Int. J. Adv. Res. Biol. Sci. 2(11): (2015): 77–86

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

SOI: http://s-o-i.org/ 1.15/ijarbs-2-11-12

Efficiency of some Bioagents and Nemastop compound in controlling damping off and root rot diseases on peanut plants

Mohamed A. Mahmoud

Plant Pathology Research Institute, Agricultural Research Center, Giza 12619, Egypt
*Corresponding author: *m.a.mahmoud75@gmail.com*

Abstract

Bacillus subtilis Cohn, *Trichoderma harzianum* Rifai (T1), *Trichoderma viride* Harz. (T2), mixture of both (T1 and T2) in addition to the experimental product Nemastop were used to control the casual organisms of damping off and root rots disease (*Rhizoctonia solani* Kuhn. *Fusarium solani* Mart) on peanut plants. The antagonistic effect of different bioagents was tested against *F. solani* and *R. solani in vitro*. All bioagents significantly reduced mycelial growth of the pathogenic fungi. *T. harzianum* gave the most reduction effect on the pathogenic fungi. The antagonistic study between different bioagents and pathogenic fungi were tested under microscopic examination by slide technique. All bioagents were destructive the mycelial growth of the pathogenic fungi by penetration, malformation and lysis compared with control treatment**.** Results obtained from field experiments were in harmony with those obtained from laboratory using biopreparations led to best effect compare with control treatment. Efficacy of the treatment which used as seed dressing at sowing achieved highest effect in controlling damping off and root rot disease and increased peanut yield. Mixture of *Trichoderma* spp. isolates were the most effective followed by Nemastop compared with control treatment under field conditions.

Keywords: Bioagents, Damping off, Root rot and Peanut.

Introduction

Peanut is a legume crop that belongs to the family of Fabaceae, genus Arachis, and botanically named as *Arachis hypogaea*. Peanuts are consumed in many forms such as boiled peanuts, peanut oil, peanut butter, roasted peanuts, and added peanut meal in snack food, energy bars and candies (Settaluri et al ., 2012).

Soil and seed borne fungi could attack the root, stem and pods causing quantitative and qualitative damages as well as increasing soil infection. Soil texture affected incidence of root and pod rot of peanut caused by several fungi, i.e. *Fusarium* spp. *Macrophomina phaseolina*, *Rhizoctonia solani* and *Aspergillus niger* as well as affected fungal growth in soil during peanut growing (Ismail and Abd EL-Momen, 2007). Damping off and root rot are considered as destructive diseases and cause great losses in many parts of the world caused by *R. solani* and *F. solani* (Celar, 2000). *F. solani* usually survives as thick-walled chlamydospores in soil (Nawar, 2007). *R. solani* Kuhn (*Thanatephorus cucumeris* Frank, Donk) causes serious losses of plants in many parts of the world. Pathogenesis has been attributed to the action of several different enzymes that degrade cell walls (Wilhelm, 1998). The fungus is an economically important pathogen of many crops worldwide.

Fungicides are widely used as seed or soil treatment to depress various root diseases. However, use of fungicides causes environmental hazards and development resistance pathogen races. Control the diseases using chemical fungicides lead to pollution of atmosphere and negatively affect the properties of treated plants. Since the end of the Second World War, there has been a great boom in the use of fungicides

throughout the world. Biological control is one of the most promising and safe measure in field of plant protection. Antagonistic microorganisms as an alternative approach to fungicides and as biocontrol agents have been used effectively, since 1980, in controlling root rot diseases (Abd El-Moity, 1981).While Trichoderma is recognized primarily as an antagonist against pathogenic fungi, it also has been reported to be antagonistic to certain bacterial pathogens. The antagonistic activity of the genus *Trichoderma* spp. is well documented as effective biological control agents of plant diseases caused by soil borne fungi (Mclean et al., 2004).

Plants treated with *Trichoderma* spp. in the root zone can produce higher levels of peroxidase, chitinase, deposition of callose-enriched wall appositions and pathogenesis-related proteins on the inner surface of cell walls. Moreover, some strains might enhance plant growth and development (Howell et al., 2000). Many researchers have demonstrated the potential of Trichoderma spp. in controlling damping-off and wilt diseases of crop plants caused by *R. solani* and *Fusarium* spp. (Dubey et al., 2007).More recent research indicated that certain strains of *Trichoderma* spp. can induce systemic and localized resistance to several plant pathogens.

T. harzianum ALL42 was capable of overgrowing and degrading the mycelia of *R. solani* coiling around the hyphae with formation of appressoria and hook-like structures. Hyphae of *T. harzianum* ALL42 did not show any coiling around Fusarium sp. a secretary analysis was used and identify some extra cellular proteins secreted by *T. harzianum* ALL42 after growth on cell wall of Fusarium sp. and *R. solani*. These proteins are Endo chitinase, Beta -glucosidase, alpha-1, 3-glucanase. (Monteiro et al., 2011).

Trichoderma spp. and *Bacillus* spp. are the most feasible biocontrol microorganisms suppress several pathogens like *F. solani*. Several reports clarified that the basic mechanisms of *B. subtilis* produced extracellular antibiotics metabolites or enzymes (e.g. proteases, chitinases and glucanases), stimulation of host defenses, incensement of plant growth, induced systemic resistance in plants, and suppression of the plant diseases (Morsy et al., 2005)

The antibiotic production by some bacteria including *Bacillus* spp. plays a role in disease suppression. The authors extracted crude antifungal substances from *B. subtilis* "SJ2" (was isolated from the sclerotia of *R. solani*). The extracted substances completely inhibited the growth of *R. solani*. The antifungal substances of *B. subtilis* "SJ2" were purified and identified as Iturin. These compounds caused an inhibition of spore germination and germ tube swellings of *R. solani* hyphae (Cook et al., 1995).

Mixing antagonists with each other's might be lead to antagonistic effect consequently decrease efficacy of treatment or lead to synergistic effect and increase the efficacy (Robinson et al., 2009). Mixture of two *T. harzianum* isolates showed synergistic effect against *R. solani*. The synergistic effect of the mixture might be due to the fact that different isolates produce their toxic substances (gliotoxin) in consequence periods, this lead to increase the establishment of *T. harzianum* in the soil and consequently increase the effect of mixture than single isolate (Yobo et al., 2011).

The present study was conducted to control the pathogens causing damping –off and root rot on peanut plants. Different biocontrol agents *T. harzianum*, *T. viride*, mixture of both and *B. subtilis* as well as Nemastop are used to improve protection effect against these diseases and to increase the peanut yield under both field and laboratory conditions in Petri dishes and under microscopic examination by slide technique..

Materials and Methods

Bioagents and biocide

Different biocontrol agents *B. subtilis*, *T. harzianum* (T1), *T. viride* (T2), mixture of both T1and T2 and a commercial biocide Nemastop were kindly obtained from central lab of Organic Agricultural Research Center, Giza, Egypt. *B. subtilis* was grown on nutrient glucose broth (NGB) suggested by (Dowson, 1957) *Trichoderma harzianum* (T1), *Trichoderma viride* (T2) were grown in liquid gliotoxin fermentation medium (GFM) (Brian and Hemming, 1945). The bioagents were allowed to grow under complete darkness for nine days just to stimulate toxin production at 28^{o}C (Abd-El-Moity and Shatla, 1981). Different bioagents were formulated as suspension using method developed by (Abd-El-Moity, 1985). Prepared suspension was adjusted to contain 30 x 10^{6}cfu /ml and mixture of them was added as (1:1). Nemastop was used as commercial biocide to compare its effect with other bioagents against damping off and root rot disease on peanut plants. Peanut shoot weight, shoot length, root weight, root length and yield were determined.

Isolation and identification of damping off and root rot causal organisms

Isolation trials from soil and naturally infected seeds and roots of peanut plants showing typical symptoms of damping off and root rot disease which grown in El-Bustan Research Station, Nubaria, Behira governorate during the growing season 2011. Seeds and roots of the infected peanut plants and soil rhizosphere samples were collected from different infested field. Diseased peanut seeds and roots showed identical symptoms were washed with tap water to remove adhering soil particles. Infected roots and seeds were surface sterilized using sodium hypochlorite solution (3%) for 3 minutes, and washed with distilled sterilized water several times. Sterilized plant materials were cut into small pieces then dried using sterilized filter paper and transferred into Petri-plates containing water agar medium. The inoculated plates were incubated at 25°C for 7 days.

One gram from the rhizosphere of collected diseased plants was added to a 250 ml flask contained 99 ml sterile water to make dilution of 1/100. Flasks were shaken on electric shaker for 1 hour. Serial dilution in sterilized water *i.e.* $10^{-4}$and 10^{-6} were prepared. One ml of each dilution was poured in sterilized petri dish; contain 10 ml of water agar medium to isolate fungi. The isolated the fungi were purified using single spore or hyphal tip technique (Dhingra and Sinclair, 1985). The developed fungi were carefully picked up and transferred to gliotoxin fermentation agar (GFA) slants medium (Brian and Hemming, 1945). Fungal cultures were purified using single spore technique adopted by (Hansen, 1926). Isolated fungi were identified according to their culture and morphological characteristics according to the keys described by (Hansen, 1926; Burnett and Hunter, 2003). The purified cultures were identified as *Rhizoctonia solani* and *Fusarium solani*. The identification was confirmed at the Taxonomy Mycological Research Department, Agricultural Research Center Giza, Egypt.

Pathogenicity test under greenhouse conditions

Pathogenicity test for the isolated fungi were carried out under greenhouse conditions in 2012 growing season to select the most pathogenic fungus. Inocula were prepared by growing each of the isolated fungi *F. solani* and *R. solani* in 500 ml. conical flask containing 200 ml of autoclaved corn meal broth media and incubated at 25ºC for 10-15 days (Abd-El-Moity, 1985). The fungal growth was blended in the blender for two minutes using sterilized water to homogenize the inocula. Plastic pots 30 cm in diameter were sterilized by immersing them in 5% formalin solution for 15 minutes, then left to dry for 7 days to ensure getting rid of and evaporation the excess poisonous of formalin. The sterilized plastic pots were filled with sterilized sandy soil. Infestation was carried out by adding the blended homogenized fungal inocula to sterilized soil at the rate of 3-5% of soil weight (v\w). The infested soil then watered and left for 15 days to ensure its distribution in the soil. Control pots were watered with the used medium free from the fungus at the same rate. Four pots were used for each particular treatment. Five seeds were sowing in each pot. All plants were observed daily and watered as needed. Pre and post emergence were recorded after 5-10 days from planting (Paternote, 1987). Healthy survival plants were recorded after 15 days from inoculation. Re-isolation of the soil borne pathogenic fungi was carried out to confirm the pathogenicity test. The most aggressive isolate of each pathogenic fungus was used *in vitro* experiments.

Effect of some bioagents on the mycelial growth of pathogenic fungi

Under laboratory conditions different bioagents were evaluated for their antagonistic effect against pathogenic fungi (*R. solani* and/or *F. solani*). Petri dishes 9.0cm in diameter each contains 15ml of GFM were used to detect the antagonistic effect between isolates fungi and pathogenic one.

On the other hand, plates containing NGB medium were used to determine the effect of bacteria against pathogenic fungi. Different plates were inoculated with discs (6mm in diameter) of pathogenic fungi obtained from the periphery of 4 days old colony. The pathogenic fungus was inoculated at one side where as the opposite side was inoculated with either disc of bioagent fungus (0.5mm in diameter) obtained from 3 days old culture or with loop full of antagonistic bacteria grown on liquid NGB medium for 48 hours. Five plates were used for each treatment. Plates only inoculated with the pathogenic fungi served as a control treatment. Inoculated plates were then incubated at 25°C. When mycelial growth covers all medium surfaces in control treatment, all plates were then examined and percentages of reduction in mycelial growth of pathogenic fungi were calculated using the next formula:

$$X = 100 – [G2- G1/G2 \times 100]$$

Where X= % of reduction
G1: growth of pathogenic fungi in control plates.
G2: growth of pathogenic fungi in treated plates.

The interaction between the pathogens antagonists:

A laboratory technique was used to illustrate the effect of different bioagents against pathogens under studies (*R. solani* and *F. solani*). In this technique a sterilized microscope glass slide was covered by a thin film sterilized diluted GFA medium (0.1N). Disc from the antagonist was inoculated at one side whereas the disc from the pathogen was inoculated at the other side of the glass slide. Inoculated slides were placed in sterilized Petri- dishes containing two filter papers (Whatman N0.1) saturated with 10 ml of sterilized distilled water just to maintain humidity around the inoculated slides. Plates with slides were incubated at 18ºC. Slides were examined periodically using light microscope with fixed camera to observe interaction between the pathogen and the antagonist**.**

Field experiments

Field experiment was carried out at botanical garden, botany and microbiology department, college of science, King Saud University in April 2012 and 2013 growing seasons. A randomized complete block design with 4 replications was used in each season. A field experiment consisted of plots (7 × 6 m); each comprised of 10 rows 20 cm distance and 30 holes/row at which were conducted with five plots as replicates for each particular treatment as well as untreated check treatment. *B. subtilis*, *T. harzianum* (T1), *T. viride* (T2), mixture (T1and T2) and nemastop were used as seed coating.Suspension was used as soaking bean seeds at the rate of 100 m/kg seed and left for 30 minutes before sowing. Plants only sprayed with water act as control treatment. Peanut seeds Giza 6 obtained from Field Crop Research Institute, Agricultural Research Centre, Giza. Pre emergence damping-off percentages were determined after a week from sowing date and post emergence damping-off percentages were also recorded after 21 days. Also, percentage of root rot and yield components per 42 m^2 were determined. All treatments received the same agricultural treatment such as amount of water, number of seeds /plot and amount of fertilizers.

Biocontrol agents were used in liquid form was applied as seed coating in the suspension for 20 minutes at five liters of each bioagent / plot. Treated plants were examined periodically for damping off till 35 days. Disease of damping off and root rot were also estimated. At harvest some peanut parameters such as shoot length (cm) shoot weight, (g) root length (cm), root weight (g) and yield of pods with kilogram per plant/plot were determined at harvest (after 150 days from planting)and the efficacy of each bioagents were recorded.

Statistical Analysis

Data obtained were subjected to statistical analysis of variance for completely randomized design (CRD) and randomized complete block design (RCBD) as outlined by Snedecor and Cochran (1980)

Results and Discussion

Isolation and identification of damping off and root rot causal organisms

Data in Table (1) show the percentage frequency of the pathogenic fungi (*F. solani* and *R. solani*) which showed identical symptoms from roots and pods peanut plants and soil samples collected through the experimental. The purified isolated pathogens were identified as *F. solani* and *R. solani*. Data in Table (1) also indicate that, three isolates from soil, infected seeds and roots were the most frequently isolated fungi. *Fusarium solani* Mart. was the most frequently isolated pathogenic fungi from infested soil (71.67%) followed by *R. solani* (63.33%) from samples roots of peanut plants collected through the experimental duration. These results are in harmony with (Ismail and Abd EL-Momen, 2007). *Fusarium solani* Mart. usually survives as thick-walled chlamydospores in soil (Nawar, 2007).

Table (1): The percentage frequency of soil borne pathogens attacked peanut plants

Isolated fungi	Frequency of isolated fungi	
	F. solani	***R. solani***
(1) from (seeds)	53.33	33.33
(2) from roots	40.00	63.33
(3) from soils	71.67	43.33
L.S.D. at 5%	20.52	23.77

Pathogenicity tests under greenhouse conditions

Data in Table (2) show that peanut plants were highly attacked by different three isolates *F. solani* and *R. solani*, the tested fungi caused damping-off disease to their respective hosts, where it significantly decreased the survival seedlings. *Fusarium solani* isolate 1 from soil was highly pathogenic while, *Rhizoctonia solani* isolate 2 was the most aggressive to all peanut plants. *Rhizoctonia solani* may infect plants at any stage of development and might cause seed decay prior to emergence (Brenneman, 1997). Root and stem rot disease caused by *Fusarium solani* is common on peanut plants (Paternote, 1987)).

Table (2): Pathogenicity test of three different isolates of *Rhizoctonia solani* and *Fusarium solani* on peanut plants

Isolates	% Disease incidence peanut plants as damping off and survival					
	Fusarium solani			*Rhizoctonia solani*		
	Pre	Post	Survival	Pre	Post	Survival
Isolate 1	30	55	15	51.67	25	23.33
Isolate 2	23.33	43.33	33.33	33.33	40	26.76
Isolate 3	41.67	30	31.67	35	36.67	28.33
L S D at %5	9.41	12.0	18.83	12.46	10.53	14.13

Effect of some bioagent on the mycelial growth of pathogenic fungi

Data in Table (3) indicat that *T. harzianum* were the most effective antagonists against the two soil borne pathogenic fungi under test which recorded 85.6 % in *F. solani* and 81.85% in *R. solani*. This high potentiality in antagonism might be due to that *Trichoderma* spp. act through different mechanisms including mycoparasitism (Abd El-Khair et al., 2010). Also through production of anti-fungal substances (Hayes, 1992). *Trichoderma* spp. also acts through production of destructive enzymes *i.e.* chitenase (Bolar et al., 2000). The antagonistic effect of *Trichoderma* spp. **(***T. harzianum* and *T. viride*) was tested against *F. solani* and *R. solani in vitro* studies. They indicated that all tested *Trichoderma* spp. significantly reduced the mycelial growth of two pathogenic fungi (Abd El-Khair et al., 2010).

Table (3): Effect of different bioagents on the percentage reduction of mycelial linear growth of *F. solani* and *R. solani*

Percentage of reduction in the linear growth		
Bioagents	***F. solani***	***R. solani***
Bacillus subtilis	57.41	54.82
Trichoderma harzianum(T1)	85.6	81.85
Trichoderma viride (T2)	83.33	81.52
Control	0.0	0.0
LSD at 5 %	13.75	8.10

Studies on the interaction between the pathogens antagonists

This technique provides a clear view for the interaction between antagonists and pathogen malformation, lysis or mycoparasitism. The morphological changes in the hyphal growth of the pathogenic fungi (*Fusarium solani* and *Rhizoctonia solani*) due to the effect of the tested antagonistic fungi (*T. harzianum* T_1 and *T. viride* T_2) and bacteria (*B. subtilis*) were illustrated in Fig. 1 (a, b and c) show penetrated, malformed and lysis hyphae of the two tested pathogens whereas Fig. (1d) show the normal hyphae of the two pathogenic fungi. Malformed mycelium changes in colors to deep dark, also increased in the wide of the infected mycelium. The same figures also show the penetrating *Trichoderma harzianum* (T_1) of developed hyphae inter and destructive the mycelial of the two pathogenic fungi. The malformation caused by *T. viride* (T_2) might be due to the effect of toxic substance produced by bioagents metabolites (Dennis and Webster, 1971). A clear lytic area started to appear in the hyphae of the pathogenic fungi due to antibiosis release from *Bacillus subtilis.* Some isolates of

T. harzianum work through different mechanisms, *i.e.* production of gliotoxin, mycoparasitism and grow quickly on many substrates, produce metabolites with demonstrable antibiotic activity and might be mycoparasitic to virulent pathogens (Abd-EL-Moity,

1981). *B. subtilis* also showed considerable effect in pathogenic fungi. This might be due to the production of more than antibiotic (Bacteriocin, Subtilisin and Antibiotics), act as inhibitors to pathogenic fungi (Nielsen, et al., 2002).

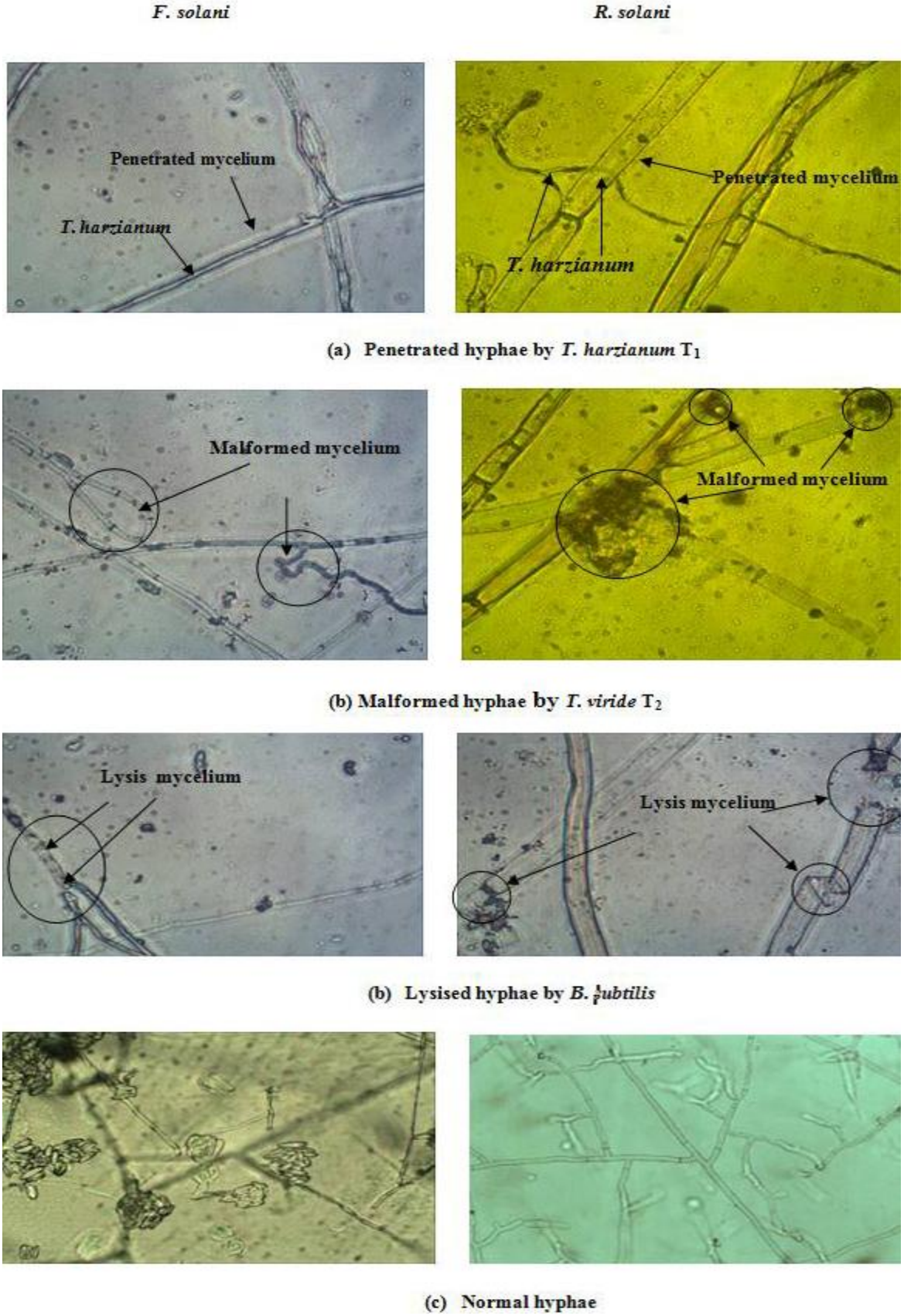

Fig. (1): Interaction between bioagents and pathogenic fungi

Using *Bacillus* spp. is a common means of biocontrol agents for fungal pathogens. These bacteria produce substances harmful to fungi, such as hydrogen cyanide, peptides, proteases, and chitinases (Farahat, 1998). It can be inhibiting the growth of *R. solani* and possibly as a result of a chemical signal which produced by bacteria to produce antifungal agents (Ghisalberti 2002; Abd-El-Moity et al., 2003).

Obtained data in Table (4) show that, all antagonists significantly reduced the percentage of disease incidence, disease severity and increased the survival seedlings of peanut compared with untreated (control). Mixture of *Trichoderma* isolates occupied the first rank in increasing survival plants (100%) followed by nemastop as commercial biocide in two tested seasons (92.5 and 96.93%) respectively. On the other hand, the percentage of root rot disease were decrease by using mixture of Trichoderma isolates which recorded in two seasons (3.4 and 2.5%) respectively. *T. harzianum* (T1) was the least effective one was recorded in the two successive seasons. The effect of mixture can be explain by (Abd-El-Moity, 1985)who stated that, this synergistic effect might be due to complementary effect between different isolates included in the mixture. This means that, one isolate produced antifungal substance (Hayes, 1992) whereas the second isolate has high potentialities in mycoparasitism (Abd-EL-Moity, 1981), while the third one induce plant resistance (Bolar et al., 2000). The combination between these different effects results in high effect in controlling diseases and nematodes. *Trichoderma* spp. are common inhabitants of the rhizosphere and well recognized as biocontrol agents of soil borne plant pathogens (Howell et al., 2000).

Table (4): Effect of different bioagents on the percentage of damping off and root rot disease incidence on peanut plants during two seasons under field conditions (2012/2013).

Treatments	Percentage of damping off and root rot disease severity							
	2012				2013			
	Pre	Post	Root rot	Survival	Pre	Post	Root rot	Survival
Bacillus subtilis	6.7	7.7	12.1	85.6	5.6	6.7	13.5	87.7
Trichoderma harzianum (T1)	8.9	9.0	16.7	82.1	8.9	7.6	18.0	83.5
Trichoderma viride(T2)	7.8	8.0	13.3	84.2	6.7	6.7	10.3	86.6
Mixture of (T1 and T2)	0.0	0.0	3.4	100.0	0.00	3.3	2.5	96.7
Nemastop	3.5	4.0	4.9	92.5	1.00	2.7	4.9	96.30
Control	10.0	12.0	53.6	78.0	13.3	12.2	53.6	74.5
LSD at 5 %	0.87	1.15	2.82	6.18	0.89	0.85	3.43	6.37

Several mechanisms have been considered to be key factors in antagonistic interactions: lysis of host cell walls, antibiosis, competition for nutrients, induced resistance in plants, and inactivation of host enzymes .The combination between these different effects results in high effect in controlling diseases and nematodes. Also treatments with bioagents contain growth regulators which increase plant nutrients uptake and plant growth (Harman et al., 2004). Mixing more than one isolate lead to increase number of antifungal, consequently increase the effect of this treatment. Thus, strains of *T. harzianum* have been commonly used as agents for the biocontrol of plant pathogenic fungi such as *R. solani* and *F. solani* (Rojo et al., 2007).

Data in Table (5) showed that caused significant differences andpositive correlation between using different bioagents as seed treatments and peanut improvement compare with control treatment. This may be due repeated treatment with bioagents which increase growth regulators and increase growing roots (Chang et al., 1986) and also may increase plant nutrients uptake [34, 37]). All treatments either bioagent alone or in combination with each other as well as commercial biocide led to high significant effects on yield**,** shoots and roots (weight and length). Increase in characteristic of yield led to significant increase in efficacy of the treatment. This increase in efficacy is due to increase establishment of the antagonist (Howell et al., 2000). Increase disease reduction led to increase in yield of treated plants. This increase in yield is due to vigorty of healthy plants, in addition to growth regulators are produced by *Trichoderma* spp. which improve photosynthesis metabolism in treated plants (Govindappa et al., 2011). This improvement led to increase in pods yield and dry matter. Bioagents do not only affect outside the treated plants but also affect metabolism inside plant and lead to changes in plant component (Hafez et al., 2012). Data at two seasons (2012/ 2013) also recorded that all agronomic characteristic increased in second seasons than first one .Mixture of *Trichoderma*

isolates occupied the first rank on increasing yield (44.58 and 57.39kg/plot) compared with control treatment. To improve and increase effects of biocontrol agent's different attempts were carried out by mixing more than one bioagent. This attempt was carried out as early by (Abd-El-Moity, 1985) who reported that using mixture of *T. harzianum* led to more effect than using single one. This may be to compatible relation between mixture of *Trichoderma* isolates, led to synergistic effect between them and increasing the uptake of macro and micro nutrients and improving all vegetative growth and yield (Robinson et al., 2009). Mixing different isolates increase the scope of mode of action consequently increase efficacy of the treatment. *Trichoderma* spp. were used in mixture a clear synergistic effect was appeared. This is due to that *Trichoderma* spp. act through different mechanisms, some isolates act as mycoparasitic (Matei and Matei, 2008) where the other act through production of antifungal substances such as Endo chitinase, Beta-glucosidase, alpha-1,3-glucanase (Monteiro et al., 2011) and trichodermin (Balode, 2010), other isolates compete for space or nutrients. Mixing more than one isolate lead to increase number of antifungal, consequently increase the effect of this treatment. This increase in yield may be due to that rhizosphere organisms produced greater amount of organic acids, such as tartaric, citric acid and lactic acid which may improve plant productivity and increase the root system (Abd-El-Moneim et al., 2006). Biocontrol agents improve the health of plants and thus contribute to overall productivity. These agents are also self-propagating under favorable conditions, and therefore, may remain in the soil for a long period. Length of intervals between treatments has significant effect on the plant health and the amount of pods yield. Results showed that, peanut plants treated with bioagents as seed treatment were more health and produce high 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 (Bolar et al., 2000). Nemastop was the most effect in increasing shoots (weight and length) and root (weight and length) at two seasons followed by mixture of *Trichoderma* isolates except *Trichoderma viride* (T2) effect on roots weight (60.46g) at season 2013. Several modes of action of the efficiency bioagents on reducing plant diseases have been described, including competition for nutrients, antibiosis, induced resistance, mycoparasitism, plant growth promotion and rhizosphere colonization capability (Abd-El-Moneim and Maisa, 2011). This relation cannot be detected in treatments with slight differences when compared with each other. Increase of growth parameters also due to two factors, healthy root system can absorb and supply adequate amount of raw nutrient substances, the second factor is syntheses of these raw nutrient material effectively in presence of high amount of chlorophyll and protein, resulted more pods yield.

Table (5): Effect of different biocontrol agents in improving agronomic characteristic on peanut plants.

Treatments	Shoots weight g.		Shoots length cm.		Roots weight g.		Roots Length cm.		Yield kg / plot	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Bacillus subtilis	71.96	133.2	35.33	40.67	31.67	37.43	28.33	30.67	26.08	37.39
*T. harzianum*T1	92.09	143.26	30.33	34.57	34.67	46.29	30.33	37.23	31.27	39.05
T. viride T2	84.41	113.67	31.33	42.00	33.90	60.64	29.00	40.00	36.00	49.63
Mixture of (T1+T2)	132.53	175.96	36.33	46.00	34.67	57.31	31.00	38.90	44.58	57.39
Nemastop	154.26	178.37	36.43	48.33	46.33	59.49	34.33	41.33	41.16	55.86
Control	35.47	55.04	22.00	24.13	24.67	32.33	19.33	28.33	13.10	14.36
LSD at 5 %	16.99	28.29	11.43	23.066	5.049	9.160	8.49	6.23	6.56	9.46

References

Abd El-Khair H., Khalifa R.Kh., Karima M.& H. E. Haggag (2010). Effect of *Trichoderma* species on damping off diseases incidence, some plant enzymes activity and nutrition status of bean plants. *J. Am. Sci*., 6(9): 486-497.

Abd-EL-Moity, T. H. (1981). Further studies on the biological control of white rot disease of onion. Ph.D. Thesis, Fac. Agric., Minufiya University.

Abd-El-Moity T.H. (1985). Effect of single and mixture of *Trichoderma harzianum* isolates on controlling three different soil borne pathogens. *Egypt. J. Microbial*., Special Issue, 111-120.

Abd-El-Moity, T.H. and Shatla M.N. (1981). Biological control of white rot disease of onion (*Sclerotium cepivorum*) by *Trichoderma harzianum*. *Phytopathology Z*., 100: 29- 35.

Abd-El-Moity T.H., Abd El-Moneim M.L., Atia M.M.M., Aly A.Z., Tohamy M.R.A (2003). Biological control of some cucumber diseases under organic agriculture. 227-236 Proc. IS on Org. Matter and Substrates Ed A.F. Abou Hadid Acta Hort., 608.

Abd-El-Moneim M.L. and Maisa. F. (2011). Integrated management of damping-off, root and/or stem rots diseases of chickpea and efficacy of the suggested formula. *Nat Sci. Biol*., 3(3): 80-88.

Abd-El-Moneim M.L., Maisa L., Seddik Wafaa M.A. (2006). Nutritional status of onion as affected by biological control of white rot disease. *J. Agric. Sci., Mansoura Univ*., 31(4): 2533-2549.

Balode, A. (2010). Effect of tricodermin, biological product against *Botrytis* in horticultural crops. *Acta Hort*., 877: 1583-1588.

Bolar J.P., Norelli J.L., Wong K.W., Hayes C.K., Harman E. & Aldwlnckle H.S. (2000). Expression of endochitinase from *Trichoderma harzianum* in transgenic apple increases resistance to apple scab and reduce vigor. *Phytopathology*, 90:72-77.

Brenneman T.B. (1997). Rhizoctonia diseases, in Compendium of Peanut Diseases, Kokalis-Burelle N., Porter D.M., Rodriguez-Kabana R., Smith D.H., Subrahmanyam P., Eds., 2nd edition, pp. 30-31, APS Press, St. Paul, Minn, USA,

Brian, P.W. and Hemming H.G. (1945). Gliotoxin as fungistatic metabolic product of *Trichoderma viride*. Ann. Appl. Biol., 32, 214-220.

Burnett H.L. and Hunter B.B. (2003). Illustrated genera of imperfect fungi.4th ed. Burgess Pub. Co. Minneapolis, Minnesota, USA.

Celar F. (2000). Cucurbit diseases. *Sodobno Kmetijstvo*, 33 (4): 162-165.

Chang Y.C., Baker R, Kleifeld O, & Chet I. (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. *Plant Dis*., 70, 145-148.

Cook R.J., Thomasow L.S., Weller D.M., Fujimoto D., Mazolla M., Bangera G. & Kim, D.S. (1995). Molecular mechanisms of defense by rhizobacteria against root disease. Proc. Natl. Acad. Sci. U.S.A., 92, 4197-4201.

Dennis C. and Webster J. (1971). Antagonistic properties of species group of *Trichoderma*. III. Hyphal interaction. *Trans. Br. Mycol. Soc.,* 57: 363-369.

Dhingra O.D. and Sinclair J.B. (1985). Basic Plant Pathology methods. CRC press, Florida, 355.

Dowson W.J. (1957). Plant diseases due to bacteria second Ed., Cambridge the university Press, London, pp23.

Dubey S.C., Suresh M. & Birendra, S. (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilts. *Biol. Control*., 40: 118-127.

Farahat, A. (1998). Biological control of some potato bacterial diseases. Ph.D. Thesis, Fac. Agric., Minufyia University.

Ghisalberti E.L. (2002). Anti-infective agents produced by the hyphomycetes Genera Trichoderma and Gliocladium. *Current Medicinal Chemistry-Anti-infective Agents*, 1(4): 343-374.

Govindappa M., Lokesh S., Naik V.R., & Raju S.G (2011). Induction of systemic resistance and management of safflower *Macrophomina phaseolina* root-rot disease by biocontrol agents. *Arch. Phytopathology Plant Protect*., 43: 1/3: 26-40.

Hafez E.E., Balbaa M.M., Kabeil S.S., El-Saadaniand M.A., & Ahmed S.A. (2012). Molecular studies on the biocontrol effect of *Trichoderma viride* and *Bacillus subtilis* on *Fusarium oxysporum* and *Rhizoctonia solani* infected tomato plants. *World Applied Sciences J*., 19(1): 89-99.

Hansen H.N. (1926). A simple method of obtaining single spore cultures. *Sci*, 64: 384-389.

Harman G.E., Petzoldt R., Comis A. & Chen, J. (2004). Interactions between *Trichoderma harzianum* strain T22 and maize intred line M017 and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola. Phytopathology,* 94: 147-153.

Hayes C.K. (1992). Improvement of Trichoderma and Gliocladium by genetic manipulation, 227- 266. In Biological Control of Plant Diseases Progress and Challenges for the future. Jamos E.C., Papavizas G.C. and Cook R.J. (Eds) Plenun Press, New York and London published in cooperation with NATO Scientific Affairs Division, 462.

Howell C.R., Hanson L.E., Stipanovic R.D. & Puckhaber L.S. (2000). Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Journal of Phytopathology*, 90: 248-252.

Ismail F.M. and Abd EL-Momen S.M (2007). Effect of some soil amendments on yield and disease incidence in peanut (*Arachis hypogaea* L.). *Egypt. J. Agric. Res*., 85(2): 379-399.

Matei G.M. and Matei S. (2008). Research on isolation, characterization and testing the interaction between *Trichoderma harzianum* and

Botrytis cinerea for biological control of gray mold in strawberry. *Horticultura*, 51: 653-657.

Mclean K.L., Dodd S.L., Sleight B.E., Hill R.A. & Stewart A. (2004).Comparison of the behavior of a transformed hygromycin resistant strain of *Trichoderma viride* with the wild type strain. N. Z. *Plant Prot*., 57: 72-76.

Monteiro S., Grondona L., Llobell M.I., & Monte A. (2011). *In vitro* antifungal activity of *Trichoderma harzianum, T. longibrachiatum, T. asperellum* and *T. atroviride* against *Botrytis cinerea* to strawberry. *Bulletin OILB/SROP*, 25(10): 253-256.

Morsy E.M., Abdel-Kawi & K.A. Khalil M.N. (2005). Efficiency of *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents against *Fusarium solani* on tomato plants. *Egyptian J. of Phytopathology*, 37(1): 47-57.

Nawar L.S. (2007). Pathological and rhizospherical studies on root rot disease of squash in Saudi Arabia and its control. *Afr. J. Biotechnol*., 6 (3): 219-226.

Nielsen T.H., Sørensen D. C., Tobiasen J.B., Andersen C., Christophersen M., Givskov A. & Sørensen J. (2002). Antibiotic and biosurfactant properties of cyclic lipopeptides produced by fluorescent *Pseudomonas* spp. from the sugar beet rhizosphere. *Appl. Environ Microbiol*., 68: 3416-3423.

Paternote S.J. (1987). Pathogenicity of *Fusarium solani* f. sp. *cucurbitae* race 1 to courgette. *Neth. J. Plant Pathol*., 93: 245-252.

Podile A.R. and Kishore G.K. (2002). Biological control of peanut diseases. In Biological control of crop disease edited by Samuel S. Gnamanickam.CRC press.

Robinson L., Jeger M.J. & Xiangming P. (2009). Management of strawberry gray mould using mixtures of biocontrol agents with different mechanisms of action. *Biocontrol Sci. Technol*., 19: 1051-1065.

Rojo F.J., Reynoso M.N., Ferez M., Chulze S.N., & Torres A.M. (2007). Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. *Crop Prot*., 26: 549-555.

Settaluri V.S., Kandala C.V.K., Puppala N., & Sundaram, J. (2012). Peanuts and their nutritional aspects-a review. *Food and Nutrition Sciences*, 3:1644-1650.

Snedecor G.W. and Cochran W.G. (1980). Statistical Methods. Oxford and J.PJ. Publishing Comp, 7th edition.

Wilhelm S. (1998). Fungal diseases of the root and crown. In: Compendium of Strawberry Diseases (Ed. Maas J.L.), The American Phytopathological Society, St. Paul, MN, 138 p.

Yobo K.S., Laing M.D. & Hunter, C.H. (2011). Effects of single and combined inoculations of selected Trichoderma and Bacillus isolates on growth of dry bean and biological control of *Rhizoctonia solani* damping off. *Afr. J. Biotechnology*, 10 (44): 8746-8756.