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Effect of some certain plant water extracts on the infection with *Fusarium solani* the causal pathogen of faba bean root-rot

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Abstract

Effect of water extracts from moringa, clove, black cumin, ginger and black pepper at 0, 1, 3, and 5% on the mycelial linear growth of *Fusarium solani* were investigated *in vitro*. The outcomes demonstrated that at 3 and 5% of the tested concentrations, all examined plant aqueous extracts effectively inhibited the linear growth of *F. solani*. In addition, the inhibitory effects increased gradually by increasing the concentration of each plant water extract tested. These extracts were used at a concentration of 5% under greenhouse and field conditions and were effective in reducing root- rot disease in faba bean.

Keywords:

Plant extracts, root rot, faba bean, *Fusarium solani*

INTRODUCTION

Faba bean (*Vicia faba* L.) is a member of the Fabaceae family and is indigenous to North Africa and Southwest Asia in addition to being widely cultivated worldwide. Faba bean is a staple item and an important part of the diet in Egypt. It is a rich source of protein, amino acids and minerals, especially for those who have limited resources. The total area cultivated with this crop in Egypt was about 26382 ha in 2021, and the gross seed yield produced was about 105051.58tons (FAOSTAT, 2021). According to several studies (Mazen, 2008; Elwakil *et al*., 2009a), the fungi *Rhizoctonia solani, Fusarium solani, Sclerotium rolfsii, Macrophomina phaseolina*, and other *Fusarium* species attack faba beans and cause root- rot disease. *Fusarium solani* is one of the most frequently found soil-borne infections that affects faba bean yields by up to 45% (PPRC, 1996), causing root-rot and black root-rot (Belete *et al*., 2015; Habtegebriel and Boydom, 2016). Plant extracts have recently shown value as potent, harmless, and readily available fungi toxicants in contrast to synthetic chemical fungicides that often impost undesirable side effects. As a result, many investigators worldwide have studied the antifungal proprieties of several plant extracts against plant diseases caused by fungal pathogens. The chemical compounds found in plant extracts, such as plant hormones, minerals, antioxidants, and osmoprotectants, help plants better withstand environmental stresses and are essential for fostering plant growth (Desoky *et al*. 2019). Plant extracts have made a substantial contribution to the improvement of seed quality and field emergence of plant seeds as well as the suppression of diseases that are transmitted through seeds. Many authors have documented the safe and efficient use of plant extracts for the management of seed-borne fungus (El-Metwally *et al*., 2010; Yoon *et al*., 2011; Ammar *et al*., 2013; Perello *et al*., 2013; Baka 2014b). Nanir and Kadu (1987) make it clear that various plant extracts and essential oils had antifungal qualities. (Stoll, 1988) said that some soilborne fungus in cereals have been successfully treated with *moringa oleifera* leaf extracts applied to the seeds. Also, (Hu and willett, 2002) reported that due to the presence of numerous chemical components in high concentrations that have antioxidant activity, clove is useful in preventing various degenerative diseases. This study aimed to investigate one of the factors that reduce root-rot disease caused by *F. solani*, such as plant extracts.

MATERIALS AND METHODS

Source of faba bean seeds:

Faba bean (*V. fabae* L.) cultivar Giza 40 used in this study, was obtained from Al-Mataana Research Station in Luxor.

Effect of some plant water extracts on the linear growth of *F. solani in vitro*:

The toxic effects of several plant water extracts, namely, leaves of moringa (*Moringa oleifera*), seeds of clove (*Syzygium aromaticum*) roots of ginger (*Zingiber officinale*), seeds of black cumin (*Nigella sativa L.*), and seeds of black pepper (*Piper nigrum L.*) on the mycelial linear growth of *F.solani* investigated. To create a fine powder, an electric blender was used to puree each dry portion of the used plant for five minutes. Then, 100 g of each plant's powder was stirred for an hour in 1000 ml of heated sterile distilled water (SDW) at 50 °C before being centrifuged at 1453 g and 5 °C for 10 minutes to create the plant extracts. When ready for use, the extract supernatant was sterilized by filtration through a 0.22 µm millipore filter and stored in sterilized dark bottles at 5 °C. Before the medium solidified, each plant extract was added to a warm (45 °C) sterilized potato dextrose agar PDA (potato 200g, sucrose 20g, agar 20g, distilled water 1000 ml) medium to get the final concentrations of 1, 3, and 5%. The control was PDA medium amended with SDW instead of plant extracts. The media were poured into 9.0 cm Petri plates, and the plates were inoculated in the center with 5 mm discs of *F.solani* obtained from the 7-day-old culture. Then plates were incubated at 28±1 ℃ till the control plates have completely covered with mycelium. Inoculated plates without plant extracts served as a control, and four plates (replicates) were used for each treatment. The

diameter of mycelial linear growth (cm) was then measured.

Effect of some selected plant water extracts on the infection with *F. solani* under greenhouse conditions:

The following experiments were conducted in the open greenhouse at the Experimental Farm, Faculty of Agriculture, Sohag, University, during the 2021/2022 and 2022/2023 growing seasons. The sowing date of both seasons was the 15th of October. Effect of plant extract, moringa, clove, ginger, black cumin, and black pepper on the incidence of root-rot disease of faba bean was studied under greenhouse conditions. *F. solani* was used in the present experiment as a model of faba bean root-rot pathogens. The loamy soil was individually infected (at a rate of 5% w:w) with the tested fungus inoculum that had previously been grown for 2 weeks on sandy barley medium (1:1, w:w and 40% water) at 28 ± 1 °C was placed in soil a week before planting. Sodium hypochlorite (2%) was used to disinfect the surface of faba bean seeds (Cultivar Giza 40), which were then rinsed multiple times with sterile water and dried between two sterile layers of filter paper. The purified faba bean seeds were coated with a different tested plant extract at a concentration of 5%. The seed coating process was carried out by placing the tested plant extract on seeds in polyethylene bags and shaking well to ensure even distribution of the additives. After treatment, the treated seeds were placed on a plastic tray to air dry. In plastic pots (25 cm in diameter) filled with variously contaminated soils, treated faba bean seeds appropriate to the particular treatment were sowed. Four identical pots were utilized for each type of treatment, and each pot contained six faba bean seeds. Untreated pots with plant extract were used as the control. Disease severity index (DSI) is calculated after 60 days.

For each replicate a DSI similar to that one described by Liu *et al*.1995 was calculated as follows:

$$\mathbf{DSI} = \frac{\boldsymbol{\Sigma d}}{\boldsymbol{d}\,\mathbf{max} \times \boldsymbol{n}} \times \mathbf{100}$$

Where d is, the disease rating possible, d max is the maximum disease rating and n, is the total number of plants examined in each replicate.

Effect of some selected plant water extracts on the infection with *F. solani* under field conditions:

The following experiments were conducted in the field at the Experimental Farm, Faculty of Agriculture, Sohag, University, during the 2021/2022 and 2022/2023 growing seasons. The sowing date of both seasons was the 15th of October. The experimental design was a complete randomized block with three replicates. The experimental was established which consisted of 3.5 × 3 m plots. Each unit included 4 rows; each row was 3.5 m in length and 60 cm width, 20 cm apart space between hills, and 10 hills in each row. One week prior to planting, *F. solani* inoculum (about 10 g) was placed in each hill. The purified faba bean seeds were coated with a different tested plant extract at a concentration of 5%. The seed coating process was carried out by placing the tested plant seeds in polyethylene bags and shaking well to ensure even distribution of the additives. The rate of two seeds per hills. DSI is calculated after 60 days.

Statistical Analysis:

All collected data were subjected to statistical analysis for each season and to combined analysis over years according to Gomez and Gomez (1984).

RESULTS

Effect of some plant water extracts on the linear growth of *F. solani in vitro*:

Effects of water extracts from moringa, clove, black cumin, ginger and black pepper at 0, 1, 3, and 5% on the mycelial linear growth of *F. solani* were investigated *in vitro*. Results represented in Table (1) and Figure (1) shows that all tested plant water extracts significantly reduced the mycelial linear growth of *F. solani* at 3 and 5% of tested concentrations. In addition, the inhibitory effects increased gradually by increasing the concentration of each plant water extract tested. The highest inhibitory effect on the growth of the fungus *F. solani* was found in

the water extract of moringa, cloves, and black cumin at a rate of 5%. They caused the highest reduction in the linear growth of *F.solani* reaching 0 cm, 0cm, and 0cm, respectively, compared to the control. In contrast, ginger water extract followed by black pepper 5% caused the lowest effect. It has an inhibitory effect and reduces the linear growth of *F. solani*, reaching 2.6 cm and 3 cm, respectively, compared to control.

Table 1: Effect of some plant water extracts on the mycelium growth of *F .solani in vitro*:

Plant water extract	Concentration (%)	Mycelium linear growth(cm)
Moringa	0 (control)	9.0[a]
	1	6.5[d]
	3	4.2[c]
	5	0[c]
	Mean	4.9
Clove	0 (control)	9.0[a]
	1	6.7[d]
	3	5.1[b]
	5	0[c]
	Mean	5.2
Black cumin	0 (control)	9.0[a]
	1	7.3[c]
	3	5.2[b]
	5	0[c]
	Mean	5.4
Ginger	0 (control)	9.0[a]
	1	8.3[b]
	3	7[a]
	5	2.6[b]
	Mean	6.7
Black pepper	0 (control)	9.0[a]
	1	8.7[a]
	3	7.3[a]
	5	3[a]
	Mean	7

Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test at 5%.

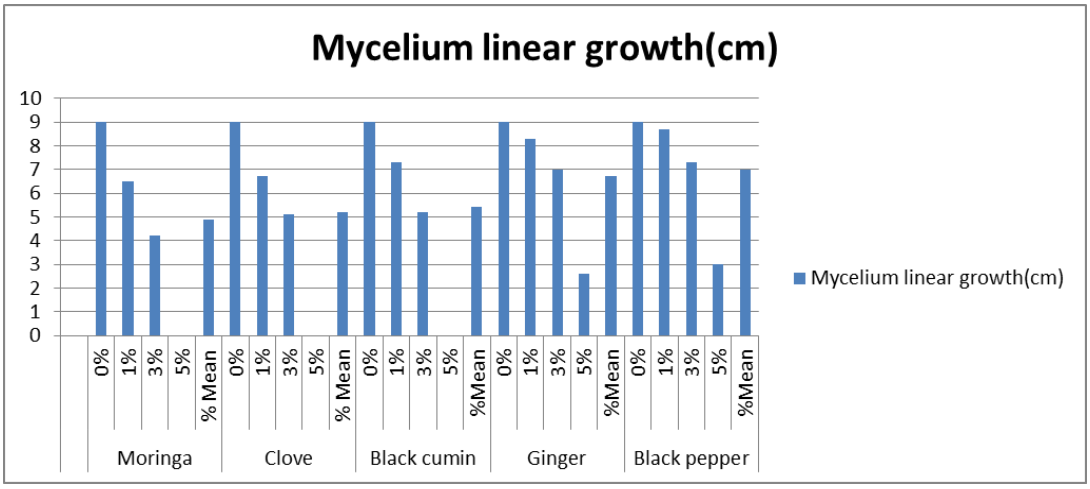

Fig. 1: Effect of some plant water extracts on the mycelial growth of *F. solani in vitro*.

Effect of some selected plant water extracts on the infection with *F. solani* under greenhouse conditions:

Effects of water extract from moringa, clove, black pepper, ginger, and black cumin at 5% on the infection with *F. solani* under greenhouse and development of root- rot disease. Data presented in Table (2) and Figure (2) show that the plant water extracts significantly varied in their effect on the infection with *F. solani*, causing root- rot disease in faba bean plants. The highest efficiency was detected for moringa, clove and black cumin water extracts, where it caused reduction in DSI of root- rot, reaching as the two-year average (14.99, 18.28 and 24.16%) respectively, compared with 86.57% in control. On the other hand, ginger and black pepper 5% water extracts caused the least efficacy as it caused root -rot with an average DSI rate over the two years (37.49% and 45 %), respectively.

Table 2: Effect of selected plant water extracts on the infection with *F. solani* under greenhouse conditions during the 2021/2022 and 2022/2023 growing seasons and development of root- rot disease:

Plant water extract	DSI 2021%	DSI 2022%	Mean%
Moringa	16.65^{d}	13.33^{d}	14.99^{c}
Clove	19.91^{cd}	16.66^{d}	18.28^{c}
Black cumin	23.33^{cd}	24.99^{cd}	24.16^{c}
Ginger	33.33^{cb}	41.66^{bc}	37.49^{b}
Black pepper	41.67^{b}	48.33^{b}	45^{b}
control	88.17^{a}	84.99^{a}	86.57^{a}

Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test at 5%.

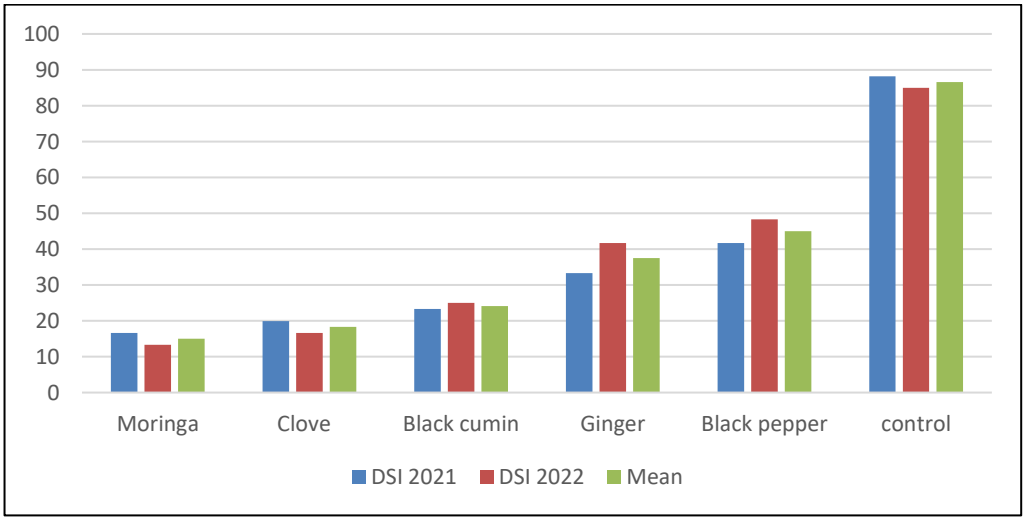

Fig. 2: Effect of selected plant water extracts on the infection with *F. solani* under greenhouse conditions during the 2021/2022 and 2022/2023 growing seasons and development of root- rot disease.

Effect of some selected plant water extracts on the infection with *F. solani* under field conditions:

Effects of water extract from moringa, clove, black pepper, ginger, and black cumin at 5% on the infection with *F. solani* under field conditions and development of root- rot disease. Data presented in Table (3) and Figure (3) show that the plant water extracts significantly varied in their effect on the infection with *F. solani*, causing root -rot disease in faba bean plants. The highest efficiency was detected for moringa, clove and black cumin water extract, where it caused reduction in DSI of root- rot, reaching as the two-year average (23.12, 25 and 31.25 %, respectively), compared with 87.49% in control. On the other hand, ginger and black pepper water extract 5% caused the least efficacy as it caused root rot with an average DSI rate over the two years (46.67% and 54.38 %, respectively).

Table 3: Effect of specific plant water extracts on the development of root- rot disease and *F. solani* infection under field conditions in 2021–2022 and 2022–2023.

Plant water extract	DSI 2021%	DSI 2022%	Mean%
Moringa	25.41^{c}	20.83^{c}	23.12^{e}
Clove	27.29^{c}	22.71^{c}	25^{e}
Black cumin	33.33^{c}	29.17^{c}	31.25^{d}
Ginger	45.63^{b}	47.71^{b}	46.67^{c}
Black pepper	51.88^{b}	56.88^{b}	54.38^{b}
control	89.16^{a}	85.83^{a}	87.49^{a}

Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test at 5%.

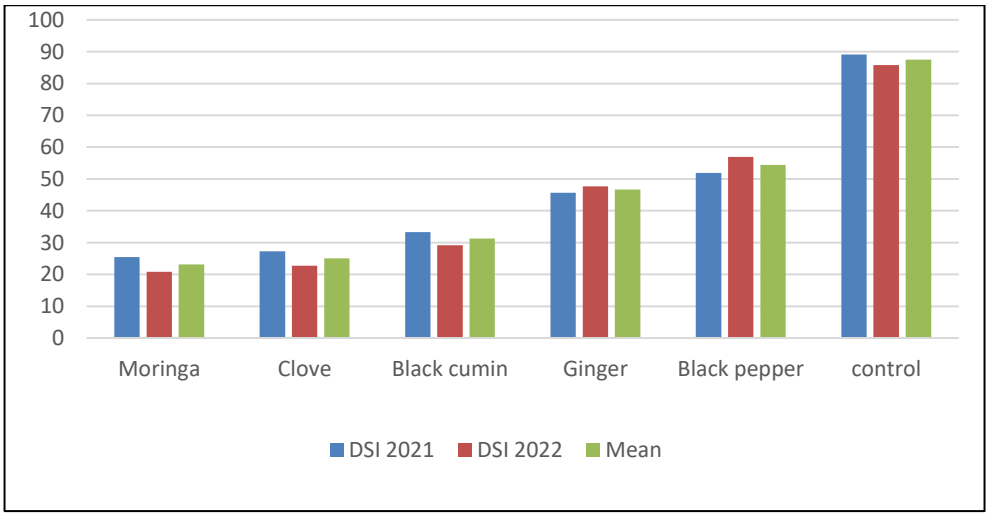

Fig.3: Effect of specific plant water extracts on the development of root- rot disease *and F. solani* infection under field conditions in 2021–2022 and 2022–2023 growing seasons.

DISCUSSION

One of the most significant crops of legumes is the faba bean *(Vicia faba* L.). The most significant fungal diseases affecting faba bean productions in Egypt are damping-off, root- rot, and wilt diseases. This causes significant output losses (Abdel-Kader *et al*., 2011).

In vitro, the effects of some plant water extracts tested at different concentrations on the mycelial linear growth of *F. solani* were also investigated. In this study, all tested plant water extracts significantly reduced the mycelial linear growth of tested fungi at 5% of concentration. The three aqueous extracts, moringa, cloves, and black cumin, had the highest inhibitory effect on fungal growth, followed by the ginger aqueous extract, then black pepper at a concentration of 5%. These results are in agreement with that obtained by (El-Korashy, 1997; O'gera *et al*., 2000) which mentioned that differences in the toxicity of the different plant extracts against *F. solani* might be due to the presence of antifungal substances such as alkaloids, essential oils, and other compounds that have antifungal activity. Under greenhouse and field conditions the application of the tested plant extract against *F.solani* gave positive results in reduction root- rot. In this study, applying plant water extracts of moringa, clove, black pepper, ginger and black cumin at 5% to faba bean plants before planting and after inoculation with *F. solani*. The highest efficiency was detected for moringa, clove and black cumin water extract, where it caused reduction in DSI of root- rot. Accrording to (Kagale *et al*., 2004; Guleria and Kumar, 2006 and Aboellil, 2007) which mentioned that treatment of some plants with plant extracts provided a control of many fungal diseases through metabolic changes in plants including induction of phenol biosynthesis enzymes, antioxidant defensive enzymes and phenol accumulation. These results are also consistent with those reported by (Jadhav *et al*., 1997) Which mentioned that due to the release of numerous chemical substances known as "allelopathy," some plants have an effect on the growth and/or development of others. These results are similar to those obtained by Goss *et al.* (2017) shown that the antifungal activities of moringa leaf and seed extracts prevent the growth of *R. solani* and *F. solani*. Data reveal that clove extract at different concentrations significantly reduced the percentage of the disease incidence in greenhouse and field trials. These data were in harmony with Huda, Ahmed, and Bayounis (2019) and Kamei *et al.*, (2022), who shown that clove water extracts were the most effective for battling soil-borne fungi like *F. oxysporum*. Additionally, other researchers discovered that the antifungal properties of some natural plant products decreased plant pathogens while increasing oxidative enzymes in plants. Oxidative enzymes can play a significant role in a plant's ability to resist disease infection, which in turn increases growth parameters and seed yield (Abdel-Monaim *et al*., 2011).

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تأثير بعض المستخلصات المائية النباتية على الأصابة بفطر *Fusarium solani* المسبب لمرض عفن جذورالفول البلدى

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الملخص العربى

أظهرت تأثير بعض المستخلصات المائية النباتية للمورينجا، القرنفل، الحبة السوداء، الجنزبيل، الفلفل الأسود عند تركيز 1،3،5% على النمو الطولى لميسيليوم الفطر المسبب *F. solani* فى المعمل أن كل المستخلصات النباتية المستخدمة أدت الى إختزال النمو الطولى لميسليوم الفطر عند 3،5 % من التركيزات المختبرة. بالأضافة لذلك زادت التأثيرات التثبيطية تدريجيا بزيادة التركيزات للمستخلصات المائية للنباتات المختبرة وتم تسجيل أعلى تأثير تثبيطى لنمو ميسليوم الفطر عند تركيز 5%. وتم أستخدام تركيز 5% من هذه المستخلصات فى الصوبة والحقل. حيث كان مستخلص المورينجا الأكثر كفاءة فى تثبيط الفطر فى المعمل وتقليل معامل شدة الاصابة فى الصوبة والحقل يلية مستخلص القرنفل يلية مستخلص الحبة السوداء وكان الأقل كفاءة مستخلص الجنزبيل يلية الفلفل الأسود.