Journal of Phytopathology and Pest Management 6(1): 78-98, 2019
pISSN: 2356-8577 eISSN: 2356-6507
Journal homepage: http://ppmj.net/

Impact of applying certain bio-agents and plant extracts to control root and pod rot peanut pathogens

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Abstract

The current study was performed to evaluate the efficacy of some bacterial and fungal bio-agents and certain plant extracts *i.e.* garlic, neem and mint against *Fusarium* spp., the causal agent of peanut root and pod rot diseases. *In vitro* tests clarified that *Penibacillus polymyxa* (BP) and *Pseudomonas fluorecensce* (PF2) achieved the highest inhibition percentage of the tested pathogenic fungi followed by *Bacillus subtilis* (BS1) and *P. fluorecensce* (PF1) while, *P. fluorecensce* (PF3) and *Bacillus megaterium* (BM2) came in the last order. Addition of suspensions of *Bacillus* (B1), *P. fluorecensce* (P11), inoculum of *T. harzianum* (T10) and combination of all bio-control agents (Mixture) to infected soil significantly increased yield of peanut plants (Giza 6 cv), such as fresh weight (gm /plant), dry weight (gm /plant), plant height (cm /plant), number of pods /pot and weight of pods /pot. Regarding plant extracts, garlic extract was the most effective plant extract in suppressing the mycelial growth of the pathogenic fungi than any other treatment. Mint extract showed the lowest effect on reduction of linear growth of the tested pathogens. Concerning root rot disease management, addition of bio-agents *in vivo* resulted in distinct disease reduction obtained by *P. fluorecensce* (PF2) and Mixture treatments followed by *Penibacillus polymyxa* (BP) and *T. harzianum* (T10), respectively. In the concern of pod rot disease, the mixture and *T. harzianum* (T10) treatments recorded the highest disease reduction as compared to control while, *P. fluorecensce* (PF2) and *Penibacillus polymyxa* (BP) came in the last order. Treated seeds of peanut (Giza 6 cv.) with certain plant extracts (garlic, neem and mint) at concentration of 30% significantly reduced the severity of root and pod rot diseases compared to control. Garlic extract gave the highest reduction of root and pod rot severities on peanut plants followed by neem extract then mint extract, respectively.

Keywords: peanut, plant extracts, *Fusarium* spp., pod rots, root rot.

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1. Introduction

Arachis hypogaea L. (Groundnut or Peanut) is one of the important crops all over the world. In Egypt it is one of the major oil seed crops and considered as one of the most important, export and edible oil crops as well as in many countries of the world. In addition to this, belonging to leguminous plants, peanut has the ability to fix nitrogen from the atmosphere biologically into the soil which enriches the soil and these benefits the succeeding crop. Peanut seeds are characterized by their high oil content (50%), which is utilized in different industries beside containing 26–28% protein, 20% carbohydrates and 5% fiber (Fageria et al., 1997). The peanut crop is vulnerable to soil borne diseases since both roots and pods of the plant grow in soil. Diseases caused by soil borne fungal pathogens reduce yields and the quality of the harvested pods and it affect the crop plant until harvest. Pathogens attack all plant parts of groundnut and restrict plant development throughout the growing season as well as reducing seed quality in post-harvest storage (Mahmoud et al., 2006). Root and pod rot diseases of peanut are serious worldwide diseases attacking roots and fruits underground (Hilal et al., 1990). *Fusarium* spp is known as a pathogen causing different symptoms of infected roots and pods (Hussin Zeinab, 2011; Marei, 2000). *Fusarium solani* is one of the most important pathogens causing brown root rot in peanuts. Under conductive conditions like drought stress, the losses could reach 95% of production in some fields. Fungicide treatments are not effective enough to control the disease beside the harmful effect of excessive and misuse of fungicides which lead to environmental pollution and causes damage to the ecosystem. Recently many researches tried to develop alternative control strategies in order to reduce dependency on synthetic fungicides. *Pseudomonas fluorescence* is an effective candidate for biological control of soil borne plant pathogens owing to its versatile nature, rhizosphere competence and multiple modes of action beside being endophytic in the plant system (Diby et al., 2001). *Pseudomonas* spp. also can induce systemic biochemical and ultra-structural changes in the roots that lead to a greater ability of the host plant to defend itself against root infecting pathogens (Sarma et al., 2000). It was noticed that in greenhouse experiments *P. flurescens* and *B. subtilis* significantly reduced the incidence of all types of peanut pod rot caused by *R. solani, S.rolfsii, M. phaseolina, Fusarium* spp. and *Aspergillus* spp. (Mahmoud, 2004). *Trichoderma* spp. are one of the most important biological control agents and one of the most frequently isolated soil fungi present in plant root ecosystems. They colonize the root and rhizosphere of plant and suppress plant pathogens by different mechanisms, such as competition, mycoparasitism, antibiosis and induced systemic resistance, improvement of the plant health by promoting plant growth, and stimulation of root growth (Mohidden et al., 2010). *Trichoderma harzianum*, *T. hamatum* and *B. subtilis* reduced the mycelial growth of *R. solani, F. solani* and *M. phaseolina*. *Trichoderma hamatum* mainly grew over the mycelium of the tested pathogens (El-Sayed et al., 2009). It is well known that plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cowan, 1999). Therefore, many scientists have reported the use of plant extracts for controlling certain fungal diseases (Touba et al., 2012; Al-Askar & Rashad, 2010). The objective of this research was the

development of alternative control strategies to reduce dependency on synthetic fungicides. Also to evaluate *Bacillus* spp., *Psedomonas florescence* and *Trichoderma* species as potential biocontrol agents to reduce the impact of the disease under greenhouse conditions.

2. Materials and methods

2.1 Seeds

Seeds of peanut that have been used in this study were cultivar Giza 6. They were obtained from Agriculture Research Center (ARC), Giza, Egypt.

2.2 Pathogenic fungi source

Virulent *Fusarium* isolates used in this study were previously isolated, identified and proved their pathogenic ability to induce root and pod rot of peanut in former study (Abdallah et al., 2016).

2.3 Isolation of biocontrol agents

Isolation trials were conducted to isolate *Pseudomonas* and *Bacillus* colonizing rhizosphere soil of peanut growing in different location of Minia, Assiut and Sohag governorates, Egypt. Isolates were picked up on the suitable media. All *Pseudomonas* and *Bacillus* isolates were purified by successive streaking on N-deficient modified king medium and nutrient agar media, respectively, using the techniques adopted by King et al. (1954) for *Pseudomonas* and nutrient agar medium (Oxoid Manual 1965) for *Bacillus*. The purified isolates were maintained on the same media and confirmed by microscope examination for Gram stained young cells (18-24 hours old). Then the purified isolates were stored at 4°C for further studies. As for *Trichoderma* spp. it was also isolated from healthy peanut plants rhizosphere using dilution plates technique according to Warcup (1955). *Trichoderma* isolates were identified according to their morphological characteristics using identification keys (Rifai, 1969), also two identified isolates obtained were from Assiut University Mycological Center, Egypt.

2.4 Antagonistic effect of isolated microorganisms against *Fusarium* isolates *in vitro*

2.4.1 Bacterial biocontrol agents

Six bacterial isolates *i.e. Pseudomonas* (PF1, PF2, PF3), *Bacillus* (BP, BS1, BM2) were investigated for their effect against the growth of *Fusarium* isolates under Lab conditions. Petri dishes (containing PDA medium) were streaked with the bacterial growth which obtained from 24hours old cultures at the periphery using sterilized needle and left for 24 hours. One disk of the pathogen was placed at the center of each plate, then plates were incubated at 27°C. Plates without bacterial inoculation were served as control treatment. Each treatment contained three replicates. When growth of the pathogen covered the plate surface (9.0 cm in diameter) of control treatment, antagonistic effect was determined by measuring the free inhibition zone, then percentage of mycelial growth inhibition was calculated according to the formula:

$$\text{Percentage of mycelial growth inhibition } \% = [A - B\ /A] \times 100$$

Where: A = length of hyphal growth of the control, B = length of hyphal growth of the treated.

2.4.2 Fugal biocontrol agents

Petri-dish was divided into equal halves. The first half was separately inoculated with standard disc (5 mm) of *Trichoderma* spp., previously isolated from peanut rhizosphere. The second half was inoculated with an equal disc of each of *Fusarium spp*. Isolates. Each treatment was replicated three times and inoculated plates with the pathogen only were used as control. All Petri-dishes were incubated at 27 °C and data were recorded when control treatments cover the plates. Antagonistic percentage was calculated according to the following scale index: 0-4, 0= no antagonism; 1= slight antagonism; 2= moderate antagonism; 3= high antagonism and 4= overgrowth (Hassan, 1992).

2.4.3 Effect of culture filtrate of antagonistic fungi on growth of *Fusarium* isolates

Trichoderma harzianum isolates (No. 2, 8 and 10) were grown on Czapek′s liquid medium. Each flask containing 100 ml of the medium was inoculated separately with 5 mm agar disc obtained from 7 days-old culture. Then, the flasks were incubated at 27°C for 30 days. The obtained cultures were used to study the effect of the culture filtrates of antagonistic fungi on mycelial growth of *F. oxysporum* (I), *F. solani* (V) and *F. solani* (VII) isolates. The mycelial growth was eliminated and the filtrate of each fungus isolate was centrifuged for 60 min at 3000 rpm to separate the fungal growth (Mohamed et al., 2008). Filtrates were sterilized by Seitz filter and added to the medium at the rate of 10, 25 and 50% (v/v), and mixed thoroughly before solidification at 40-50°C. Petri-dishes were inoculated with equal discs *Fusarium oxysporum* (I), *Fusarium solani* (V) and *Fusarium solani* (VII). Petri dishes received medium only without culture filtrate were used as control. Four replicates were used for each treatment. All treatments were incubated at 27°C for 7days. Data were recorded as diameter of linear growth when the control plates were completely covered by the fungal mycelium. Percentage of reduction in growth was calculated according to the following formula (Abd El-Khair & Haggag Wafaa, 2007):

Growth inhibition % = ((Growth in control – Growth in each treatment) / Growth in control) × 100

2.5 Effect of aqueous plant extracts on growth of pathogenic fungi

Aqueous plant extracts were prepared from cloves of garlic (*Allium sativum*), leaves of Neem (*Azadirachta indica*) and leaves of mint (*Mentha*). The plant parts were washed several times with sterilized distilled water , cut into small pieces, then 100 gram of each were macerated in 100 ml distilled water by using mortar, resulting extract was squeezed twice through four layers of cheese cloth, then, centrifuged at (4000 rpm for 9 min), and sterilized using Seitz filtrate (Hassan, 2006). Sterilized filtrates of aqueous plant extracts were kept in dark bottles in refrigerator until use. Flasks (250 ml) contained 100 ml of sterilized PDA medium were melted. After that, plant extracts were added to the PDA medium to obtain concentrations 10, 20 and 30%, mixed and poured in sterilized Petri dishes (20 ml /plate). Plates were

inoculated with equal discs (6 mm in diameter) of *Fusarium* isolates taken from 4 days old cultures. Four replicates were used for each treatment. Control treatment was obtained by culturing the tested fungi on PDA medium without addition of aqueous plant extracts. The inoculated plates were incubated at 27 ±2°C until the fungal growth covered the plate surface of the control treatments. The percentage of mycelial growth reduction was calculated using the following formula:

$$\text{Percentage of growth reduction} = [(\text{growth in control} - \text{growth in treatment}) / \text{growth in control}] \times 100$$

2.6 Greenhouse experiments

2.6.1 Effect of applying biocontrol agents to control peanut root and pod rot diseases

Pot experiments were carried out under greenhouse conditions in Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut branch), Assiut, Egypt during 2015 and 2016 growing seasons. It was done to study the effect of *Penibacillus polymyxa* (BP), *Pseudmonas fluorescens* (PF2) and *Tichoderma harzianum* (T10) on controlling root and pod rot of peanut. Each bacterial suspension ($1x10^8$ cfu/ml) was prepared by dilution plate assay as described by Callan et al. (1990). Bacterial cells from agar cultures of each isolate were inoculated into nutrient broth (NB) and centrifuged at 3000 rpm for 5 min, the supernatant was discarded and the precipitate was re-suspended in 100 ml sterilized distilled water. The suspension was re-centrifuged for 5 min. and the precipitate was finally suspended in sterilized distilled water. Bacterial concentrations were determined according to their turbidity using spectrophotometer. Inoculum of *Trichoderma harzianum* was prepared according to Abdel-Moneem (1996) by inoculating sterilized conical flasks (1000 ml) which contain 75 g sorghum cereal, 25 g clean sand, 2 g sucrose and 200 ml water with equal discs (0.5 cm) taken from 7 days old cultures grown on PDA medium at 27°C. The inoculated flasks were incubated at 27°C for two weeks. Bacterial isolates and *T. harzianum* were applied as soil treatment by adding 100 ml of bacterial suspensions (10^8 cfu /ml) and *T. harzianum* at the rate of 2% (w/w) for each pot. Inocula of the pathogenic fungal isolates were prepared by growing them in sterilized conical flasks (1000 ml) containing sand and sorghum medium, then incubated at 27°C for 21 days. Sterilized pots (30 cm in diameter) were filled with infested sand clay soil at the rate 2% (w/w) 7 days before sowing. Pots were left for one week, watered and mixed thoroughly to ensure distribution with the tested fungi. Eight seeds of Giza 6 cv. were sown in each pots and the biocontrol agents were applied as mentioned early. Untreated pots were served as control. Each treatment was replicated four times. Disease severity of root rot was recorded after 90 days from sowing. The arbitrary (0-5) disease index scale as described by Grunwald et al. (2003) and Hussin Zeinab (2011) was adopted, where: 0= No visible symptoms, 1= slight hypocotyls lesions, 2= lesions coalescing around epicotyls and hypocotyls, 3= lesions starting to spread into the root system with root tips starting to be infected, 4= epicotyls, hypocotyls and root system almost

completely infected and 5= completely infected root**.** Pod rot severity was recorded by adopting the scale based on area of spots covered on pods and percent disease index (PDI), calculated mean disease index. Pods were grouped into six categories described by Wheeler (1969), Where 0=non disease symptoms (disease free), 1=spots cover 1-10%, 2= spots cover 10-30%, 3= spots cover 30-50%, 4= spots cover 50-70% and 5= spots cover >70%. The percentage of disease severity of root rot and the percentage of pod rot disease were estimated using the following formula:

$$\text{Disease severity (\%)} = \sum [(n \times V)/5 \times N)] \times 100$$

Where, n= number of root or pod within each infection category, V= numerical values of infection categories, N= total number of root or pods examined, 5= constant, the highest numerical value.

At the end of the experiment some growth parameters such as plant fresh and dry weights, plant height, and number of pods and weight of pods were evaluated.

2.6.2 Effect of aqueous plant extracts on the incidence of peanut root rot and pod rot diseases under greenhouse conditions

This experiment was conducted to study the effect of aqueous plant extracts of garlic, neem and mint on the incidence of root and pod rots caused by *Fusarium* spp. on peanut plants cv. Giza 6, under greenhouse conditions during 2015 and 2016 growing season. Preparation of aqueous plant extracts has been done as mentioned before. Seeds of cv. Giza 6, were immersed in the highest concentration (30%) of each extract for 15 minutes. Sterilized pots (30 cm in diameter) were filled with sterilized sand clay soil. Infested soil with each tested pathogen was carried out as previously mentioned. Four replicates were used for each treatment. Each replicate contained four pots (8 seeds/ pot). Untreated seeds with aqueous plant extracts were used as control. After 90 days, diseases severity was recorded as mentioned before. At the end of the experiment several growth parameters such as plant fresh and dry weights, plant height, and number of pods and weight of pods were estimated.

2.7 Statistical analysis

Comparison of means was performed using Fisher's protected least significant difference (LSD) at $p \leq 0.05$ (Gomez & Gomez, 1984) and the standard error was calculated using the statistical analysis software "CoStat 6.4" (CoStat, 2005)

3. Results and Discussion

3.1 Identification of the biocontrol agents

Bacterial biocontrol agents isolated from peanut soil rhizosphere were identified according to their morphological and biochemical characteristics as: 3 isolates as *Bacillus* spp., one isolate as *Penibacillus polymyxa* (BP), one isolate as *Bacillus subtilis* (BS1), one isolate as *Bacillus megaterium* (BM2) and 3 isolates as *Pseudomonas florescence* (BF1, BF2 and BF3). Fungal biocontrol agents were also isolated from peanut soil rhizosphere; eight isolates were obtained from rhizosphere soil of peanut plants plus two isolates obtained from Assiut University Mycological Center

(AUMC), Egypt. Fungal isolates were identified as *Trichoderma harzianum* (1, 2, 5, 7, 8, 9 and 10) and *Trichoderma hamatum* (3, 4 and 6) according to their cultural and microscopical characters as described by Rifai (1969).

Table 1: Effect of some antagonistic bacteria on the mycelial growth of the tested fungi *in vitro*.

Bacterial isolates	Code	Mycelial growth inhibition (%)		
		F. solani (V)	*F. solani* (VII)	*F. oxysporium* (I)
Bacillus spp.	BP	58.5	33.6	63.8
	BS1	40.7	30	52.3
	BM2	12.3	7.8	49.5
Pseudomonas fluorescence	PF1	21.2	18.3	47.8
	PF2	41.8	35	62.2
	PF3	14.3	8.6	33
Control		0	0	0
L.S.D at 5%		2.01	2.20	1.64

3.2 Antagonistic capability of bacterial bioagents against mycelial growth of the pathogenic fungi *in vitro*

Data in Table (1) and Figure (1) indicated that the antagonistic isolates of bacteria were able to inhibit the mycelial growth of *F. solani* (V), *F. solani* (VII) and *F. oxysporum* (I) as compared with the control. *Penibacillus polymyxa* (BP) gave the greatest reduction of mycelial growth of *F. solani* (No. V), followed by isolates *Pseudomonas fluorescens* (PF2), *Bacillus subtilis* (BS1) and *P. fluorescens* (PF1) as reached 58.5%, 41.8%, 40.7% and 21.2% respectively. *Bacillus megaterium* (BM2) showed the lowest mycelial growth inhibition of *F. solani* (V), followed by *P. fluorescens* (PF3) as recorded 12.3% and 14.3% respectively. *Pseudomonas fluorescens* (PF2), *Penibacillus polymyxa* (BP) followed by *B. subtilis* (BS1) showed the highest degree in suppressing mycelial growth of *F. solani* (VII), as reached 35%, 33.6% and 30% respectively. *P.fluorescens* (PF1) showed moderate effect of inhibiting the mycelial growth of *F. solani* (VII) while, *B. megatirum* (BM2) followed by *P. fluorescens* (PF3) gave slight effect on the mycelial growth of the same fungus as reached 7.8% and 8.6% respectively. In the same context, *Penibacillus polymyxa* (BP), *P.fluorescens* (PF1) and *Bacillus subtilis* (BS1) gave the greatest inhibition of mycelial growth of *F.oxysporium* (I) as recorded 63.8%, 62.2% and 52.3% respectively. *B.megatirum* (BM2) and *P.fluorescens* (PF1) exhibited moderate effect of inhibition of the same fungus as reached 49.5% and 47.8%, followed by *P.fluorescens* (PF3) as recorded 33%. These results are similar to those obtained by El-Mougy *et al.* (2011) who examined the influence of the antagonistic isolates of *B. subtilis* and *P. fluorescens* and their culture filtrates against soil-borne root rot pathogens *R. solani* and *F. solani in vitro*. The tested antagonists reduced the linear growth of fungal pathogens. Pandya *et al.* (2009) evaluated some antagonists (*B. subtilis*, *T. viride* and *T. harzianum* against mycelial growth of *F. solani in vitro*. All the antagonists were effective against *F. solani* and might be very useful as potential biological control agents. Also,

Mahmoud *et al*. (2006) tested seventeen bacterial isolates *in vitro* for their antagonistic effect against the pathogens *F. solani* and *M. phaseolina*. The most effective isolates in reducing the mycelium growth of pathogenic fungi were *P. fluorescens* (Pf2) followed by *B. subtills* (BS1) and *Bacillus* spp. (BP). In this respect, Abdel-Monaim (2011) tested certain bio-control agents (*B. subtilis, B. megaterium, T. viride* and *T. harzianum)* which isolated from chickpea rhizosphere, against *R. solani, F. solani* and *S. sclerotiorum*. *Bacillus subtilis, B. megaterium, T. viride* and *T. harzianum* gave the highest effect against the tested fungi *in vitro*.

3.3 Effect of *Trichoderma harzianum* and *T. hamatum* on linear growth of the pathogenic fungi

3.3.1 Dual culture

Seven isolates of *Trichoderma harzianum* and three isolates of *T. hamatum* were tested to study their effect to inhibit mycelial growth of *F. solani* (V), *F. solani* (VII) and F. *oxysporum* (I) *in vitro* on Czapek's agar medium. Data in Figure (2) indicate that all the tested isolates of both *Trichoderma* species significantly reduced the mycelial growth of *F. solani* (V), *F. solani* (VII) and *F. oxysporum* (I) although exhibited different degrees of reaction. The mycelium of *Trichoderma* spp. grew rapidly over the mycelium of the tested pathogens and prevented their development. *Trichoderma harzianum* T2, T8 and T10 were the most effective in inhibiting the pathogenic fungal growth, while isolate T9 exhibited the lowest effect. On the other hand, *T. hamatum* isolates showed moderate effects on the pathogenic fungi mycelial growth. *Trichoderma harzianum* T2, T8 and T10 were selected for further studies. Such results are in line with those reported by Ha (2010). Antagonistic effect might be due to direct influence of antagonistic fungi against pathogens through coiling their hyphae around the hyphae of the pathogens to prevent their continued growth (Adekunle et al., 2006) and/or production of antagonistic substances which can play an important role in lyses of cell wall components of the pathogenic fungi to help the antagonists to penetrate the host hyphae and grow on it as a hyper parasite (Papavizas et al., 1984). This can be explained in the light of results recorded by Abd El-Moity (1981), who stated that *T. harzianum* works through different mechanisms, *i.e.* production of gliotoxin, mycoparasitism and growing very fast and act as barrier between susceptible plant tissues and virulent pathogens. Mycoparasitism by *T. harzianum* is a complex process, involving recognition of the host, attachment to the mycelium, coiling round the hyphae, partial degradation of the cell wall and penetration of the host mycelium (Seema and Devaki, 2012). Scanning electron microscopic observation of parasitism of *T. harzianum and T. hamatum* on *R. solani* revealed that the hyphae of *Trichoderma* coil around the host. *T. harzianum* attached to host mycelium by forming hooks and *Trichoderma* produces appressoria at the tips of short branches.

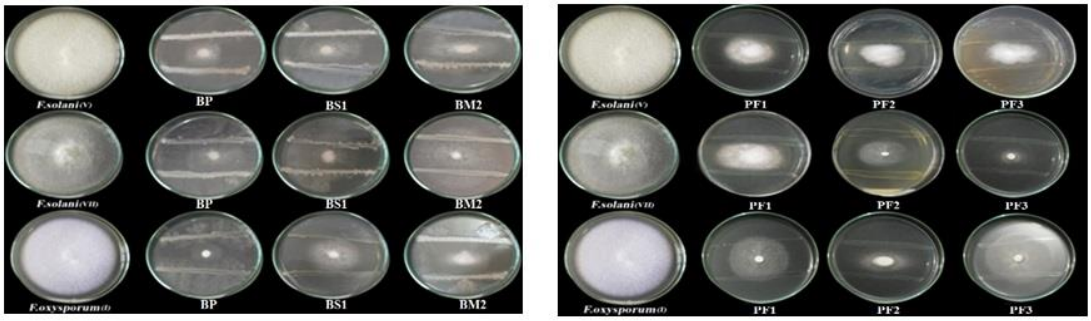

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Figure 1: (A) Effect of *Bacillus* spp. isolates on mycelial growth of the tested fungi *in vitro* where BP=*Penibacillus polymexa,* BS1=Bacillus substiles and BM2= *Bacillus megaterium.* (B) Effect of *Pseudomonas fluorescence* isolates on mycelial growth of the tested fungi *in vitro*.

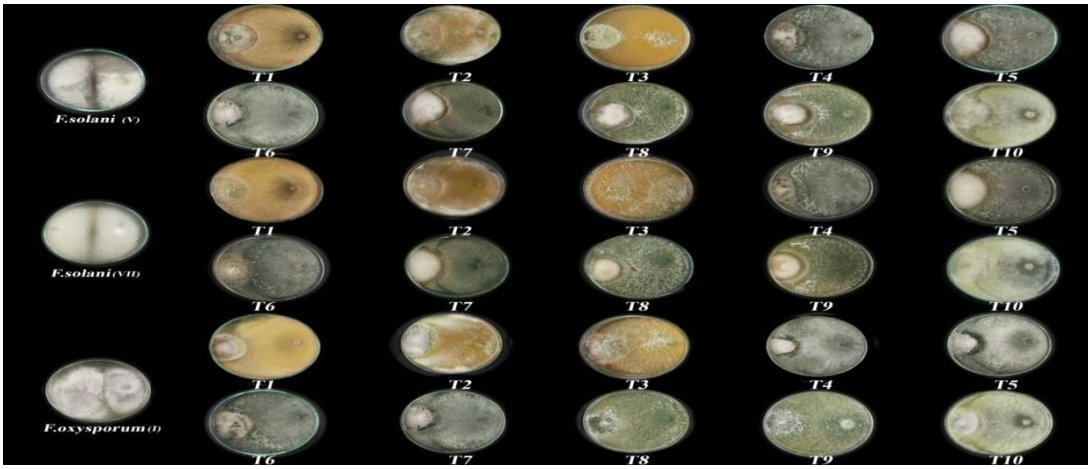

Figure 2: Effect of *Trichoderma harzianum* (isolates 1, 2, 4, 5, 7, 8, 9 and 10) and *T. hamatum* (isolates 3, 4 and 6) on inhibiting mycelial growth of the tested pathogenic fungi.

3.3.2 Effect of culture filtrates of *T. harzianum* isolates on linear growth of *Fusarium* isolates *in vitro*

Isolates of *F. solani* and *F. oxysporum* were used to study their reaction against toxin produced by *T. harzianum*. Data in Table (2) that the addition of culture filtrate of *T. harzianum* isolates T2, T8 and T10 separately to the medium significantly affected the mycelial growth of the tested fungi. The reduction of the mycelial growth of the tested fungi increased with increasing culture filtrate concentration. The culture filtrate at the concentration of 50% gave higher reduction of the mycelial growth of the *Fusarium* isolates. *Fusarium oxysporum* was significantly reduced for a large degree with different concentrations of *T. harzianum* culture filtrates comparing with isolates of *F. solani* (V) and *F. solani* (VII). Generally, the best isolate of *T. harzianum* which affected the mycelial growth of the pathogenic fungi was *T. harzianum* (T10) with all tested concentrations followed by *T. harzianum* (T2). While, the lowest reduction was obtained by using *T. harzianum* isolate (T8). These results are in conformity

with the results obtained Haran et al. (1995). *T. harzianum* is known to produce relatively high concentrations of cell-wall degrading enzymes as β-1, 3-glucanasea and different chitinolytic enzymes. Several enzymes have been purified and characterized *in vitro*. Howell (2003) noted that enzymes such as chitinases and glucanases produced by the biocontrol agents are responsible for suppression of the plant pathogens. These enzymes function by breaking down the polysaccharides, chitin, and β-glucanase that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity.

Table 2: Effect of culture filtrates of *T. harzianum* isolates on mycelial growth of the tested pathogenic fungi.

Isolate No.	Conc.	Reduction of mycelial growth (%)			Mean
		F. solani (V)	*F. solani* (VII)	*F.oxysporum* (I)	
Trichoderma harzianum No. 2	10%	15	17	23	18.3
	25%	29	25	40	31.3
	50%	33	33	43	36.3
Trichoderma harzianum No. 8	10%	20	17	28	21.6
	25%	24	19	31	24.6
	50%	34	30	45	36.3
Trichoderma harzianum No. 10	10%	18	19	27	21.3
	25%	19	21	32	24
	50%	34	34	47	38.3
Control		0	0	0	-
LSD at 5%	Isolate (I)	3.03	3.78	2.19	-
	Conc. (C)	1.36	1.84	2.24	-
	(I) X (C)	2.73	3.68	4.49	-

3.4 Effect of certain plant extracts on the mycelial growth of the tested fungi *in vitro*

Plant extracts of garlic, neem and mint were tested to investigate their ability to inhibit the mycelial growth of *F. solani* (V), *F. solani* (VII) and *F. oxysporum* (I) *in vitro*. Data in Table (3) and Figure (3) showed that all tested plant extracts reduced the mycelial growth of *F. solani* (V), *F. solani* (VII) and *F. oxysporum* (I), with the tested 10, 20 and 30 % concentrations. The largest percentage of reduction was obtained with 30% concentration, while the lower effect of plant extracts on growth of pathogenic fungi was obtained with concentration of 10%. Treatment with garlic extract at concentration of 30% gave the highest effect on the reduction of the mycelial growth of *F. solani* (V), *F. solani* (VII) and *F. oxysporum* (I), isolates which recorded 90.5, 90.8 and 91.8% respectively. Neem extract with the concentration 30% recorded higher effect on *F. solani* (V), *F. solani* (VII) and *F. oxysporum* (I) isolates, where the values reached it 80.2, 77.8 and 88.9% respectively. Mint extract at concentration 30% recorded the lowest reduction on the mycelial growth of the pathogenic fungi as values of 60.2, 60.7 and 65.6% respectively. These results are in line with results of El-Sharkawy (2006) and Mukhtar (2007). The maximum reduction of the mycelial growth by plant extracts may be due to the presence of antifungal compounds in the extracts (Anusha, 2003).

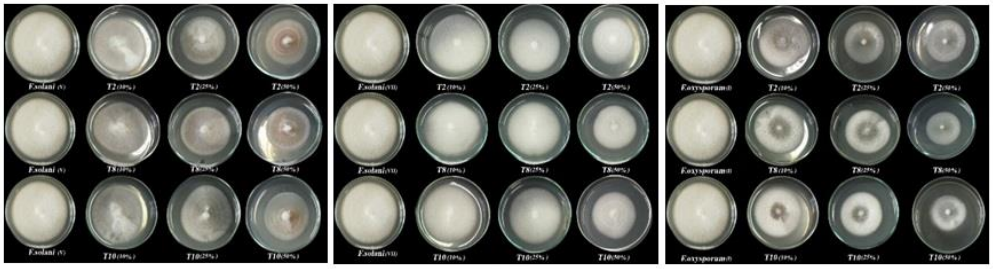

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Figure 3: Effect of culture filtrates of *Trichoderma harzianum* isolates on the mycelial growth of a) *F. solani* (V), b) *F.solani* (VII) and c) *F.oxysporium* (I) *in vitro*.

Table 3: Effect of different plant extracts on mycelial growth of the tested pathogenic fungi *in vitro*.

Plant extracts	Conc.	Reduction of pathogenic fungi mycelial growth (%)			Mean
		F. solani (V)	*F. solani* (VII)	*F. oxysporum* (I)	
Garlic	10%	47.3	45.7	50	47.6
	20%	56.5	52.8	60	56.4
	30%	90.5	90.8	91.8	91
Neem	10%	38	37.5	40.4	38.6
	20%	51.5	52	56.2	53.2
	30%	80.2	77.8	88.9	82.3
Mint	10%	9.3	8.5	12.5	10.1
	20%	19.4	18.5	22.3	20
	30%	60.2	60.7	65.6	62.2
Control		0	0	0	-
LSD at 5%	Concentration (A)	3.48	2.87	3.09	-
	Treatments (B)	4.05	4.78	4.02	-
	Interaction (A × B)	6.96	5.75	6.18	-

Ahmed and Sultan (2000) mentioned that garlic and onion extracts showed toxic effects on mycelial growth of the *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium* spp. *in vitro*. Shalin and Dohroo (2003) studied the activity of 13 plant extracts of broccoli leaves, eucalyputs, neem, pine, ginger, turmeric rhizome, chili seeds, tulci seeds, ocimum sanctum, cotton, sarson caka, basil and garlic cloves *in vitro* against *Fusarium oxysporium*. These extracts gave different effects on mycelial growth of *Fusarium oxysporum* f.sp. *pisi* causing the wilt of pea plants.

3.5 Estimation of different bio-agents effect on incidence of root rot disease of peanut under greenhouse conditions

Data presented in Table (4) showed that treated soil with bacterial bio agents significantly reduced the disease severity of root rot disease of peanut compared with the control. The soil treated with the mixture of bio-bacteria and *P. fluorescens* (PF2) gave higher effects in decreasing root rot disease incidence caused with *F. solani* (V) and *F. solani* (VII) more than the other bio-agents under greenhouse conditions during 2015 and 2016 growing seasons. *Penibacillus polymyxa* (BP), followed by *T. harzianum* (T10) showed moderate effects in decreasing disease severity caused with the tested

pathogenic fungi. Concerning *F. oxysporum* (I), *Penibacillus polymyxa* (BP) exhibited the highest disease reduction followed by mixture whereas, *P. fluorescens* (PF2) gave moderate effect in the term of disease reduction while, the lowest disease reduction was obtained by *T. harzianum* (T10). Such results are in line with those reported by El-Habbaa et al. (2001; 2002) who suggested that addition of *Rhizobium* and *B. subtilis* to soil before sowing peanut seeds significantly reduced root-rot disease of peanut caused by *S. rolfsii*, *R. solani*, *M. phaseolina* and *F. solani*. Ibrahim *et al.* (2008) tested the effects of certain bacterial isolates against *R. solani*, *Sclerotium rolfsii*, *F. solani* and *M. phaseolina* causing root rot disease of peanut, in greenhouse experiments. The most effective isolates in reducing the diseases of peanut were *P. fluorescens* followed by *B. subtills* and *Bacillus* sp. Kishore and Podile (2002) also found that different isolates of *Trichoderma* spp. were identified as biocontrol agents of groundnut stem rot and other soil-borne diseases. Vargas et al. (2008) mentioned that inoculation with *Trichoderma* spp. recorded the lowest incidence of peanut root rot disease caused by *F. solani*.

3.6 Effect of different bio-agents on incidence of pod rot disease of peanut under greenhouse conditions

It was shown from data in Table (5) that the soil treated with each of tested bio-agents significantly reduced the disease severity of pod rot disease of peanut compared with the control. The infested soil with mixture of bacteria gave the highest reduction of pod rot disease of peanut as compared with the control and the other bio-agents tested separately during 2015 and 2016 growing seasons. At the same time, *T. harzianum* (T10) and *P. fluorescens* (PF2) exhibited moderate effects in reducing pod rot disease caused with all tested pathogenic fungi during two successive seasons. *Penibacillus polymyxa* (BP) gave the lowest reduction of pod rot disease severity during two growing seasons. The obtained data revealed that *F. oxysporum* was affected with bioagents for a large degree compared with *F. solani* fungus, so the reduction of pod rot disease was clear during growing seasons.

Table 4: Effect of different bio-agents on root rot disease of peanut under greenhouse conditions during 2015 and 2016 growing seasons.

Biocontrol agents	Root rot disease severity (%)								
	F. solani (V)			*F. solani* (VII)			*F. oxysporum* (I)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
Penibacillus polymyxa (BP)	36	35.1	35.55	35	33.8	34.4	22	20.8	21.4
Pseudomonas fluorescens (PF2)	25	24	24.5	29.2	28	29	30	29.2	29.6
Trichoderma harzianum (T10)	37.5	36.4	36.95	37.5	36.7	37.1	30	38.8	34.4
Mixture	29	27.8	28.4	26	25	25.5	24.4	23	23.7
Control	73	74	73.5	77	78	77.5	65.5	64	64.75
L.S.D at 5%	2.63	2.04	-	2.78	2.52	-	1.78	2.4	-

Table 5: Effect of different bio-agents on pod rot disease of peanut under greenhouse conditions during 2015 and 2016 growing seasons.

Biocontrol agents	Pod rot disease severity (%)								
	F. solani (V)			*F. solani* (VII)			*F. oxysporum* (I)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
Penibacillus polymyxa (BP)	32	31	31.5	32.8	32.1	32.45	27	26.8	26.9
Pseudomonas fluorescens (PF2)	23.5	22.2	22.85	25	23.8	24.4	20	21	20.5
Trichoderma harzianum (T10)	23.5	22	22.75	21	21.8	21.4	20.5	20	20.25
Mixture	16.5	15.3	15.9	17.5	16.8	17.15	16	15	15.5
Control	56	58	57	66	65	65.5	52	54	53
L.S.D at 5%	2.78	2.58	-	1.97	1.89	-	3.26	2.7	-

The results obtained are in agreement with both Mahmoud (2004) who found that *B. subtilis* and *P. fluorescens* significantly reduced incidence of all types of pod rots caused by *Fusarium* spp. Ahmed (2006) and Ibrahim et al. (2008) revealed the superiority of *T. harzianum* followed by commercial products Rhizo-N and Plant-guard which showed high reduction of pod rot disease of peanut caused by the *R. solani, M. phaseolina, S. rolfsii* and *F. moniliforme*.

3.7 Effect of bio-agents on growth parameters of peanut infected with *F. solani* (V) under greenhouse conditions

Data in Table (6) showed that adding mixture of biocontrol agents to infested soil with *F. solani* isolate (V) gave the best result in increasing shoot vigor as increased both fresh and dry weight (g /plant) followed by *Trichoderma harzianum* and *Pseudomonas fluorescens*. It also gave the best result in increasing plant height (cm /plant) followed by *Trichoderma harzianum*, *Penibacillus polymyxa* while *Pseudomonas fluorescens* gave the lowest value. In the same time the highest number of pods /pot was achieved by the application of mixture treatment followed by *Pseudomonas fluorescens* and *Trichoderma harzianum*. Mixture of bioagents achieved the highest weight of pods /pot followed by *Pseudomonas fluorescens* and *Penibacillus polymyxa*. Generally, mixture treatment gave the highest values of growth parameters of peanut plants infected with *F. solani* (V). Such results are in line with those reported by Bolar et al. (2000) who reported that peanut plants treated with bioagents as seed treatment were more health and produced higher yield compare with control plants. This might be due to that bioagent act through different mechanisms. These mechanisms include nutrient and growth regulator substances and some of these antagonists when sprayed on plant surface, prior real infect led to stimulate plant resistant and enforce treated plants to produce some metabolites which depress pathogens (Abd-El-Moneim & Maisa, 2011). Several modes of action of the efficiency of bioagents on reducing plant diseases have been described, including competition for nutrients, antibiosis, induced resistance, mycoparasitism, plant growth promotion and rhizosphere colonization capability.

Table 6: Effect of bio-agents on growth parameters of peanut infected with *Fusarium solani* (V) under greenhouse conditions during 2015 and 2016 growing seasons.

Biocontrol agents	Plants infected with *F. solani* (V)														
	Fresh weight (g/plant)			Dry weight (g/plant)			Plant height (cm/plant)			Number of (pods/pot)			Weight of (pods/ pot)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
Penibacillus polymyxa	17	19	18	6	6.2	6.1	29	34	31.5	33	31	32	57	55	56
Pseudomonas fluorescens	20.5	22	21.25	6.9	7	6.95	34	38	36	23	26	24.5	45.5	48	46.75
Trichoderma harzianum	16	19	17.5	5.7	6	5.85	33	37	35	23	26	24.5	48	50	49
Mixture	31	33	32	9.5	7.5	8.5	39	42	40.5	35	37	36	65	67	66
Control	9	10	9.5	3	3.3	3.15	23	21	22	10	11	10.5	20.5	22	21.25
L.S.D at 5%	4.49	4.08	-	0.4	0.51	-	4.11	4.58	-	4.72	4.45	-	4.59	5.58	-

3.8 Effect of bio-agents on growth parameters of peanut infected with *F. solani* (VII) under greenhouse conditions

It′s shown from data in Table (7) that adding bioagents mixture treatment in infested soil with *F. solani* isolate (VII) gave the highest values of both fresh and dry weights (g /plant), followed by *Pseudomonas fluorescens* and *Penibacillus polymyxa*. Data also showed that mixture treatment gave the best results in plant height (cm/plant) followed by *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Penibacillus polymyxa*, respectively. In this respect, treatment with mixture of bioagents was the best among all other treatments in increasing yield represented in both number and weight of pods followed by *Penibacillus polymyxa*, *Trichoderma harzianum* and *Pseudomonas fluorescens* respectively. In general, mixture treatment was the most effective treatment applied when added in soil infested with *F. solani* (VII) to enhance growth parameters of peanut plants as compared with other treatments and the control. Zafari et al. (2008) reported that using beneficial micro-organisms as biocontrol agents led to enhancement of plant growth parameters. Such enhancement may be due to induce plant resistance**,** produce extracellular enzymes and antifungal or antibiotics, which decrease biotic stress on plant, and produce growth promoter's substances (Szczech and Shoda, 2004). In addition, Egamberdiyeva (2005) hypothesized that there are several mechanisms by which rhizosphere bacteria may stimulate plant growth, such as production of plant growth substances, nitrogen fixation, phytohormones, vitamins, solublizing minerals besides, their role in direct inhibition of pathogen growth and suppression of diseases caused by micro-organisms and increased plant growth and yield.

Table 7: Effect of bio-agents on growth parameters of peanut infested with *F. solani* (VII) under greenhouse conditions during 2015 and 2016 growing seasons.

Biocontrol agents	Plants infected with *F. solani* (VII)														
	Fresh weight (g/plant)			Dry weight (g/plant)			Plant height (cm/plant)			Number of (pods/pot)			Weight of (pods/ pot)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
Penibacillus polymyxa	18	17	17.5	6.5	6	6.25	28.5	34	31.25	23	27	25	50.5	58	54.25
Pseudomonas fluorescens	21	22	21.5	7	7.3	7.15	29	31	30	28	28	28	63.5	60	61.75
Trichoderma harzianum	20	23	22	7	7.4	7.2	29	34	31.5	26	29	27.5	48.5	46	47.25
Mixture	31.5	30	32.25	8	9	8.5	32	34	32	36	34	35	70	64	67
Control	9	11	10	3.2	3.7	3.45	25	21	23	6	9	7.5	12.5	16	14.25
L.S.D at 5%	3.89	3.67	-	0.68	0.52	-	3.84	4.46	-	3.89	2.72	-	5.22	5.17	-

3.9 Effect of applying bio-agents on growth parameters of peanut infested with *F. oxysporum* (I) under greenhouse conditions

Data in Table (8) exhibited that mixture treatment gave the best increase fresh and dry weights (g /plant) followed by *Pseudomonas fluorescence*, *Trichoderma harzianum* and *Penibacillus polymyxa*. Concerning plant height (cm /plant), the best record was achieved also by treatment with mixture of bioagents followed by *Penibacillus polymyxa*, *Pseudomonas fluorescence* then *Trichoderma harzianum*. Mixture treatment gave the highest number of pods /pot followed by *Trichoderma harzianum* and *Penibacillus polymyxa* as compared with control. *Pseudomonas fluorescence* gave moderate effects. Also mixture treatment was the best treatment in increasing weight pods/pot followed by *Penibacillus polymyxa*, *Trichoderma harzianum* and *Pseudomonas fluorescence*. In general treatment plants with mixture of biocontrol agents gave the highest values of growth parameters of peanut plants infected with *F. oxysporum* (I). Application of microorganisms to control diseases, which is a form of biological control, is an environment-friendly approach (Lugtenberg & Kamilova, 2009). In general, competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production are the chief modes of biocontrol activity in PGPR**.** Many rhizobacteria have been reported to produce antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2, diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin (Bhattacharyya & Jha, 2012). Interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi, and viruses.

Table 8: Effect of bio-agents on growth parameters of peanut infected with *F. oxysporum* (I) under greenhouse conditions during 2015 and 2016 growing seasons.

Biocontrol agents	Plants infected with *F. oxysporum* (I)														
	Fresh weight (g/plant)			Dry weight (g/plant)			Plant height (cm/plant)			Number of (pods/pot)			Weight of (pods/ pot)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
Penibacillus polymyxa	18	21	19.5	6	7	6.5	31	40	35.5	26	28	27	48	50	49
Pseudomonas fluorescens	23	24.5	23.75	7	7.5	7.25	30	33	31.5	21	21	21	43	48	45.5
Trichoderma harzianum	20	21	20.5	6.8	7.2	7	29	31	30	28	32	30	44	50	47
Mixture	32	35	33.5	9.5	8.5	9	47	40	43.5	38	34	36	66	60	63
Control	10	9	9.5	3.5	3.3	3.4	26	27	26.5	10	10	10	18.5	15	16.75
L.S.D at 5%	5.22	4.62	-	0.51	0.33	-	4.59	4.81	-	4.63	4.08	-	4.99	6.09	-

3.10 Effect of applying plant extracts on root and pod rot diseases of peanut *in vivo*

Peanut seeds cv. Giza 6 were treated with aqueous solution of three plant extracts, *i.e.* garlic, neem and mint to study their effects with the highest concentration 30% on root and pod rot diseases incidence caused with *F. solani* (V), *F. solani* (VII) and *F. oxysporum* (I) isolates under greenhouse conditions during 2015 and 2016 growing seasons. Data represented in Tables (9 and 10) revealed that soaking peanut seeds before sowing in each of tested plant extract significantly reduced root and pod rots diseases severity compared with the control. Garlic extract gave higher reduction of root and pod rot diseases

severity followed by neem and mint extracts. Treated seeds with each plant extract and sown in infested soil with *F. oxysporum* (I) showed the highest disease severity of root rot disease followed by *F. solani* (VII) then *F. solani* (V). On the contrary, seeds treated with each plant extract and sown in infested soil with *F. solani* (VII) showed the highest disease severity percentage of pod rot disease followed by *F. solani* (V), while *F. oxysporum* (I) gave the lowest disease severity of pod rot disease. Such results are in agreement with those reported by Syed *et al.* (2012). They reported that plant extracts have important roles in biologically based management strategies for controlling plant diseases. Hassan (2006) studied the effects of five plant extracts (camphor, garlic cloves, basil, *Lantana camara* and neem) against *F. solani* and *F. oxysporum* caused root rot disease of pea plants. All plant extracts at any concentration reduced the mycelial growth of the tested pathogenic fungi. Under greenhouse conditions, garlic extract gave the highest effect of reducing root rot disease on pea plants.

Table 9: Effect of plant extracts on root rot disease of peanut under greenhouse conditions during 2015 and 2016 growing seasons.

Plant extracts	Root rot disease severity (%)									Mean
	F. solani (V)			*F. solani* (VII)			*F. oxysporum* (I)			
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	
Garlic	37.5	35	36.25	43	41	42	44	43	43.5	40.58
Neem	41	40.5	40.75	44.15	42.5	43.3	45	44	44.5	42.85
Mint	37	40	38.5	41.6	40	40.5	53	52	52.5	43.9
Control	73	75	74	77	79	78	65.5	65	65.25	71.42
L.S.D at 5%	3.76	2.42	-	4.03	2.80	-	4.7	2.94	-	-

Table 10: Effect of plant extracts on pod rot disease of peanut under greenhouse conditions during 2015 and 2016 growing seasons.

Plant extracts	Pod rot disease severity (%)									Mean
	F. solani (V)			*F. solani* (VII)			*F. oxysporum* (I)			
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	
Garlic	29	28	28.5	29.5	28.7	29.1	24	23.4	23.7	27.1
Neem	32.5	32.3	32.4	34.5	34.5	34.5	31	32	31.5	32.8
Mint	36	35	35.5	36.5	36.2	36.35	29	29.5	29.25	33.7
Control	56	58	57	66	68	67	52	54	53	59
L.S.D at 5%	2.48	5.15	-	6.05	5.79	-	9.08	5.68	-	-

3.11 Effect of different plant extracts on growth parameters of peanut plants

This study was carried out using plant extracts on fresh weight of shoots, dry weight, plant height, number of pods and weight of pods. Data in Table (11) revealed that fresh and dry weights of plant shoot increased to varying degrees when sprayed with plant extracts. Garlic extract gave higher increases of fresh and dry weights compared with neem and mint extracts as well as control plants, which infected with the same pathogens. Also, mint extract gave moderate effect of fresh and dry weight, while neem extract came in the last during 2015 and 2016 growing seasons. Concerning the effect of plant extracts on plant height, data exhibited that the used concentration of plant extracts was more effective, wherever the height of peanut plants

obviously increased compared with control plants and infected with the tested fungi during two growing seasons. The same trend was obtained by adding garlic extract followed by neem extract, while mint extract gave the lowest plant height.

Table 11: Effect of plant extracts on growth parameters of peanut plants under greenhouse conditions during 2015 and 2016 growing seasons.

Plant extracts	Pathogenic fungi	Fresh weight g/plant			Dry weight g/ plant			Plant height cm /plant			Number of pods / pot			Weight of pods/pot		
		2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
Garlic	*F. solani* (V)	17	21	19	6	6	6	28	34	31	28	32	30	47	52	49.5
	F. solani (VII)	17	23	20	6.3	6.5	6.4	26	30	28	20	25	22.5	41	50	45.5
	F. oxysporum (I)	17	23	20	6.3	7	6.65	35	40	37.5	18	22	20	35	43	39
Neem	*F. solani* (V)	16	14	15	5.8	6	5.9	30	31	30.5	21	23	22	46	46	46
	F. solani (VII)	14	15	14.5	4.8	5	4.9	26	27	26.5	18	22	20	43.5	45	44.25
	F. oxysporum (I)	19	19	19	6.5	7	6.75	36	37	36.5	18	21	19.5	36	40	38
Mint	*F. solani* (V)	14	16	15	4.5	5	4.75	29	30	29.5	25	23	24	51	46	48.5
	F. solani (VII)	17	19	18	6	6	6	31	31	31	25	22	23.5	46	44	45
	F. oxysporum (I)	17	17	17	6	6.2	6.1	36	35	35.5	15	17	16	31	33	32
Control	*F. solani* (V)	9	9	9	3.1	3	3.05	23	22	22.5	10	11	10.5	20.5	22	21.25
	F. solani (VII)	9	8	8.5	3.4	3	3.2	25	22	23.5	6	9	7.5	12.5	18	15.25
	F. oxysporum (I)	10	9	9.5	3.5	3	3.25	26	23	24.5	10	9	9.5	18.5	18	18.25
LSD at 5%	Plant extract (A)	1.44	2.66	-	0.28	0.32	-	3.31	2.48	-	2.14	2.83	-	1.90	3.52	-
	Pathogen (B)	1.47	1.75	-	0.25	0.23	-	1.79	1.80	-	1.69	2.06	-	2.64	2.92	-
	Interaction (A×B)	2.94	3.49	-	0.49	0.46	-	3.57	3.6	-	3.39	4.13	-	5.27	5.84	-

On the other hand, data indicated that number of pods /pot increased when peanut plants received plant extracts. In this respect, garlic extract gave a positive effect on number of pods, followed by mint extract, while neem extract exhibited lower effect in increasing pods/ pot. So, the plant extracts of garlic followed by neem when added on peanut plants positive by affected weight of pods /plant more than mint extract and control plant. Generally, plant extracts exhibited the higher increase of fresh, dry weight of peanut plants, plant height number of pods and weight of pods when compared with growth parameters of control.

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