab}	109.8 ± 5.8^{f}	101.1 ± 8.0^{de}	24.4 ± 0.8^{a}	109.0 ± 9.8^{e}
Pomegranate		200.0 ± 9.6^{a}	257.0 ± 15.5^{cd}	179.0 ± 10.9^{abc}	27.0 ± 2.8^{a}	197.2 ± 9.9^{ef}	206.0 ± 8.08^{ab}	225.2 ± 8.5^{cd}	112.5 ± 4.3^{cde}	25.7 ± 1.4^{a}	138.1 ± 10.4^{e}
Halfa bar	Acetone	190.0 ± 41.3^{a}	286.0 ± 3.0^{abc}	169.0 ± 22.1^{abc}	26.0 ± 4.6^{a}	132.1 ± 1.8^{gh}	198.0 ± 0.5^{ab}	216.2 ± 4.7^{cd}	99.9 ± 16.6^{e}	25.0 ± 5.1^{a}	110.0 ± 6.3^{e}
Pomegranate		221.0 ± 26.0^{a}	333.0 ± 14.5^{a}	190.0 ± 35.0^{ab}	32.5 ± 51.1^{a}	581.2 ± 46.2^{a}	228.0 ± 17.6^{a}	427.8 ± 20.2^{a}	237.7 ± 11.6^{a}	28.7 ± 2.6^{a}	351.7 ± 6.0^{a}
Acacia	Ethanol	220.0 ± 15.7^{a}	322.0 ± 18.3^{ab}	214.0 ± 19.2^{a}	29.2 ± 3.6^{a}	443.0 ± 23.7^{b}	224.0 ± 9.2^{ab}	386.3 ± 17.1^{a}	199.8 ± 5.7^{b}	28.0 ± 2.8^{a}	335.6 ± 7.8^{a}
Halfa bar		197.0 ± 27.6^{a}	290.0 ± 27.8^{abc}	174.0 ± 16.5^{abc}	26.0 ± 3.0^{a}	155.1 ± 17.7^{fg}	200.0 ± 23.3^{ab}	216.3 ± 7.5^{cd}	104.4 ± 4.4^{cde}	25.4 ± 3.4^{a}	110.3 ± 3.4^{e}
Pomegranate		189.0 ± 22.7^{a}	299.0 ± 14.4^{abc}	104.4 ± 13.0^{d}	24.5 ± 5.5^{a}	87.5 ± 6.8^{h}	191.0 ± 10.2^{ab}	158.6 ± 17.2^{e}	62.4 ± 3.75^{f}	23.7 ± 3.6^{a}	109.4 ± 15.8^{e}
Acacia	Water	199.0 ± 6.3^{a}	306.0 ± 8.0^{abc}	195.0 ± 31.7^{ab}	27.5 ± 0.8^{a}	293.4 ± 4.3^{d}	210.0 ± 5.7^{ab}	278.1 ± 4.6^{b}	128.8 ± 5.0^{c}	26.0 ± 2.3^{a}	272.6 ± 4.3^{c}
Halfa bar		196.0 ± 3.4^{a}	278.0 ± 8.6^{bc}	178.0 ± 16.1^{abc}	26.0 ± 2.4^{a}	188.1 ± 6.9^{fg}	205.0 ± 13.8^{ab}	222.4 ± 1.9^{cd}	107.0 ± 6.8^{cde}	25.5 ± 2.0^{a}	136.5 ± 7.7^{e}
Pomegranate		210.0 ± 28.8^{a}	210.0 ± 11.5^{de}	7.0 ± 1.1^{e}	28.2 ± 1.8^{a}	356.2 ± 15.1^{c}	212.0 ± 9.8^{ab}	284.2 ± 3.0^{cd}	150.3 ± 7.0^{g}	26.7 ± 2.7^{a}	314.4 ± 9.4^{ab}
Commercial biofungicide (*Bacillus subtilis*)		183.0 ± 15.6^{a}	170.3 ± 10.8^{ef}	140.0 ± 14.6^{bcd}	23.6 ± 5.6^{a}	248.9 ± 34.4^{de}	180.0 ± 28.9^{ab}	201.7 ± 19.7^{d}	126.6 ± 9.6^{cd}	26.7 ± 1.8^{a}	288.5 ± 36.2^{bc}
Control		150.0 ± 25.1^{c}	156.0 ± 28.0^{f}	96.0 ± 7.8^{d}	21.7 ± 1.4^{b}	75.0 ± 11.8^{h}	171.0 ± 20.5^{b}	156.2 ± 26.7^{e}	108.9 ± 6.0^{cde}	22.0 ± 1.5^{d}	110.0 ± 3.7^{a}

*Data with the same letter are not significantly different at $P > 0.0001$, according to Duncan's Multiple Range Test (Duncan 1955)

**The recorded values are the means ± standard deviations

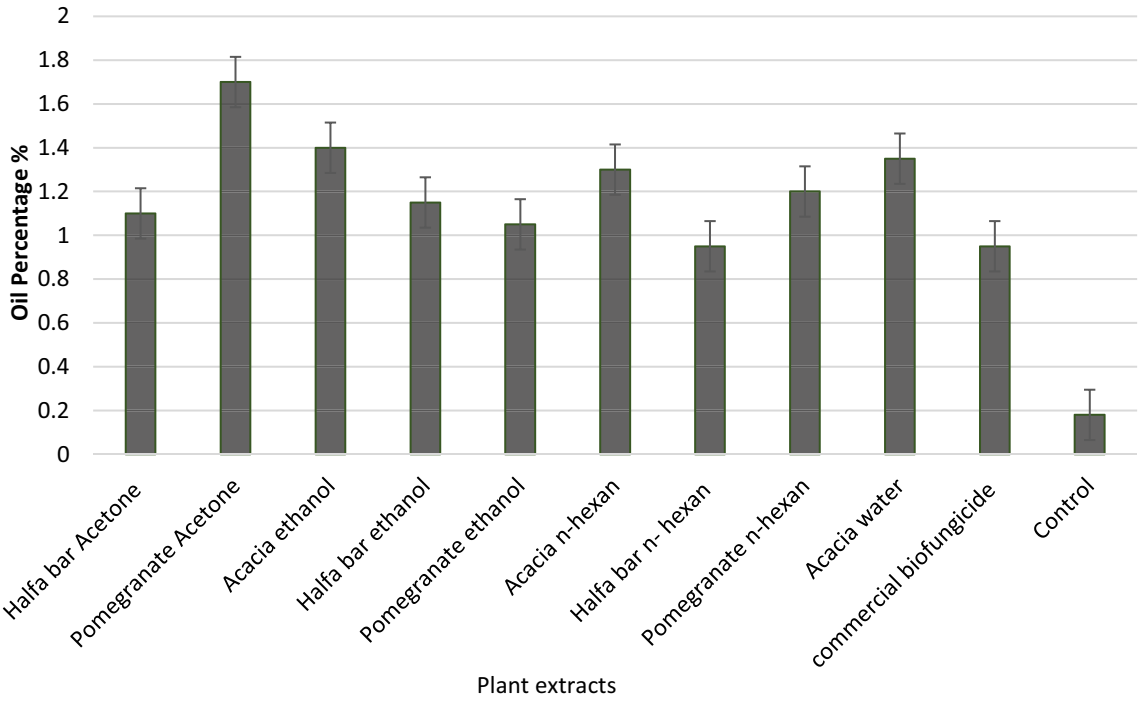

Fig. 4 Impact of plant extracts on the percentage of fennel seed volatile oil

Halfa bar and commercial biofungicide (*Bacillus subtilis*) gave moderate effect in the improvements plant growth and yield in both 2021/2022 growing season (Table 2).

Oil percent

Data shown in (Fig. 4) demonstrate that, the volatile oil percent in fennel seeds increased for both pomegranate peel extracted by acetone and acacia seeds extracted by ethanol. Plants treated with pomegranate peel extracted by acetone showed the highest oil content, rising from 0.18 to 1.7% for volatile oil. Furthermore, ethanol-extracted Acacia seeds raised the oil content from 0.18 in the untreated control to 1.4%, meanwhile biocide gave less effect (0.95%).

Oil Frequency

The information in Table 3 demonstrates that, when plant extracts applied as a seed soaking, all produced discernible variations between the values obtained from the control treatment and those of Estragole and Anethole. In comparison to the untreated control (77.17%) and biocide (86.76%), the results indicated a considerable increase in etragole (87.81 and 84.92%) when pomegranate peel extracted with acetone and Acacia seeds treated with ethanol were utilized.

Discussion

A variety of fungal diseases can affect fennel crops, including damping off and root rot of seedlings caused by *M. phaseolina, Pythium spp.,* and *Fusarium spp.* (Khalequzzaman 2020; Khder Sara et al. 2022; Haggag Wafaa et al. 2023). To control plant infection, bioelcitors and natural plant extracts can be used instead of commercial fungicides (Haggag Wafaa et al. 2017; Khder Sara et al. 2022). In vitro, plant extracts prepared from pomegranate peel, halfa bar, and acacia seeds using acetone, ethanol, n-hexane, and water showed a substantial reduction in linear growth of the three tested fungi. The pomegranate peel and acacia seed ethanol extracts proved to be the most effective.

The results of greenhouse experiments demonstrated that applying the tested plant extracts—pomegranate peel, half a bar plant, and acacia seeds—at different concentrations to combat disease incidence caused by three pathogens reduced the incidence of fennel diseases. The best treatments were pomegranate peel extracted with acetone and acacia seeds extracted with ethanol. Pangallo et al. (2017); Belgacem et al. (2021) have stated that pomegranate peel extract demonstrates potent inhibitory activity in vitro against numerous common plant fungal pathogens, including *Alternaria alternata, Colletotrichum acutatum* and *Stemphyliumn botryosum*. Similar results were reported by El Khetabi et al. (2020) who discovered that the bacteria that cause brown rot, *M. fructigena* and *Monilinia laxa*, were susceptible to

Table 3 Impact of different plant extracts treatments on the percentage of fennel seed volatile oil compounds

Compounds	Area %												
	Control	Commercial biofungicide (*B. subtilis*)	Acacia n-hexan	**Halfa n-hexan	*Pomg n-hexan	**Halfa acetone	*Pomg acetone	Acacia ethanol	**Halfa Ethanol	*Pomg Ethanol	Acacia water	**Halfa water	*Pomg water
α-Pinene	0.28	0.31	0.73	0.29	0.57	0.43	1.78	1.23	0.44	0.42	0.81	0.54	1.06
à-Phellandrene	0.15	0.17	0.37	0.15	0.35	0.16	0.54	0.52	0.26	0.14	0.44	0.27	0.5
D-Limonene	4.59	4.81	9.76	4.75	9.20	6.66	16.58	11.20	7.52	5.40	10.54	8.81	10.81
Eucalyptol	0.11	0.123	0.33	0.12	0.13	0.11	0.74	0.52	0.13	0.11	0.40	0.14	0.41
trans- à –Ocimene	0.15	0.26	0.42	0.15	0.42	0.23	0.57	0.53	0.37	0.18	0.45	0.41	0.50
ç-Terpinene	0.11	0.19	0.13	0.12	0.13	0.11	0.27	0.21	0.12	0.16	0.14	0.12	0.16
L-Fenchone	6.12	6.41	7.12	6.68	6.72	5.90	9.73	9.51	5.90	5.73	7.87	6.22	8.78
Limonene oxide	0.05	0.05	0.11	0.06	0.10	0.09	0.15	0.14	0.09	0.08	0.12	0.09	0.13
Trans-Limonene Oxide	–	–	0.05	–	0.04	0.03	0.07	0.07	0.03	–	0.07	0.04	0.07
Camphor	–	–	0.06	–	0.06	–	0.07	0.06	–	–	0.06	0.05	0.06
Estragole	77.1	86.76	81.57	73.42	81.08	79.81	87.81	84.92	80.03	75.97	83.26	80.31	83.26
Fenchyl acetate	0.07	0.09	0.11	0.08	0.11	0.5	0.24	0.18	0.8	0.07	0.11	0.9	0.13
d-Carvone	–	–	0.06	–	0.02	–	0.13	0.07	–	–	0.06	–	0.07
Anethole	1.35	1.53	1.13	1.12	1.39	1.10	1.38	1.26	1.12	1.48	1.14	1.15	1.23

*Pomg = Pomegranate peel

**Halfa = halfa

the pomegranate peel aqueous extract in vivo. This suggests that pomegranate peel aqueous extract could be a viable alternative management strategy. Belgacem et al. (2021) report that applying a methanolic pomegranate peel extract as a seed or soil treatment under a green house significantly reduced soil borne, root rot and wilt diseases of tomatoes caused by *Fusarium oxysporum*. *Acacia nilotica* was used by Hosni et al. (2021) as an antibacterial and antifungal agent due to the presence of active chemical components. These substances consist of proanthocyanidins, sterols, terpenes, anthocyanins, tannins, flavonoids, phenolics, alkaloids, xanthonoids, and anti-microbials (Jayaprakash and Sangeetha 2016; Guerrero-Solano et al. 2020). There is strong evidence that plant extracts not only directly combat bacteria and fungi but also induce resistance in plant tissues. Our results showed that all plant extracts stimulated the activities of antioxidants as peroxidase, chitinase, and polyphenyl oxidase as well as soluble proteins in inoculated fennel plants with three pathogens. The greatest activators of polyphenol oxidase, peroxidase, chitinase, and soluble proteins were pomegranate peel extracted with acetone and acacia seeds extracted with ethanol. Pomegranate peel extraction has been demonstrated to increase the quantity of polyphenolic chemicals that bind with the proteins in the fungal cell membrane, decrease the pH gradient surrounding the membrane, and increase permeability, all of which contribute to the eventual death of the cell (Akhtar et al. 2015; Rongai et al. 2018). Pomegranate peel extract enhanced plant defensive responses in citrus fruits inoculated with *P. digitatum* and *P. italicum*, and olive inoculated with *C. acutatum*, according to studies by Pangallo et al. (2017); Belgacem et al. (2019). Inducing a route and correlating the activation of these enzymes as oxidative enzymes to fight microbial infections may be the main mechanism of action of pomegranate peel extract. The induction of pathways is linked to the activation of these enzymes as oxidative enzymes.

Under field conditions, the pomegranate peel extracted by acetone (250 ppm) proved to be the most effective treatment in terms of reducing the incidence of damping off and root-rot diseases, as well as increasing the survival of fennel plants during the two successive growing seasons (2021–2022) compared to commercial biocide and the untreated control. In tomato plants grown under field conditions, pomegranate peel extraction suppresses *F. oxysporum* caused pre- and post-emergence damping off when treated as a seed or soil treatment (Belgacem et al. 2021; Leontopoulos et al. 2022). All growth parameters and yield output of fennel plant, as well as the percentage of volatile oil and its frequency two seasons were found to increase simultaneously when pomegranate peel extracted with acetone, ethanol, n-hexan, and water (250 ppm) was applied as a seed treatment. Disease control as well as induced resistance were cited as reasons for this. Plant growth, yield, and the proportion of volatile oil increased highest in the pomegranate peel that was extracted with acetone during two seasons. The root weight, yield/plot, sucrose content, and percentage of total soluble solids all increased in response to pomegranate extract treatments, according to Osman et al. (2021).

According to this study, pomegranate peel and acacia seed extracts can be used as natural, eco-friendly fungicide substitutes at 250 ppm concentrations to reduce plant diseases, encourage and stimulate systemic resistance, and increase fennel plant growth and yield.

Data availability The authors confirm that the data supporting the findings of this study are available within the article. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Conflict of interest The authors have no competing interests.

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