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Effectiveness of Plant Extracts as Safe Control Means against Damping-off and Root Rot Diseases in Faba Bean Plants

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Faba bean root rot caused by *Fusarium solani* and *Rhizoctonia solani* is one of the most important biotic stresses in the major growing areas in Egypt. In this study, aqueous extracts and organic solvent extracts of three wild medicinal plants (*Lawsonia inermis*, *Eugenia jambolana* and *Moringa oleifera*) were evaluated *in vitro* and *in vivo* for protection of faba bean plants against damping-off and root rot diseases. Under laboratory conditions, all aqueous and organic solvent extracts significantly suppressed linear growth of *F. solani* and *R. solani*. *E. jambolana* extracts recorded the highest inhibition to the linear growth of both pathogenic tested fungi. Also, organic solvent extracts were more active than aqueous extracts of all tested plants. Under greenhouse and field conditions, all tested extracts significantly reduced damping-off and root rot incidence as well as increased some crop parameters in the field compared with untreated control. Faba bean seeds treated with *E. jambolana* extracts gave the highest protection against damping-off and root rot as well as recorded the highest crop components compared to the other extracts. In physiological studies, activity of defense-related enzymes, including peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD) and catalase (CAT), as well as content of phenolic compounds were increased in plants treated with aqueous or organic solvent extracts grown in soil infested with any of *F. solani* and *R. solani*, compared with untreated plants (control). In general, *E. jambolana* extracts recorded the highest increase in the activity of all enzymes as well as the content of total phenolic compounds. The organic solvent extracts increased the activity of all assessed enzymes and total phenolic compounds more than aqueous extracts of all tested plants. These results suggested that aqueous and organic solvent extracts may play an important role for controlling faba bean damping-off and root rot diseases.

Keywords: Crop parameters, damping-off, faba bean, oxidative enzymes, plant extracts, and total phenolic compounds.

Faba beans are among the oldest crops in the world. The Chinese used them for food almost 5,000 years ago; they were cultivated by the Egyptians 3,000 years ago and a little later by the Greeks and Romans. Today, faba bean is a major crop in many countries including China, Ethiopia and Egypt and is widely grown for human food throughout the Mediterranean region and in parts of Latin America (Singh *et al.*, 2013). Moreover, faba bean is the most important leguminous crop used for human nutrition in Egypt and is an excellent source of protein, calcium, phosphorus, lysine, methionine, cystein, crabohydrates, dietary fibers, choline, lecithin, and

minerals (Wang *et al*., 2014). However, there are many soil-borne fungi which are able to attack faba bean plants in different growth stages causing damping-off, root rot, collar rot and wilt diseases. Soil borne fungal pathogens, *i.e*. *F. solani* and *R. solani* are considered the most important pathogens that cause serious diseases, *i.e.* damping-off and root-rot diseases, which affect crop productivity by reducing both quality and quantity of the yield (Abdel-Monaim, 2013). There are many methods which are presently being used to control various plant pathogens including root rot pathogens such as physical, chemical, biological, cultural etc., but to some extent effective control of plant diseases is generally achieved by the use of fungicides. Due to increased awareness about the risks involved in the use of pesticides, much attention is being focused on the alternative methods of pathogen control. The recurrent and indiscriminate use of fungicides have posed a serious threat to human health and to the existing human ecogeographical conditions as some of them have already proved to be either mutagenic, carcinogenic or tetretogenic (Sadda and Varma, 2015). Moreover, the spiralling up cost of chemical fungicides particularly in the countries, where pesticides are imported; pollution to soil, ground water and air by the accumulation of obnoxious chemicals residues due to continuous use of fungicides. in addition, development of resistance races to these chemicals are therefore now facing the scientists to look for methods which are ecologically, friendly, safe and specific for pathogens (Joseph *et al*., 2008).

Using of some plant products in plant disease control seems to be an effective method to control many plant diseases. Aqueous and organic solvent extracts of several plants showed inhibitory effects against the cause of damping-off and root rot diseases *in vitro* and *in vivo* (Abded-Monaim *et al*., 2011). Plant extracts are considered as a mixture of various compounds that have the ability to induce systemic resistance (ISR) (Kagale *et al*., 2011). To date, extracts of at least few plant species have been reported to contain allelopathic substances that can act as elicitors and induce systemic resistance in host plants resulting in reduction or inhibition of disease development. Also, induce accumulations of pathogenesis related proteins (PR-proteins) in many plants (Kagale *et al*., 2011). 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 (Kagale *et al*., 2004; Guleria and Kumar, 2006 and Aboellil, 2007).

The present investigation aims to evaluate the effectiveness of certain plant extracts against the fungal growth *in vitro* and suppression of damping-off and root rot diseases of faba bean under greenhouse and field conditions. Also, investigate the effect of these treatments on some crop parameters and protein content in faba bean seeds under field conditions. The work was expanded to evaluate the activity of oxidative reductive enzymes and accumulation of phenolic compounds in faba bean plants.

Materials and Methods

Source of faba bean seeds and growth of plants:

Seeds of Faba bean (*Vicia fabae* L.) cultivar Misr 1 used in this study were obtained from Legume Crop Res. Dep., Field Crop Res. Inst., Agric. Res. Center, Ministry of Agric. and Land Reclamation, Egypt. Seeds were planted in plastic pots (30 cm in diameter), filled with a pasteurized mixture of soil and sand (4:1 w/w). Five seeds were sown in each pot and these pots were irrigated every three days.

Source of fungal pathogens:

Pathogenic isolates of *Fusarium solani* Snyder & Hansen and *Rhizoctonia solani* Kühn isolated from diseased faba bean plants collected from New Valley governorate were used in this study (Abdel-Monaim, 2013). The fungi were identified on the basis of cultural properties and microscopic morphological characters according to Sneh *et al*. (1991) and Leslie and Summerell (2006). Subcultures of the obtained isolates were kept in PDA slants and stored at 5 ±1°C for further studies.

Preparation of fungal inoculum

The inocula of *F. solani* and *R. solani* isolates were prepared from one-week old culture grown on 50 ml potato dextrose broth (PDB) medium in conical flasks (250 ml) and incubated at 25 ± 1°C. The content of each flask was homogenized in a blender for one min. Plastic pots were filled with pasteurized soil and mixed with the fungal inoculum at the rate of 100 ml homogenized culture per pot, seven days before sowing (Abdel-Monaim, 2013).

Preparation of plant extracts:

A. Aqueous plant extracts:

Plants belonging to three species in Table 1, were collected from different localities in El-Kharga, New Valley governorate. The selected leaves of different plants were cut into small pieces and washed several times with running tap water, then washed with sterile water and dried at room temperature (≈25 °C) for 15 days. Plant materials were ground to fine powders in a grinder, then 100 g of each one were blended in 1 L of distilled water for 48 h. The macerated materials were squeezed through double cheesecloth sheets and then filtered through a Whitman No. 1 filter paper. The fresh extracts were applied just after preparation at the rate of 20% from the original ones (Abdel-Monaim *et al*., 2011).

Table 1. English name, family and scientific name of the tested plants

English name	Family name	Scientific name
Henna	Lythraceae	*Lawsonia inermis*
Stopper	Myrtaceae	*Eugenia jambolana* (Syn. *Syzygium cumini* or *Syzygium jambolana* or *Eugenia cuminii*)
Moringa	Moringaceae	*Moringa oleifera*

B. Solvents of plant extracts:

In this experiment, some organic solvents, *i.e.* ether, ethanol and acetone were used for preparation of plant extracts. The method was the same to the aqueous plant extracts except using organic solvents instead of distilled water. The extracted materials with each of the solvents were concentrated with rotary vacuum evaporator at 50°C for 6 hrs. The final concentration was prepared as those of water extracts (20%) by adding water for each organic solvent extract (Abdel-Monaim *et al*., 2011).

Evaluation of plant extracts:

A. In vitro antifungal activity:

Ten milliliters of aqueous and/or organic solvent extract prepared from any of the plant samples were mixed with 40 ml PDA medium (20%), poured into sterilized Petri plates at 15 ml/plate and allowed to solidification. The plates were then inoculated with disk (9-mm in diameter) of 10-day old culture of any of *F. solani* and *R. solani* grown on PDA and incubated at 25 ±1 °C. The percentage of radial growth inhibition of the pathogen was calculated. Each treatment was replicated three times with five plates per replicate. The mycelial growth inhibition (%) of colony diameter grown on each extract was calculated as follows:

$$\text{Mycelial growth inhibition (\%)} = 100\ (C\text{-}T / C)$$

Where, C= growth in control and T = growth in treatment.

B. Under greenhouse conditions:

The fungal inocula of *F. solani* and *R. solani* were prepared as described before. Plastic pots (30 cm diameter) were packed with pasteurized sandy clay soil infested with fungal inocula at the rate of 100 ml homogenized culture per pot, seven days before planting. Disinfested faba bean seeds (cv. Misr 1) were soaked in the solution of each extract (aqueous or organic solvent extracts) for 12h (Somda *et al*., 2007), then sown in infested soil at the rate of 5 seeds/pot. While in control treatment, faba bean seeds soaked in water for the same time were sown in infested soil with the pathogen at the same rate. Four pots were used per treatment as replicates. Percentage of damping-off was recorded 30 days after planting. Moreover, severity of root rot was determined 90 days after planting using a rating scale of 0 to 5 on the basis of root discoloration or leaf yellowing as follows: 0, neither root discoloration nor leaf yellowing; 1= from <0 to25% root discoloration or one leaf yellowed; 2= from <25 to 50% root discoloration or more than one leaf yellowed; 3= from <50 to 75% root discoloration plus one leaf wilted; 4= up to 75% root discoloration or more than one leaf wilted; and 5, completely dead plants. For each replicate, a disease severity index (DSI) similar to that suggested by Liu *et al*. (1995) was followed:

$$DS = \Sigma d/(d\ max \times n) \times 100$$

Whereas: 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.

C. Under field conditions:

This experiment was carried out in the Farm of El-Kharga Agric. Station, New Valley governorate during growing seasons of 2014/15 and 2015/16. The effect of water, ether, ethanol and acetone extracts of *L. inermis*, *E. jambolana* and *M. oleifera* leaves on incidence of damping-off and root rot diseases of faba bean was evaluated under natural infection as well as their effect on growth and some crop parameters under field conditions. Healthy faba bean (cv. Misr 1) seeds were soaked in the solutions of the selected extracts for 12h at the rate of 20% (Somda *et al*., 2007). All treatments were arranged in a complete randomized block design with three plots as replicates. A plot was 3 × 3.5 m with five rows; seeds were sown in hills 25 cm apart on both sides of the row ridge, with two seeds per hill. In the control treatment, faba bean seeds were soaked in water for the same time and sown with the same method. Percentages of damping-off and root rot disease index severity were calculated as well as some crop parameters, *i.e.* plant height (cm), number of branches, pods and seeds plant^{-1}, weight of 100 seeds (seed index) and total yield per feddan were recorded. Total crude protein (%) in faba bean seeds was determined. The previously determined nitrogen of dry seeds was used for calculating total crude protein by multiplying N- values by 6.25 (Anonymous, 2000).

Biochemical changes in faba bean plants due to plant extract treatments

After 15 days from planting, 1 g fresh samples were taken from treated and untreated faba bean plants grown in soil infested and un-infested with *F. solani* and *R. solani* individually then extracted according to Maxwell and Bateman (1967). Then the extracts were used for assaying biochemical changes associated with the tested treatments of plant extracts, the activities of peroxidase enzyme (Hammerschmidt *et al*., 1982), polyphenoloxidase enzyme (Gauillard *et al*., 1993), phenylalanine ammonia lyase enzyme (Cavalcanti *et al*., 2007), superoxide dismutase (Dhindsa *et al*., 1981) and catalase (Aebi, 1984) were determined.

Protein content:

Total protein content of the samples was quantified according to the method described by Bradford (1976).

Determination of phenolic compounds:

To assess phenolic content, 1 g fresh plant sample was homogenized in 10 ml 80% methanol and agitated for 15 min. at 70°C. One ml of the extract was added to 5 ml of distilled water and 250 µl of 1 N Folin-Ciocalteau reagent and the solution was kept at 25°C. The absorbance was measured with a spectrophotometer at 725 nm. Catechol was used as a standard. The amount of phenolic content was expressed as phenol equivalents in µg/ gm fresh tissue (Saikia *et al*., 2006).

Statistical analysis:

All experiments were performed twice. Analyses of variance were carried out using MSTAT-C program version 2.10 (Anonymous, 1991). Least significant difference (LSD) was employed to test for significant difference between treatments at $P \leq 0.05$ (Gomez and Gomez, 1984).

Results

In vitro antifungal activity:

The obtained results show that all the tested extracts suppressed growth of *F. solani* (Fig. 1) and *R. solani* (Fig. 2) with different percentages. *E. jambolana* extracts gave the highest inhibition percentage for both fungi more than *L. inermis* and *M. oleifera* extracts. On the other hand, the organic solvent extracts of all tested plants highly suppressed both the pathogenic fungi more than aqueous extracts. Acetone extract recorded the highest inhibition of radial growth of both tested pathogenic fungi.

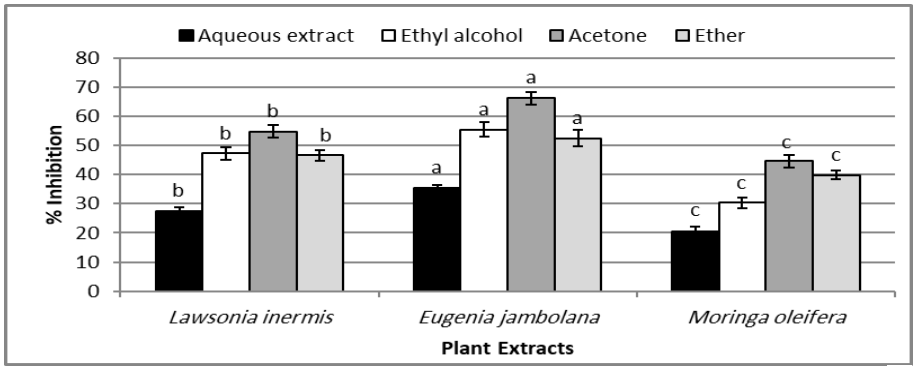

Fig. 1. *In vitro* effect of aqueous and organic solvent extracts of *L. inermis*, *E. jambolana* and *M. oleifera* individually on *F. solani* growth. Different letters indicate significant differences among treatments within the same color column according to the least significant difference test (P ≤ 0.05). Bars indicate the standard deviation.

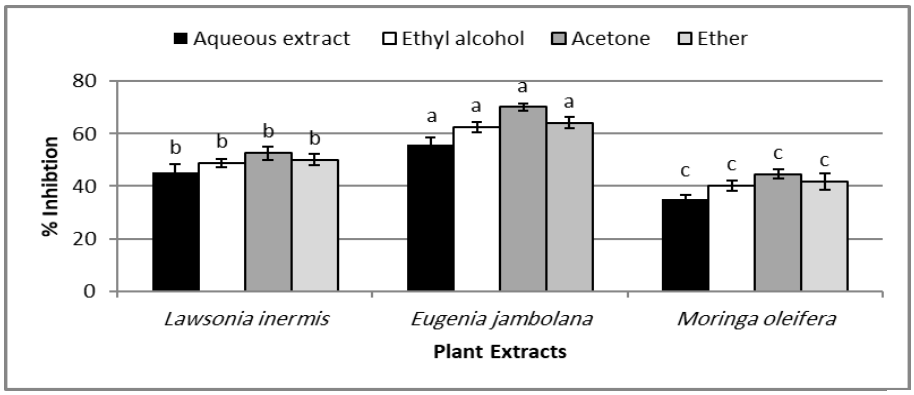

Fig. 2. *In vitro* effect of aqueous and organic solvent extracts of *L. inermis*, *E. jambolana* and *M. oleifera* individually on *Rhizoctonia solani* growth. Different letters indicate significant differences among treatments within the same color column according to the least significant difference test (P ≤ 0.05). Bars indicate the standard deviation.

Greenhouse experiment:

The efficacy of aqueous and organic solvent extracts of *L. inermis*, *E. jambolana*, *M. oleifera* against damping-off and root rot diseases caused by *F. solani* and *R. solani* of faba bean is shown in Table (2). Faba bean seeds treated with any the tested plant extracts recorded significant reduction of damping-off and root rot incidence caused by the two tested fungi compared with untreated seeds (control) in pots. The extracts of *E. jambolana* recorded the highest reduction in both damping-off and root rot diseases caused by any of the tested fungi followed by *L. inermis* extract. Meanwhile, *M. oleifera* extract recorded the lowest protection against damping-off and root rot incidence in this respect. On the other hand, the organic solvent extracts of all tested plants suppressed damping-off and root rot incidence more than aqueous extracts. Acetone and ether extracts of all tested plants were more effective for suppressing damping-off and root rot incidence than aqueous and Ethanol extracts.

Table 2. Effect of seed soaking in aqueous and organic solvent plant extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on the incidence of faba bean damping-off and root rot diseases caused by *F. solani* and *R. solani* under greenhouse conditions

Source of plant extract	Solvent type	*F. solani*		*R. solani*	
		% Damping-off	% Root rot	% Damping-off	% Root rot
L. inermis	Aqueous extract	20.0	23.75	25.0	15.75
	Ethanol	20.0	18.75	15.0	11.25
	Acetone	10.0	12.25	10.0	6.25
	Ether	15.0	14.25	10.0	8.75
	Mean	16.3	17.25	15.0	10.50
E. jambolana	Aqueous extract	15.0	18.25	15.0	10.00
	Ethanol	10.0	14.25	10.0	7.25
	Acetone	5.0	5.75	5.0	4.00
	Ether	5.0	10.75	10.0	5.00
	Mean	8.8	12.25	10.0	6.56
M. oleifera	Aqueous extract	25.0	27.25	30.0	17.75
	Ethanol	20.0	24.25	20.0	12.25
	Acetone	15.0	13.75	10.0	6.75
	Ether	20.0	17.00	10.0	8.25
	Mean	20.0	20.56	17.5	11.25
Control		45.0	40.25	60.0	30.33
LSD at 0.05 for:					
Plant extracts (A) =		2.08	0.98	2.08	0.99
Solvent types (B) =		3.57	1.28	3.09	1.16
Interaction (A×B) =		6.19	2.22	5.35	2.01

Field experiments:

Soaking faba bean seeds in the aqueous and organic solvent extracts (Ethanol, acetone and ether) of *L. inermis, M. oleifera* and *E. jambolana* leaves before planting resulted in significant reduction of damping-off and root rot diseases incidence in the field during seasons of 2014/2015 and 2015/2016 as compared with the control (Table 3). Extracts of *E. jambolana* leaves and those of *M. oleifera* showed the lowest percentage of damping-off and root rot incidence without significant differences in both growing seasons and acetone extract was the most efficient one in both growing seasons, being 4.67 and 3.33% damping-off and 2.75 and 3.26% root rot in growing seasons 2014/15 and 2015/16, respectively compared with the other extracts.

Table 3. Effect of seed soaking in aqueous and organic solvent extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on the incidence of faba bean damping-off and root rot diseases under field conditions, during 2014-2015 and 2015-2016 growing seasons

Source of plant extract	Solvent type	Season 2014-2015		Season 2015-2016	
		% Damping-off	% Root rot	% Damping-off	% Root rot
L. inermis	Aqueous extract	15.00	8.33	17.33	10.26
	Ethanol	12.00	7.52	13.67	8.00
	Acetone	8.67	5.49	10.00	5.00
	Ether	10.33	6.96	12.67	7.67
	Mean	11.50	7.08	13.42	7.73
E. jambolana	Aqueous extract	8.00	6.67	10.67	7.96
	Ethanol	8.67	4.29	6.67	5.49
	Acetone	4.67	2.75	3.33	3.26
	Ether	8.67	5.49	10.00	4.67
	Mean	7.50	4.80	7.67	5.35
M. oleifera	Aqueous extract	12.00	7.26	14.33	8.49
	Ethanol	10.33	5.78	12.33	5.26
	Acetone	6.33	6.67	7.67	7.25
	Ether	11.67	8.29	13.67	9.67
	Mean	10.08	7.00	12.00	7.67
Control		30.00	17.45	35.26	15.78
LSD at 0.05 for:					
Plant extracts (A) =		2.20	1.18	2.93	1.78
Solvent types (B) =		0.84	0.53	1.08	0.60
Interaction (A×B) =		3.02	1.72	4.09	2.43

Effect on some parameters and protein contents:

Data in Tables 4 and 5 indicate that faba bean seeds treated with any of the tested extracts significantly increased plant growth (plant height and number of branches/ plant) and yield component (No. of pods/plant, No. of seeds /plant, seed index, total yield /feddan) as well as protein content in the seeds during 2014/15 and 2015/16 growing seasons compared with untreated seeds (control). *E. jambolana* extracts recorded the highest increase of plant growth and yield components in both growing seasons followed by *L. inermis* extracts then the extracts of *M. oleifera*. On the other hand, organic solvent extracts increased plant growth and yield components more than the aqueous extracts of all the tested plants in both growing seasons. Generally, acetone extract was more effective in this respect than other extracts, especially in case of *E. jambolana*. Also, the results recorded in season 2015-16 were nearly similar to those of 2014-15 growing season.

Table 4. Effect of soaking faba bean seeds in aqueous and organic solvent extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on some crop parameters and protein content under field conditions, during 2014-2015 growing season

Source of plant extract	Solvent type	Plant height (cm)	No. of branches /plant	No.of pods/ plant	No. of seeds/ plant	Seed index	Total yield (Kg/fed.)	Protein content %
L. inermis	Aqueous extract	99.30	3.50	17.40	50.30	76.90	1563.50	30.40
	Ethanol	110.20	4.10	20.60	60.90	78.20	1689.30	31.20
	Acetone	117.60	5.40	24.10	70.40	79.50	1875.30	31.40
	Ether	115.70	5.10	22.70	65.40	77.90	1715.80	30.90
	Mean	110.70	4.53	21.20	61.75	78.13	1710.98	30.98
E. jambolana	Aqueous extract	105.70	4.40	19.30	56.80	77.80	1725.60	30.20
	Ethanol	119.50	5.30	22.30	63.50	80.20	1836.90	31.40
	Acetone	124.60	6.10	27.60	80.10	81.40	2142.30	31.80
	Ether	118.40	5.70	25.00	70.20	80.00	2001.20	30.20
	Mean	117.05	5.38	23.55	67.65	79.85	1926.50	30.90
M. oleifera	Aqueous extract	90.70	3.20	15.90	40.90	76.50	1452.30	29.20
	Ethanol	100.40	4.00	17.50	48.20	78.50	1545.90	30.20
	Acetone	114.40	4.80	19.90	55.40	79.50	1648.90	31.00
	Ether	110.90	4.20	18.70	51.40	78.40	1548.90	30.70
	Mean	104.10	4.05	18.00	48.98	78.23	1549.00	30.28
Control		80.50	2.60	11.50	30.50	70.20	896.30	27.50
LSD at 0.05 for:								
Plant extracts(A) =		3.87	0.38	1.55	2.49	5.47	60.26	1.88
Solvent types(B) =		3.66	0.18	1.18	2.13	1.95	64.21	1.65
Interaction(A×B)=		7.12	0.53	2.75	4.72	7.02	130.21	3.24

Table 5. Effect of soaking faba bean seeds in aqueous and organic solvent extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on some crop parameters and protein content under field conditions, during 2015-2016 growing season

Source of plant extract	Solvent type	Plant height (cm)	No. of branches /plant	No. of pods /plant	No. of seeds/ plant	Seed index	Total yield (Kg/feddan)	Protein content (%)
L. inermis	Aqueous extract	106.80	3.20	18.40	47.20	75.90	1508.9	29.50
	Ethanol	112.40	4.20	21.60	59.60	77.40	1654.4	30.80
	Acetone	119.80	5.30	25.40	70.90	78.90	1825.4	31.00
	Ether	115.70	5.40	23.80	63.70	76.40	1689.7	29.70
	Mean	113.68	4.53	22.30	60.35	77.15	1669.6	30.25
E. jambolana	Aqueous extract	100.00	4.60	20.40	54.50	77.60	1731.8	30.40
	Ethanol	113.50	5.40	21.70	61.40	79.50	1802.6	31.20
	Acetone	122.40	6.00	28.50	79.30	80.20	2100.3	31.40
	Ether	116.80	5.80	26.70	68.70	79.10	1985.7	29.70
	Mean	113.18	5.45	24.33	65.98	79.10	1905.1	30.68
M. oleifera	Aqueous extract	93.50	3.30	16.00	38.90	75.40	1426.8	28.70
	Ethanol	102.40	4.20	17.40	48.70	77.40	1502.4	30.00
	Acetone	113.80	4.60	20.10	53.70	78.60	1625.7	30.70
	Ether	111.20	4.00	17.90	50.20	78.40	1504.3	30.40
	Mean	105.23	4.03	17.85	47.88	77.45	1514.8	29.95
Control		77.50	2.70	10.90	31.40	70.20	887.6	27.80
LSD at 0.05 for:								
Plant extracts (A) =		5.18	0.36	1.74	3.27	2.67	61.89	1.62
Solvent types (B) =		3.32	0.20	1.34	2.26	1.90	59.95	1.36
Interaction (A×B) =		9.21	0.58	3.12	5.62	4.62	125.42	3.21

Biochemical changes in faba bean plants treated with plant extracts:
Peroxidase activity:

Results in Table 6 clearly show that all tested extracts of the three plant species increased activity of peroxidase (PO) enzyme in faba bean plants developed from treated seeds planted in artificially infested soil with any of *F. solani* and *R. solani*, compared to the control. Plants treated with *E. jambolana* extracts recorded the highest increase followed by *L. inermis* extracts. The lowest values of PO enzyme were recorded in plants treated with *M. oleifera*. On the other hand, treatments of faba bean seeds with organic solvent extracts increased PO enzyme activity more than those treated with aqueous extracts. Acetone and ether extracts of all tested plants recorded the highest increase compared with aqueous and Ethanol extracts. Also, activity of PO was lower in plants grown in soil infested with *F. solani* more than those grown in soil infested with *R. solani* (Table 6).

Table 6. Effect of soaking faba bean seeds in aqueous and organic solvent extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on the activity of peroxidase in plants grown in artificially infested soil with any of *F. solani* or *R. solani* under greenhouse conditions

Source of plant Extract	Solvent type	PO activity (Enzyme unit min^{-1} mg $protein^{-1}$)			
		F. solani		*R. solani*	
		Activity	% Increase [a]	Activity	% Increase
L. inermis	Aqueous extract	0.988	54.86	1.096	45.74
	Ethanol	1.172	83.70	1.165	54.92
	Acetone	1.205	88.87	1.221	62.37
	Ether	1.288	101.88	1.263	67.95
E. jambolana	Aqueous extract	1.025	60.66	1.156	53.72
	Ethanol	1.196	87.46	1.259	67.42
	Acetone	1.251	96.08	1.349	79.39
	Ether	1.305	104.55	1.359	80.72
M. oleifera	Aqueous extract	0.901	41.22	1.025	36.30
	Ethanol	1.148	79.94	1.141	51.73
	Acetone	1.198	87.77	1.196	59.04
	Ether	1.263	97.96	1.215	61.57
Control		0.638	0.00	0.752	0.00

[a] Increase to untreated control plants

Polyphenoloxidase activity:

The activity of polyphenoloxidase (PPO) was increased in plants grown from seeds treated with any of the tested plant extracts compared with untreated seeds under artificial infestation with *F. solani* or *R. solani* (Table 7). *E. jambolana* followed by *L. inermis* extracts recorded the highest increase in activity (%) of PPO in plants grown in soil infested with any of the two tested fungi. While, *M. oleifera* extracts gave the lowest increase in this respect. Ether extract of all tested plant species recorded higher increase compared with the other extracts (aqueous, Ethanol and acetone) either in plants inoculated with *F. solani* or *R. solani*. Generally, ether extract of *E. jambolana* recorded the highest increase of PPO activity, being 65.05 and 61.16% increase of activity in case of soil infestation with *F. solani* and *R. solani*, respectively.

Phenyalalanine ammonia lyase activity:

The activity of phenyalalanine ammonia lyase (PAL) was increased due to treating faba bean seeds with aqueous and organic solvent (Ethanol, acetone, ether) extracts of *L. inermis, E. jambolana* and *M. oleifera* leaves of plants grown under artificially infested soil with any of *F. solani* or, *R. solani* (Table 8). The obtained data indicate that *E. jambolana* extracts recorded the highest increase in all types of extracts compared with those of *L. inermis* and *M. oleifera*. Organic solvent extracts increased activity of PAL enzyme more than aqueous extract in all tested plants. Also, acetone extracts of all tested plants recorded the highest increase in activity of PAL enzyme compared with the other types of extracts.

Table 7. Effect of soaking faba bean seeds in aqueous and organic solvent extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on the activity of polyphenoloxidase (PPO) in plants grown in soil infested with any of *F. solani* or *R. solani* under greenhouse conditions

Source of plant extract	Solvent type	PPO activity (Enzyme unit min^{-1} mg $protein^{-1}$)			
		F. solani		*R. solani*	
		Activity	% Increase	Activity	% Increase
L. inermis	Aqueous extract	1.028	24.30	1.148	18.60
	Ethanol	1.148	38.81	1.256	29.75
	Acetone	1.196	44.62	1.325	36.88
	Ether	1.251	51.27	1.345	38.95
E. jambolana	Aqueous extract	1.099	32.89	1.199	23.86
	Ethanol	1.189	43.77	1.298	34.09
	Acetone	1.254	51.63	1.396	44.21
	Ether	1.365	65.05	1.560	61.16
M. oleifera	Aqueous extract	1.000	20.92	1.025	5.89
	Ethanol	1.144	38.33	1.196	23.55
	Acetone	1.201	45.22	1.241	28.20
	Ether	1.224	48.00	1.428	47.52
Control		0.827	0.00	0.968	0.00

Table 8. Effect of soaking faba bean seeds in aqueous and organic solvent extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on the activity of Phenyalalanine ammonia lyase (PAL) in plants grown in soil infested with any of *F. solani* or *R. solani* under greenhouse conditions

Source of plant extract	Solvent type	PAL activity (Enzyme unit min^{-1} mg $protein^{-1}$)			
		F. solani		*R. solani*	
		Activity	% Increase	Activity	% Increase
L. inermis	Aqueous extract	4.396	30.87	4.256	36.19
	Ethanol	5.575	65.97	5.021	60.67
	Acetone	6.354	89.16	5.624	79.97
	Ether	6.124	82.32	5.472	75.10
E. jambolana	Aqueous extract	5.214	55.22	4.856	55.39
	Ethanol	5.895	75.50	5.635	80.32
	Acetone	6.742	100.71	6.256	100.19
	Ether	6.235	85.62	6.124	95.97
M. oleifera	Aqueous extract	4.214	25.45	3.968	26.98
	Ethanol	4.365	29.95	4.256	36.19
	Acetone	5.326	58.56	4.869	55.81
	Ether	5.124	52.55	4.789	53.25
Control		3.359	0.00	3.125	0.00

Superoxide dismutase activity:

Data presented in Table 9 indicate that all plant extracts either aqueous or organic solvent extracts of the tested plant species increased activity of superoxide dismutase (SOD) enzyme compared with untreated seeds (control) under artificial

soil infestation with any of *F. solani and R. solani*. Faba bean seeds treated with *E. jambolana* extract recorded the highest activity compared to the other tested plants under artificial soil infestation with the two tested fungi.

Table 9. Effect of soaking faba bean seeds in aqueous and organic solvent extracts of *L. inermis*, *E. jambolana* and *M. oleifera* individually on the activity of superoxide dismutase (SOD) in plants grown in soil infested with any of *F. solani* or *R. solani* under greenhouse conditions

Source of plant extract	Solvent type	SOD activity (Enzyme unit min^{-1} mg $protein^{-1}$)			
		F. solani		*R. solani*	
		Activity	% Increase	Activity	% Increase
L. inermis	Aqueous extract	6.589	44.24	8.268	32.61
	Ethanol	7.598	66.33	9.784	56.92
	Acetone	9.002	97.07	12.478	100.13
	Ether	10.472	129.25	11.457	83.75
E. jambolana	Aqueous extract	8.457	85.14	9.856	58.08
	Ethanol	10.247	124.32	10.478	68.05
	Acetone	11.240	146.06	13.427	115.35
	Ether	11.012	141.07	13.024	108.89
M. oleifera	Aqueous extract	7.012	53.50	7.986	28.08
	Ethanol	8.012	75.39	8.967	43.82
	Acetone	9.214	101.71	10.002	60.42
	Ether	10.427	128.26	11.424	83.22
Control		4.568	0.00	6.235	0.00

On the other hand, organic solvent extracts recorded the highest increase compared with aqueous extracts of all the tested plants under soil infestation with any of the two tested pathogenic fungi. Also, acetone extract of *E. jambolana* gave the highest activity of SOD enzyme, being 146.06 and 115.35% in case of soil infestation with *F. solani* or *R. solani*, respectively. The activity of SOD enzyme in plants grown in pots inoculated with *R. solani* was higher than those grown in soil infested with *F. solani*. In contrary, the increase of SOD enzyme activity was higher in case of *F. solani* than that of *R. solani* in all treatments.

Catalase activity:

Data presented in Table 10 show that all tested plant extracts increased activity of catalase enzyme (CA) compared with untreated seeds (control) under artificial inoculation with *R. solani* or *F. solani*. Aqueous extracts of all tested plants recorded the lowest increase in activity of the CA enzyme compared with the organic solvent extracts (Ethanol, acetone, ether) in all tested plants. The *E. jambolana* extracts recorded the highest increase of activity in both cases of inoculation with *R. solani* and *F. solani*. However, CA activity in faba bean plants grown in pots inoculated with *R. solani* was higher than the activity of the CA enzyme in plants grown in pots inoculated with *F. solani* in all tested plant extracts.

Table 10. Effect of soaking faba bean seeds in aqueous and organic solvent extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on the activity of catalase (CA) in plants grown in soil infested with any of *F. solani* or *R. solani* under greenhouse conditions

Source of plant extract	Solvent type	**CA** activity (µmoles H_2O_2 decomposed mg^{-1} protein min^{-1})			
		F. solani		*R. solani*	
		Activity	% Increase	Activity	% Increase
L. inermis	Aqueous extract	11.210	36.14	17.245	53.36
	Ethanol	12.412	50.74	18.256	62.35
	Acetone	13.421	62.99	21.142	88.01
	Ether	14.215	72.64	22.145	96.93
E. jambolana	Aqueous extract	12.475	51.51	18.241	62.21
	Ethanol	14.124	71.53	21.421	90.49
	Acetone	16.524	100.68	23.457	108.60
	Ether	16.523	100.67	24.124	114.53
M. oleifera	Aqueous extract	9.362	13.70	13.214	17.51
	Ethanol	10.214	24.05	14.256	26.78
	Acetone	11.245	36.57	16.547	47.15
	Ether	12.020	45.98	17.025	51.40
Control		8.234	0.00	11.245	0.00

Total phenol contents:

The content of total phenolic compounds was greatly increased in plants treated with different aqueous and organic solvent extracts, compared with untreated plants (Table 11). Maximum increase in total phenols was recorded in plants treated with *E. jambolana* under artificial soil infestation with any of *F. solani* and *R. solani*. The organic solvent extracts of all tested plants increased the phenolic contents in faba bean plants more than in the plants treated with aqueous extracts. Ether extracts of all tested plants recorded the highest increase of phenolic contents in faba bean plants grown in soil infested with any of the two pathogenic fungi compared with the other plant extracts.

Table 11. Effect of soaking faba bean seeds in aqueous and organic solvent extracts of *L. inermis, E. jambolana* and *M. oleifera* individually on phenolic compounds in plants grown in soil infested with any of *F. solani* and *R. solani* under greenhouse conditions

Source of plant extract	Solvent type	Total phenolic contents (µg gm^{-1} fresh weight)			
		F. solani		*R. solani*	
		TPC contents	% Increase	TPC contents	% Increase
L. inermis	Aqueous extract	2.312	17.66	2.656	32.01
	Ethanol	2.752	40.05	2.965	47.37
	Acetone	2.865	45.80	3.512	74.55
	Ether	3.021	53.74	3.968	97.22
E. jambolana	Aqueous extract	2.546	29.57	3.324	65.21
	Ethanol	2.965	50.89	3.996	98.61
	Acetone	3.568	81.58	4.826	139.86
	Ether	3.985	102.80	4.965	146.77
M. oleifera	Aqueous extract	2.201	12.01	2.302	14.41
	Ethanol	2.513	27.89	2.614	29.92
	Acetone	2.825	43.77	2.954	46.82
	Ether	2.968	51.04	3.324	65.21
Control		1.965	0.00	2.012	0.00

D i s c u s s i o n

Plant extracts may contain natural antimicrobial compounds, and these can be used for seed disinfection as an alternative to fungicide treatments. Using of plant extracts against plant pathogenic fungi is relatively a recent approach. In this study, aqueous and organic solvent extracts (Ethanol, acetone and ether) of *L. inermis*, *E. jambolana* and *M. oleifera* were evaluated for controlling damping-off and root rot diseases caused by *F. solani* and *R. solani* in faba bean plants *in vitro* and *in vivo*.

The obtained data indicated that all the tested plant extracts significantly reduced the linear growth of the tested fungi *in vitro*. Organic solvent extracts were more effective for suppressing linear growth of both tested pathogenic fungi than aqueous extracts of all tested plant species. *E. jambolana* extracts recorded the highest reduction in growth of *F. solani* and *R. solani*.

Also, under greenhouse and field conditions, faba bean cv. Misr 1 treated with any of the tested extracts of the three plant species significantly reduced damping-off and root rot incidence compared with untreated seeds (control) as well as they increased plant growth (plant height and No. of branches/plant) and yield components (No. of pods/plant, No. of seeds/plant, seed index, seed yield weight /feddan and protein content in seeds) during growing seasons of 2014-15 and 2015-2016. *E. jambolana* extracts caused the highest reduction of damping-off and root

rot incidence and gave the highest increase in plant growth and yield components compared with the other two tested plants. On the other hand, organic solvent extracts were more effective than aqueous extracts for all the tested plant species under greenhouse and field conditions.

This may be due to the solvent that has potential to extract the different constituents that having antimicrobial activity. The inhibitory effect of the tested extracts might be due to natural bioactive materials present in these extracts (Abdel-Monaim *et al*., 2011 and Atta *et al*., 2013). In this respect, the hydroalcoholic extract of *E. jambolana* leaves is shown to possess antifungal effects against *Candida albicans* and *C. krusei* (De Oliveira *et al*., 2007). The aqueous and methanolic seed extracts inhibited growth of dermatophytic fungi *C. albicans*, *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum* (Chandrasekaran and Venkatesalu 2004). The aqueous, ethanol and n-hexane extracts from the leaves, fruits, root-bark and stem-bark possess growth inhibitory effects on *Ascochyta rabiei*, the causative agent of blight disease in chickpea (*Cicer arietinum* L.) suggesting its use as a non-toxic agent effective in preventing food infestation by fungi. The aqueous extracts of all the four *Syzygium cumini* (L.) parts showed significant antifungal activity (Jabeen and Javaid, 2010). However, the essential oil from the leaves of *E. jambolana* is shown to contain the phytochemicals pinocarveol, α-terpeneol, myrtenol, eucarvone, muurolol, α-myrtenal, cineole, geranyl acetone, α-cadinol and pinocarvone (Shafi *et al*., 2002). On the other hand, *L. inermis* (Henna) has been used for more than 4000 years as a cosmetic by Mediterranean, Middle Eastern, and Asian cultures. Aqueous extract of *L. inermis* (Henna) leaves was tested for antifungal activity against eight important isolated species of *Aspergillus* from sorghum, maize, and paddy seed samples. *A. flavus* showed high sensitivity toward henna (Raveesha *et al*., 2007). Ethanol, methanol, and aqueous extracts of *L. inermis* leaves were involved also in defensive mechanism against spore germination of *Drechslera oryzae* (Natarajan and Lalitha, 1987).

Some authors reported that the origin of the antimicrobial activity of *L. inermis* leaves to gallic acid or naphthoquinones (lawsone) (Cowan, 1999 and Ahmed *et al*., 2000). It was reported that lawsone isolated from the leaves of *L. inermis* has shown a significant antifungal effect (Tripathi *et al*., 1978 and Dixit *et al*., 1980). However, Rahmoun *et al*. (2013) thought there is a role of polyphenols in the antifungal activity showed by the ethanol extract. The polyphenols isolated from the ethanol extract are: naphthoquinones derivatives, lawsoniaside, luteolin, acacetin, cosmosiin, lalioside, lawsoniaside, syringinoside, daphneside, daphnorin, agrimonolide pyranoside derivatives and isoscutellarin (Takeda and Fatope, 1988 and Cuong *et al*., 2010). Moreover, ethanolic extract of *M. oleifera* leaves showed antifungal activity against a number of dermatophytes (Chuang *et al*., 2007). Leaves of *M. oleifera* contain Phytochemicals like tannins, sterols, saponins, trepenoids, phenolics, alkaloids and flavanoids like quercitin, isoquercitin, kaemfericitin, isothiocyanates and glycoside compounds (Gopalakrishnan *et al*., 2016).

Also, other investigators found an antifungal activity of some natural plant products suppressed plant pathogens with an increase of oxidative enzymes in plants that can play an important role in the resistance to infection with diseases and

consequently increasing growth parameters and seed yield (Mohamed and El-Hadidy, 2008; Abdel-Monaim *et al*., 2011 and Goel and Paul, 2015). Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in them may either act on the pathogen directly (Baraka *et al*., 2006). In this study, all tested extracts resulted in increasing the activity of oxidative enzymes, *viz*. peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL), superoxide dismutase (SOD) and catalase (CA) as well as total phenolic contents in faba bean plants compared with untreated seeds. *E. jambolana* extracts gave the highest activity of all tested oxidative enzymes and total phenols content. Also, organic solvent extracts of all tested plant species increased the activity of these enzymes and phenols more than aqueous extracts. Many researches showed that the application of some plant extracts can induce systemic resistance in many plants through accumulations of oxidative enzymes, pathogenesis related proteins (PR-proteins) and phenolic compounds (Kagale *et al*., 2004 and 2011; Farag *et al*., 2011 and Baka, 2015).

As a conclusion, our study demonstrated that some plant extracts can be used as alternative source of antifungal agents for protecting faba bean plants from damping-off and root rot diseases. Thus, this method can contribute to minimizing the risk and hazard of toxic fungicides.

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فاعلية المستخلصات النباتية كوسيلة آمنة في مكافحة أمراض سقوط البادرات وأعفان الجذور في الفول

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تُعتبر أمراض سقوط البادرات وأعفان الجذور المتسببة عن الفطرين الفيوزاريوم سولانى والريزوكتونيا سولانى من أهم الأمراض التي تصيب الفول في مناطق زراعته فى مصر. في هذه الدراسة تم استخدام المستخلص المائي والعضوي لثلاثة أنواع من النباتات الطبية وهى أوراق الحنة والبامبوزيا والمورينجا تحت ظروف المعمل والصوبة والحقل ضد مسببات أمراض سقوط البادرات وأعفان الجذور في الفول. تبين من النتائج المتحصل عليها تحت ظروف المعمل أن جميع أنواع المستخلصات النباتية ثبطت بدرجة معنوية النمو القطرى للفطرين الممرضين وكان مستخلص البامبوزيا أعلى تثبيطاً لنمو كلا الفطرين. أيضا كانت المستخلصات العضوية أكثر كفاءة فى تثبيطهما عن المستخلصات المائية. تحت ظروف الصوبة والحقل تبين أن جميع المستخلصات النباتية المستخدمة أدت إلى انخفاض معنوي في شدة الإصابة بأمراض سقوط البادرات وأعفان الجذور بالإضافة إلى زيادة بعض الصفات المحصولية مقارنة بالكنترول. أعطت بذور الفول المعاملة بمستخلص أوراق البامبوزيا أعلى نسبة وقاية من الإصابة بأمراض سقوط البادرات وأعفان الجذور بالإضافة إلى زيادة صفات النمو والمحصول مقارنة بالمستخلصات الأخرى. تبين من الدراسات البيوكيميائية أن المعاملة بهذه المستخلصات أدت إلى زيادة نشاط الإنزيمات المرتبطة بمقاومة النباتات للأمراض والتى من أهمها إنزيمات البيروكسيديز والبولى فينول أوكسيديز والفنيل الآنين أمونيا ليبز وسوبر أوكسيد ديزميتيز والكتاليز بالإضافة إلى زيادة محتوى النباتات من الفينولات الكلية فى النباتات النامية تحت ظروف العدوى الصناعية بالفطرين الممرضين مقارنة بالكنترول. عموماً سجل مستخلص البامبوزيا أعلى نشاط لإنزيمات البيروكسيديز والبولى فينول أوكسيديز والفنيل الآنين أمونيا ليبز وسوبر أوكسيد ديزميتيز والكتاليز بالإضافة إلى محتوى النباتات من الفينولات الكلية . كما سجلت المستخلصات العضوية أعلى نشاط لتلك الإنزيمات والفينولات الكلية محل الدراسة عن المستخلص المائى لكل النباتات المختبرة. هذه النتائج المتحصل عليها تبين أن استخدام المستخلصات المائية والعضوية ممكن أن تلعب دوراً هاماً في مكافحة أمراض سقوط البادرات وأعفان الجذور عن طريق استحثاث المقاومة في نباتات الفول.