Biocontrol of some cowpea soil-borne diseases and its relation to nitrogen fixing bacteria (*Brdyrhizobium* sp.)

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ABSTRACT

Cowpea is exposed to damping off diseases caused by *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium solani*, and *Macrophomina phaseolina*. Two antagonistic strains were isolated from soil rhizosphere samples of healthy cowpea plants. These antagonistic strains exhibited antifungal activity against all the tested fungal pathogens. One out of the predominant antifungal bacterial isolate was *Bacillus subtilis* and this strain produced the maximum of its metabolites at the end of logarithmic phase. The other antagonist was *Trichoderma longibrachiatum*. *T. longibrachiatum* inhibited completely sclerotia germination of *S. rolfsii*.

Results of in vitro and in vivo experiments confirmed the compatibility of these antagonists with *Bradyrhizobium* sp. Neither the growth of *Bradyrhizobium* sp. nor its N_2-fixation ability was negatively affected by the antagonistic strains. The fungal pathogens affected *Bradyrhizobium* sp. and N_2-fixation parameters.

Seed (peat- based inoculum) or soil (bio-enhanced compost) treatments with each of these antagonists at the time of planting in soil infested (artificially or naturally) under greenhouse or field conditions with any of the pathogens decreased the disease incidence on cowpea plants, increased survival plants and restore of biological symbiotic N_2-fixation.

The effects were similar in more cases to those of Rizolex-T 50, which increased germination; emergence and reduced root rot severity but affected adversely *Bradyrhizobium* sp. and the symbiotic N_2-fixation parameters.

Keywords: Biological control, Soil - borne diseases, Cowpea *Bradyrhizobium* sp.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.), is a major food crop in many countries including Egypt, where its leaves, green

pods and grains are eaten as a dietary source of protein. The cowpea seed contains about 23 % protein and 57% carbohydrate, while the leaves contain between 27 - 34% protein (Belane and Dakora, 2009). Cowpea proved vulnerable to root rot diseases caused by *Fusarium solani* Mart sacc., *Rhizoctonia solani* Kuhn, *Macrophomina phaseolinae*, *Sclerotium rolfsii* and *Pythium* sp., which attack roots causing damping-off and root rot diseases, these diseases cause substantial losses to cowpea crop (Shihata and Gad El-Hak, 1989; Ushamalini *et al.,* 1993; Rauf, 2000; Satish *et al.,* 2000; El-Mohamedy *et al.,* 2006).

Controlling soil borne pathogens depends mainly on fungicidal applications, that causing hazards to the human health and environment (Rauf, 2000). Fungicidal seed treatment is not desirable for disease control due to some adverse effects on the environment and ecosystem, such as harm to nontarget organisms especially beneficial microorganisms such as *Rhizobium*, bioagents, animals, and plants. Fungicides also may induce pathogen resistance, making their effects variable and short lived. In addition, fungicides are expensive in comparison with the relatively low commodity price of field cowpea. Hence, there is a need for an improved soil-borne management system with reduced fungicide use (Cook and Baker, 1983; Belal *et al.,* 1996; Xue, 2003).

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Cook and Baker, 1983; Osman *et al.,* 1986). Several fungi and bacteria have been reported to have antagonistic effects to soil-borne pathogens. Harman (1991); Nelson *et al.,* (1988) reported the use of *Trichoderma hamatum* for the control of *Pythium* seed rot and *Rhizoctonia* root rot in pea. Xi *et al.,* (1996) noted that *Pseudomonas cepacia, P. fluorescens* and *Bacillus subtilis* were effective against the different pathogenic fungi such as *Pythium*, *Aphanomyces*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Fusarium oxysporum f.* spp. when applied as seed treatment (Papavizaz and Lewis, 1984; Callan *et al.,* 1990; Lewis *et al.,* 1995; Belal *et al.,* 1996; Nemec *et al.,* 1996). Soil amendment treatments are gaining importance in management of many plant pathogens as another alternative to chemical fungicides in recent times. Also,

seed coating with bio-control agents was the most effective treatment for controlling root rot diseases as shown by (Loeffez *et al.,* 1986; Ushamalini *et al.,* 1993; Mitra and Nandi, 1994; Abdel-Kader and Ashour, 1999).

Therefore, the present investigation was designed to investigate the potential of seed or soil treatment with the biocontrol agents to reduce damping-off disease incidence and its relation to *Bradyrhizobium* sp.

MATERIALS AND METHODS

Pathogens

Samples of cowpea plants showed typical damping-off and root rot symptoms were collected from different localities of Elbehira governorate, Egypt. All samples were subjected for isolation trials of causal organisms. The purified isolated fungi were identified according to cultural and microscopically characters described by Barnett and Hunter, (1972); Booth, (1977) and Nelson *et al.,* (1988). Pathogenicity tests were performed on cowpea plants and reisolated pathogenic fungi were maintained on PDA slants at 4 °C for further experiments according to the method described by Khalifa, (1991). Pots (30 cm diameter) containing sterilized sand loam soil infested individually with inoculums of each fungus, which was grown on corn meal sand medium (95 gm clean moistened sand : 5 gm corn meal) for two weeks at 28 ± 2 °C. Three pots were used for each fungus. Surface sterilized seeds of cowpea were sown at the rate of 5 seeds / pot. Degrees of disease incidence were recorded as percentages of pre- and post- emergence damping off, and percentages of survival plants were also recorded after 15 and 90 days from sowing. Check treatment (control) was prepared without addition of the tested fungi.

Antagonists

Screening and isolation

Antagonists were isolated from rhizosphere soil samples of healthy cowpea plants by the methods described by Elad *et al.,* (1980) and Belal *et al.,* (1996). The relative power for bacterial antagonists of antibiosis (RPA) was estimated for each isolate as described by Ibrahim *et al.,* (1987), where: RPA = Z/C, Z = diameter of inhibition zone, C = diameter of spotted antagonistic isolate. Estimation of the antagonistic efficiency of the fungal strains were determined according to Bell *et al.,* (1982).

Identification

The most effective antagonists were identified according to Parry *et al.,* (1983) for bacterial strain and Rifai, (1969) for fungal strain.

***Bradyrhizobium* sp. isolation**

Bradyrhizobium sp. was isolated from active nodules on healthy cowpea plants collected from Elbehira Governorates in Egypt according to Vincent, (1970). Brady*rhizobium* – like colonies were subjected to different cultural, biochemical and plant inoculation test for identification according toSomasegaran and Hoben, (1985).

Cultivation of *Bacillus subtilis* (EB8) in nutrient liquid medium for metabolites (antibiotics) production

One hundred ml nutrient liquid medium inoculated with 1 ml of a cell suspension of *Bacillus subtilis* (EB8) (nutrient broth medium, 10^7 cfu / ml, incubated at 30 °C and 150 rpm for 4 days), The culture was incubated at 30 °C and 150 rpm for 7 days. Cells number of the bacterial strain was determined by plating appropriate dilutions of liquid medium onto nutrient agar medium.

The test was standardized as follows: potato dextrose agar (PDA) medium was poured into Petri dishes (9 cm diameter, 15 ml per dish). After solidification, wells were punched in each plate. The plates were inoculated in the side with a disc (5 mm diameter) bearing the mycelium growth from each fungal pathogen culture (five day- old culture). After that, 50 µl of the supernatant were placed in the punched holes (5 mm diameter) in the PDA, where the culture broth was obtained by culturing *Bacillus subtilis* (EB8) in nutrient broth at 30 °C and 150 rpm for 6 days. The culture broth was passed through a sterile membrane filter (0.2 µm). On the other hand, 50 µl of the sterilized liquid medium were placed in the punched holes and this sample was then used for the control treatments. The experiments were performed in three replicates. The plates were incubated at 28 °C until full growth of the control treatments had been achieved. The diameter of the inhibition zone in which growth was inhibited (in mm), surrounding each hole was recorded. The production of metabolites (antibiotics) was determined daily by determination of diameter of the inhibition zone (mm) against the tested pathogens on potato

dextrose agar (PDA), then the inhibition percent was calculated comparing to control.

Effect of *T. longibrachiatum* on sclerotial germination of *Sclerotium rolfsii*

The method was used to evaluate effect of *T. longibrachiatum* based on its ability to infect sclerotia (Whipps and Budge, 1990; Elzahaby and Belal, 2008). An experiment was performed at room temperature (30 °C) in which spores suspension of *T. longibrachiatum* grown on PDA was prepared in distilled sterile water (10^7 spore / ml). Ten sclerotia of *S. rolfsii* were immersed in the spore suspension of *T. longibrachiatum* for 30 min. or in sterile distilled water , then the sclerotia were placed either on water agar or on moist sterile sand in Petri dishes and incubated for 2 weeks. Thereafter, the sclerotia were then assessed to get the percentage of the destroyed sclerotia (not germinated) as a result of treatment with the antagonist comparing with controls free of spore suspension of *T. longibrachiatum.*

Interaction between *Bradyrhizobium* sp. and the tested antagonists

In Vitro

Effects of antagonistic strains on the growth of *Bradyrhizobium* sp. and on symbiotic N_2-fixing efficiency in cowpea plant were tested as described by Belal *et al.,* (1996). To detect any antagonistic effect between bradyrhizobial and antagonistic strains, yeast extract mannitol agar containing bromothymol blue or congo red was poured into Petri dish. Solid medium was cut and removed. The rest half of plate was filled with potato dextrose agar for fungal strain or nutrient agar for bacterial strain and allowed to solidify. Plates were inoculated with *Bradyrhizobium* sp. and the fungal or bacterial antagonistic strain (each on the proper medium) and incubated at 30 °C for 6 days. Three replicates were used for each treatment. The presence or absence of inhibition zones between *Bradyrhizobium* and antagonists were determined.

In Leonard's Jars

Leonard's jars were used for an *in vivo* to evaluate *Bradyrhizobium* sp. isolate and determination of the symbiotic N_2-fixation parameters (No. of nodules/plant, fresh and dry weight of nodules / plant, N_2 % and total N_2 in the shoot system) under sterilized condition after 45 days from seed planting. Jars were filled with clean sand which had bean treated with 0.1 M

HCl and washed several times with tap water and the Jars were autoclaved. Seeds of cowpea were surface-sterilized using standard methods (Somasegaran and Hoben, 1985). The brdyrhizobium inoculation was carried out as seed treatment by a peat – based inoculum at sowing time or soil treatment with bio-enhanced compost. Inoculum preparation and inoculation process were made in the same manner of peat based antagonistic inocula which is described below.

Treatment with antagonists

Seed treatment with peat- based noculum

Antagonists were applied at the time of planting as seed treatment. Peat-based antagonistic inocula were prepared as described by Vincent, (1970) for *Rhizobium* as follow: Blended liquid cultures of antagonists adjusted to 10^7 cfu/ ml were used to impregnate sterilized peat at the rate of 1 ml / g peat. Mixed peat was allowed to mature at room temperature (30 °C) for 48 h before packing in polyethylene bags and stored at 4 °C. Seeds were witted with 10 % Arabic gum water solution, were thoroughly mixed with an amount of peat –based antagonistic inoculum enough to obtain 10^7 cfu / per gram of seeds. Seeds were kept to air drying and then were inoculated with *Bradyrhizobium* sp. before sowing (Belal *et al.*, 1996). Cowpea seeds were dressed with Rizolex-T 50 % at the recommended dose (3 g/kg seeds) then sown.

Soil treatment with bio-enhanced compost

Compost-based antagonistic inocula were prepared as follow: Blended liquid cultures of antagonists adjusted to 10^7 cfu/ ml were used to impregnate compost at the rate of 10^7 cfu / per gram / g compost. Mixed compost was allowed to mature at room temperature (30 °C) for 48 h before packing in polyethylene bags and stored at 4 °C. Soil amended with bio-enhanced compost at the rate of 10 % (w/w of soil) at sowing time. Inoculation with *Bradyrhizobium* sp was carried out as seed treatment as described before. Each treatment was represented by 3 replicates. Jars were irrigated with sterilized water and fertilized with nitrogen free nutrient solution prepared as described by (Allen, 1959). N_2 % and total N_2 were assayed in the shoot by the Kjeldahl methods (AOAC, 1990). Each treatment was represented by 3 replicates. Total nitrogen content = N_2 % × dry weight of plants (Belal *et al.*, 1996).

Control of the tested pathogens on cowpea plants

Antagonists, exhibiting broad spectra and high relative power of antagonism with no effect on both growth of *Bradyrhizobium* sp. and its N_2-fixing efficiency on cowpea plants were used to control the tested pathogens under greenhouse and field applications.

Greenhouse experiment

Pots (30 cm in diameter) were used in the season were filled with clay soil and cowpea seeds were planted (5 seeds/pot). Pathogens were added to soil one week before planting. Inocula of pathogens were prepared by growing on corn meal sand medium at 28-30°C for 15 days. Soil infestation was carried out one week before planting at the rate of 2% (Khalifa, 1991 and Belal *et al.,* 1996). Infested soil was mixed thoroughly and moistened every other day. Antagonists were applied at the time of planting as seed or soil treatment as described before.

Field experiment

One field experiments were carried out in naturally heavily infested soil with cowpea root rot pathogens at Elbehira Governorate. Antagonists and *Bradyrhizobium* sp. were inoculated as described before. Field experiment consisted of 30 plots (2 m x 5 m) each comprised 5 rows and 12 pits/holes/row, which were conducted in randomly complete block design with 3 replicates (plots) for each particular treatment as well as control (check treatment). Cowpea seeds were sown in all treatments at the rate of 3 seeds/pit. All plots were sown on the first March, summer cultivation (plantation) season. Cultivated plots received the traditional agricultural practices. Non-treated (healthy seeds) of cowpea were sown in infested soil with pathogenic fungi and were used as control. Degrees of disease incidence and N2-fixation parameters were recorded as described above.

Fungicide seed dressing

Cowpea seeds was dressed with Rizolex-T 50 % at the recommended dose (3 g/kg seeds) then sown in infested soil and served as a comparison treatment.

Effect of different pesticides on growth of antagonists

One hundred µl (72 hour - old culture) for bacterial strain (10^8 cfu/ml) or (10^7 spore / ml) for fungal antagonist (one week - old culture) were spread by using glass spreader on nutrient agar or potato dextrose agar plates, respectively. Then, 50 µl from each pesticide (malathion Insecticides), Punch or Rizolex-

T 50% as fungicides, Stomb as herbicide) with recommended dose were putted in wells (5 mm in diameter) in nutrient agar or potato dextrose agar media for bacterial r fungal strains, respectively and then the plates were incubated at 30 °C and examined daily. The inhibition zone was recorded between the tested strains after 5 days as incubation periods. Three replicates were used for each isolate.

Effect of different pH value on growth of antagonists

To determine the effect of pH on the growth of the tested antagonists, 50 ml Chapek- Dox medium supplemented with 2% glucose/L for fungal isolates and 1% for bacterial ones were used in Chapek- Dox medium. Chapek- Dox medium was inoculated by 1 ml fungal suspension at 10^6 spore / ml or bacterial cell suspension at 10^7 cfu / ml, respectively. To determine the optimum pH, experiments were carried out at pH 5, 6, 7, 8 and 9. Cultures were incubated on a rotary shaker at 30°C and 150 rpm up to 3 and 7 days for bacterial and fungal isolates, respectively. The growth of fungal isolates was determined as mycelia dry weight of biomass (g) after 7 days. Cells number of the bacterial strain was determined by plating appropriate dilutions of liquid medium onto nutrient agar medium.

Statistical analysis:

The obtained data were subjected to the proper statistical procedures for analysis of variance according to Gomez and Gomez, (1984).

RESULTS AND DISCUSSION

Isolation and identification of the causal organisms

Isolation trials were carried out on damping-off, root-rotted cowpea plants, collected from different regions at Elbehira Governorate, Egypt, resulted in the isolation of four fungal isolates belonging to the genera *Rhizoctonia, Sclerotium, Fusarium and Macrophomina* as shown by preliminary microscopic examination. The fungal pathogens were identified as *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani* and *Macrophomina phasolina*. Results in (Table 1) show that, *R. solani* and *Sclerotium rolfsii* caused a highly significant effect at pre- and post-emergence dampin- off for cowpea plants followed by *F. solani* and *M. phaseolina*. As the least percent of survival plants were recorded with *R. solani* and *Sclerotium rolfsii* followed by *Fusarium solani and Macrophomina*

phasolina. However, cowpea is subjected to the attack of several soil-born pathogenic fungi. Many investigators noted that *R. solani*, *S. rolfsii*, *F. solani*, *M. phaseolina*, *F. oxysporum* and *Pythium* spp. are considered among the main pathogens causing root rot diseases of cowpea (Shihata and Gad El-Hak, 1989; Lagrange and Aveling, 1998; Rauf, 2000; Satish *et al.*, 2000 and El-Mohamedy *et al.*, 2006).

Table 1. Pathogenicity tests of the isolated pathogenic fungi.

Treatments	Diseases expression		
	% pre-emergence damping off	% post-emergence damping off	% Survival plants
Control (uninoculated)	5^{a}	5^{a}	90^{e}
Rhizoctonia solani	50^{ed}	40^{bc}	10^{a}
Sclerotium rolfsii	45^{cd}	40^{bc}	15^{ab}
Fusarium solani	30^{bc}	35^{b}	35^{c}
Macrophomina phasolina	25^{b}	35^{b}	40^{d}

Means followed by the same letter within a column do not differ significantly at $P < 0.05$, Duncan's multiple-range test.

Screening and identification of the antagonists

The initial screening of more than 80 microbial comprising fungi and bacteria originated from different rhizosphere-soil samples of healthy cowpea plants, resulted in the isolation of 20 isolates exhibiting obvious antagonistic action on plates against one or more of the tested pathogens of cowpea. Only two isolates could antagonize all the tested pathogens of cowpea. One of them was a bacterium andwhich was identified according to its morphological and physiological characteristics as *Bacillus subtilis* (EB8), and the other isolate was a fungus which was identified according to its morpholological and microscopicall characteristics as *Trichoderma longibrachiatum* (EB7). The mechanism of action of the antagonism for the two antagonists was differed. *Bacillus subtilis* has antibiosis phenomenon thereby which produced many antibiotics which inhibit growth of the tested plant pathogens (Figure 1).

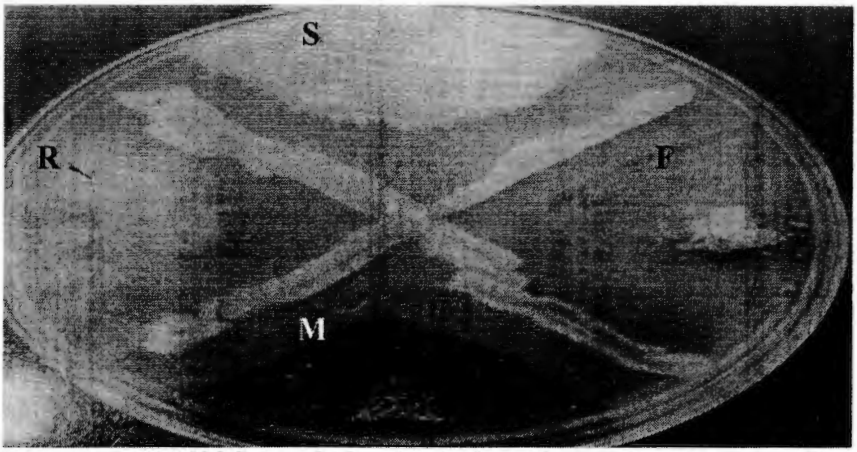

Figure 1. Inhibition of the tested pathogenic fungi by *B. subtilis* (EB8), where R: *R. solani*, S: *S. rolfsii*, F: *F. solani* and M: *M. phaseolina*.

Characteristics of the metabolites (antibiotic) system of *Bacillus subtilis* (EB8) and its growth behaviour on nutrient broth medium

At the beginning the general growth behaviour for *Bacillus subtilis* (EB8) which produced the metabolites (antibiotic) was achieved. A series of experiments were carried out to study the growth behaviour of *Bacillus subtilis* (EB8) strain in nutrient broth medium and its metabolites effect on suppression of the tested fungal pathogens. The results in (Figure 2) show the growth was higher after 3 days (logarithmic phase). A maximum of growth was obtained after 3 days. Figure 2 illustrate that the metabolites formation started when the organism grew on the medium. The highest accumulation of metabolites exhibited in the fifth days of cultivation. The maximum accumulation of metabolites occurred at the end of stationary phase and then the accumulation of metabolites decreased at the end of the stationary growth phase. Metabolites accumulation coincided with the increase in the specific growth rates of the cultures. The maximum accumulation of *B. subtilis* metabolites occurred at the end of stationary phase. Metabolites accumulation coincided with increase in the specific growth rates of the cultures and the increase of suppresion of the tested pathogens by formation wider inhibition zone.

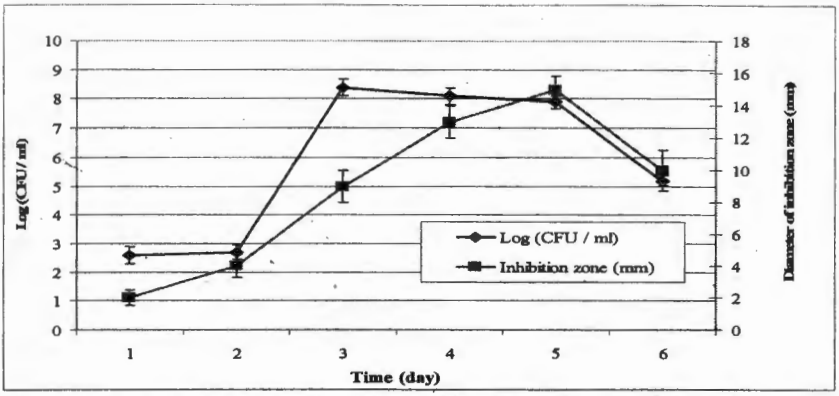

Figure 2. production of metabolites (antibiotics) by *B. subtilis* (EB8) strain.

Mechanism of action of *T. longibrachiatum* against the tested pathogens

On the other hand, *T. longibrachiatum* has antbiosis and hyperparsitism, where it inhibited growth of *F. solani* and over grew on *the pathogen* (Figure 3). The microscopical examination of the hyphae of *F. solani* showed that lysis and destruction of the pathogen hyphae (Data not shown).

Figure 3. Inhibition of *F. solani* by *T. longibrachiatum*, where F: *F. solani* and T: *T. longibrachiatum*.

Efficacy of *T. longibrachiatum* on sclerotia germination of *S. rolfsii*

Results in (Table 2) showed the highest ability of *T. longibrachiatum* in destroying the sclerotia of *S. rolfsii* after 2

weeks at 30 °C temperature. It was also observed that there was no difference between the destroying of *S. rolfsii* sclerotia on water agar and on moistened sandy soil. The obtained results show the possibility of use of *T. longibrachiatum* in biological control of root rot disease in cowpea.

Table 2. Effect of *T. longibrachiatum* on sclerotia viability of *S. rolfsii* on water agar and in sandy soil.

Treatment	% Not destroyed (germinated)	% Destroyed (not germinated)
T. longibrachiatum + Water agar	5^{c}	95^{a}
Water agar		
T. longibrachiatum + moistened	97^{a}	3^{c}
sandy soil	6^{b}	94^{b}
moistened sandy soil		
	98^{a}	2^{c}

Means followed by the same letter within a column do not differ significantly at $P < 0.05$, Duncan's multiple-range test.

Compatibility between antagonists and *Bradyrhizobium* sp. of cowpea

Results *in vitro* experiments indicated that *Bradyrhizobium* sp. was capable to grow and survive on plates side by side with selective antagonists (Figure 4). Inhibition zones could not be detected between *Bradyrhizobium* sp. and the antagonist.

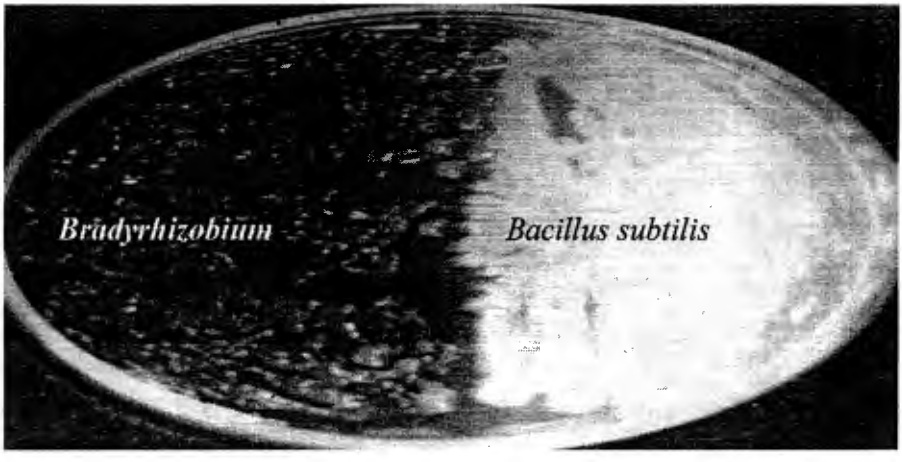

Figure 4. *Bradyrhizobium* sp. growing side by side with *Bacillus subtilis*.

Results in (Table 3) show the N_2- fixing parameters of *Bradyrhizobium* sp. on cowpea as affected by the tested antagonists (*Bacillus subtilis* and *T. longibrachiatum*) compared to a fungicide Rizolex-T 50 % dose (3 g/kg seeds). Results clearly indicate that the N_2- fixing performance of the bradyrhizobium on cowpea plants has not been negatively affected with the tested antagonists. The obtained results in (Table 3) shows similar levels of bradyrhizobial nodulation in sand inoculated or un inoculated with the antagonists. On the other hand, seed dressing with Rizolex-T 50 % dose (3 g/kg seeds) significantly reduced the nitrogen fixation parameters.

Table 3. Effect of the antagonists *Bacillus subtilis* and *T. longibrachiatum* on symbiotic N_2-fixing parameters of cowpea inoculated with *Bradyrhizobium* sp.

Treatments	N_2- fixation parameters after 45 days				
	No. of nodules / plant	Dry weight of nodules (g) / plant	Dry weight of shoots (g) / plant	% N_2 plant	Total N_2 (mg) / plant
Seed treatment as peat-based culture					
Control (un-ioculated)	0	0	0.18^{d}	0.6^{c}	1.1^{bc}
Bradyrhizobium sp.	26^{a}	0.36^{a}	0.94^{a}	2.4^{a}	22.6^{a}
Bradyrhizobium sp. + Rizolex-T 50 % dose (3 g/kg seeds)	5^{b}	0.002^{c}	0.3c	1.2^{b}	3.6^{b}
Bradyrhizobium sp. + *B. subtilis*	25^{a}	0.35^{b}	0.94^{b}	2.3^{a}	21.6^{a}
Bradyrhizobium sp. + *T. longibrachiatum* (10^{7} spore / g soil)	24^{a}	0.34^{b}	0.93^{b}	2.3^{a}	21.4^{a}
Soil treatment as bio-enhanced compost					
Bradyrhizobium sp. + *B. subtilis*	24^{a}	0.34^{b}	0.93^{b}	2.4^{a}	22.32^{a}
Bradyrhizobium sp. + *T. longibrachiatum*	23^{a}	0.33^{b}	0.92^{b}	2.3^{a}	21.2^{a}

Means followed by the same letter within a column do not differ significantly at $P < 0.05$, Duncan's multiple-range test.

Effect of certain environmental factors on growth of the tested antagonists

Disease suppression depends on the prevailing environmental conditions such as soil pH and biological components, including all root-colonizing plant -beneficial bacteria and fungi. The influence of pH on growth of the antagonists is shown in (Figure 5). Generally, pH 7 was the optimal for the antagonists. The maximal growth of both

antagonists was recorded at pH 7. Most of the bacterial strains are known to prefer the neutral pH. The bacterial strain can tolerate alkali pH till pH 8, but the fungal strain can grow on acidic pH and can not tolerate alkali pH. The measured pH of the used soil in this work was pH 7.8. Therefore, it can be deduced from the results that the pH is considered an important environmental factor in the rhizosphere which affect on the efficiency of the bioagents.

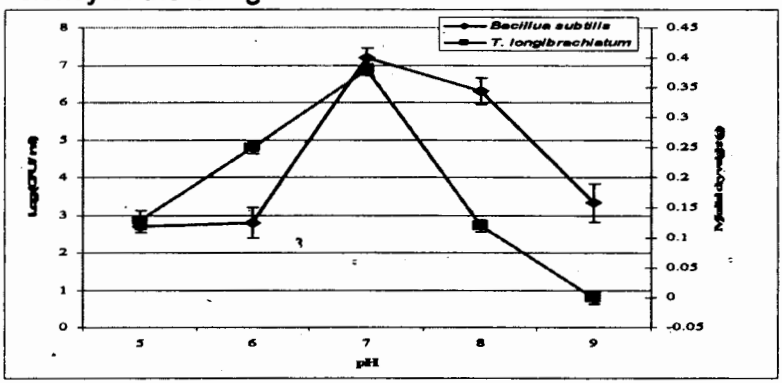

Figure 5. Effect of pH on growth of the antagonists.

Effect of some pesticides on growth of antagonists

Application of pesticides has a pronounced harmful effect on many beneficial organisms such as antagonists. Results in (Table 4) show the effect of some pesticides such as Insecticide (Silicron and Malathion), fungicide (Punch and Rizolex-T 50%) and herbicide (Stomb) applied at recommended doses on growth of the antagonists grown on nutrient or potato dextrose agar plates by recording of inhibition zone (mm). Generally, growth of all the tested antagonists was inhibited by the tested pesticides.

Table 4. Inhibition antagonists by some pesticides.

Antagonists	Inhibition zone (mm)				
	Silicoron	Malathion	Punch	Rizolex-T 50%	Stomb
Bacillus subtilis	10	7.5	7.5	10	9
T. longibrachiatum	13	8.5	10	15	11
Control	0	0	0	0	0

Efficacy of antagonists against soilborne fungal pathogens under greenhouse conditions

Under greenhouse conditions, the selected antagonists exhibited their efficacy to control the damping – off and root rot

disease on cowpea plants. Data presented in Tables (5, 6, 7, and 8) clearly show the beneficial effect of bradyrhizobial inoculation of legumes. It increased the total N_2 of cowpea plants recorded after 90 days from 36.8 to 167.2 mg / plant. This indicates the importance of bradyrhizobial inoculation for cowpea plants.

However, inoculation of Bradyrhizobium sp. in soli infested with *R. solani*, *S. rolfsii*, *F. solani* or *M. phaseolina* was almost meaningless, beside the harmful effect of pathogens (s) on cowpea, the total N_2 of survived palnts (90 days after planting) dropped from 167.2 to 32.4, 27.2, 23.8, 22.4 mg/plant in soil infested with *R. solani*, *S. rolfsii*, *F. solani* and *M. phaseolina*, respectively (Tables 5, 6, 7, and 8).

Although the seed dressing with Rizolex-T 50% at rate of 3 g/kg seeds significantly reduced the disease incidence, its harmful effects on N_2-fixation parameters was remarkable significantly, the total N_2 of survived palnts (90 days after planting) dropped from 167.2 to 40.8, 40.8, 42.5 and 36 in soil infested with *R. solani*, *S. rolfsii*, *F. solani* and *M. phaseolina*, respectively (Tables 5, 6, 7, and 8).

Since the efficiency of the selected antifungal strains to achieve levels of antagonism against soil borne pathogens, and their biological compatibility towards *Bradyrhizobium* sp. had been confirmed in previous experiments, it was necessary to evaluate their performance in the presence of both the pathogen (s) and bradyrhizobia. Both achievable levels of protection and symbiotic N_2- fixation parameters were determined over period 90 days. Two forms of application of the selected antagonists were used such as seed inoculation as peat culture and soil inoculation with bio-enhanced compost. Treatment of cowpea seeds or soil treatment with bio-enhanced compost with the antagonists increased emergence of cowpea seedlings grown in soil infested with each of *R. solani, S. rolfsii, F. solani*, and *M. phaseolina.* These effects were significant for all pathogens and were similar to more or less than with those obtained with the fungicide Rizolex. Although the tested bioagents appeared to reduce severity of root rots caused by most of the tested pathogens, the effect was statistically significant only for seed treatment with *B. subtilis* and *T. longibrachiatum.* Both antagonists were more efficient antagonists in controlling damping – off of cowpea plants. Data showed also that seed inoculation and soil treatment with bio-

enhanced compost of cowpea at the time of planting significantly increased the percentage of survival plants with all fungal pathogens. The application of the fungicide however, has led to a significant and drastic decrease in all N_2- fixation parameters. The latter was significantly different from those of uninoculated plants. It is obvious that applications of the fungicide Rizolex negatively affects *Bradyrhizobium* sp. and deprives the legumes from any beneficial effects of bradyrhizobial inoculation. Seed inoculation or soil treatment with bioenhanced compost at the time of planting in soil infested with *R. solani* (Table 5), the percentage of survival plants increased from 10 to 85 % as well as total N_2 also significantly increased from 32.4% to 85%. In soil infested with *S. rolfsii* (Table 6) the percentages of survival plants significantly increased from 10 to 85 % and the total N_2 significantly increased from 27.2 to 79.2. Similar results were obtained in soil infested with *F. solani* (Table 7) where percentage of survived plants increased from 25 to 90 % in addition the total N_2 increased from 23.8 to 74.4 %. On the other hand, the percentage of survival plants increased from 35 to 90 % in soil infested with *M. phaseolina* (Table 8) and the total N_2 increased from 22.4 to 88.8 %. Only the application of the selected antifungal strains *Bacillus subtilis* and *T. longibrachiatum* at the time of planting could achieve high levels of protection against pathogen (s) and permit meanwhile reasonable symbiotic N2-fixation. Although the total N_2 dropped from 167.2 to (63.8 – 85), (61.6 – 79.2), (53.2 – 74.4) and (52.5 - 88.8) in soil infested with *R. solani*, *S. rolfsii*, *F. solani* and *M. phaseolina*, respectively (Tables 5, 6, 7, and 8), it was still large than of unprotected plants as well as protected plants by Rizolex-T 50%.

Table 5. Effect of seed or soil treatment with *Bacillus subtilis* and *Trichoderma longibrachiatum* on disease incidence and symbiotic N_2- fixing parameters of cowpea inoculated with *Bradyrhizobium* sp. in the presence of *Rhizoctonia solani*.

Treatments	Disease expression			N_2- fixation parameters after 90 days				
	% pre-emergence damping off	% post-emergence damping off	% Survival plants	No. of nodules / plant	Dry weight of nodules (g) / plant	Dry weight of shoots (g) / plant	% N_2 / plant	Total N_2 (mg) / plant
Seed treatment as peat-based culture								
Control (un-inoculated)	0	0	100^{a}	8^{fg}	0.001^{h}	2.3^{f}	1.6^{eg}	36.8^{fg}
Bradyrhizobium sp.	0	0	100^{a}	45^{a}	0.3^{a}	4.4^{a}	3.8^{a}	167.2^{a}
R. solani	60^{a}	30^{a}	10^{f}	3^{h}	0.001^{h}	1.1^{h}	1.1^{h}	15.4^{i}
R. solani + *Bradyrhizobium* sp	50^{ab}	25^{b}	25^{e}	11^{f}	0.003^{fg}	1.8^{g}	1.5^{eg}	32.4^{gh}
R. solani + *B. subtilis* + Bradyrhizobium sp	15^{cd}	10^{d}	75^{d}	33^{bc}	0.12^{bc}	3.2^{bc}	2.4^{bc}	74.4^{c}
R. solani + *T. longibrachiatum* + *Bradyrhizobium* sp	15^{cd}	5^{e}	80^{bc}	35^{b}	0.14^{b}	3.4^{b}	2.5^{b}	85^{b}
R. solani + Rizolex-T 50 % dose (3 g/kg seeds) + *Bradyrhizobium* sp.	10^{de}	5^{e}	85^{b}	18^{e}	0.004^{f}	2.4^{f}	1.7^{e}	40.8^{f}
Soil treatment as bio-enhanced compost								
R. solani + *B. subtilis* + *Bradyrhizobium* sp	20^{c}	10^{d}	70^{de}	28^{d}	0.1d^{e}	2.7d	2.1^{cd}	63.8^{e}
R. solani + *T. longibrachiatum* + *Bradyrhizobium* sp	15^{cd}	15^{c}	70^{de}	27^{d}	0.11^{cd}	2.8^{de}	2.2^{cd}	71.3^{cd}

Means followed by the same letter within a column do not differ significantly at $P < 0.05$, Duncan's multiple-range test.

Table 6. Effect of seed or soil treatment with *Bacillus subtilis* and *Trichoderma longibrachiatum* on disease incidence and symbiotic N_2- fixing parameters of cowpea inoculated with *Bradyrhizobium* sp. in the presence of *Sclerotium rolfsii*.

Treatments	Disease expression			N_2- fixation parameters after 90 days				
	% pre-emergence damping off	% post-emergence damping off	% Survival plants	No. of nodules / plant	Dry weight of nodules (g) / plant	Dry weight of shoots (g) / plant	% N_2 / plant	Total N_2 (mg) / plant
Seed treatment as peat-based culture								
Control (un-inoculated)	0	0	100^{a}	8^{ef}	0.001^{hi}	2.3^{e}	1.6^{f}	36.8^{fg}
Bradyrhizobium sp.	0	0	100^{a}	45^{a}	0.3^{a}	4.4^{a}	3.8^{a}	167.2^{a}
S. rolfsii	55^{a}	35^{a}	10^{g}	4^{g}	0.002^{h}	1.3^{fg}	1.3^{h}	16.9^{j}
S. rolfsii + *Bradyrhizobium* sp.	45^{ab}	25^{ab}	30^{f}	10^{ef}	0.003^{fg}	1.6^{f}	1.7^{g}	27.2^{h}
S. rolfsii + *B. subtilis* + Bradyrhizobium sp.	20^{c}	10^{c}	70^{cd}	31^{bc}	0.12^{bc}	3.3^{b}	2.3^{b}	75.9^{cd}
S. rolfsii + *T. longibrachiatum* + *Bradyrhizobium* sp.	10^{de}	10^{c}	80^{bc}	32^{b}	0.13^{b}	3.3^{b}	2.4^{b}	79.2^{b}
S. rolfsii + Rizolex-T 50 % dose (3 g/kg seeds) + *Bradyrhizobium* sp.	10^{de}	5^{cd}	85^{b}	9^{ef}	0.004^{f}	2.4^{e}	1.7^{f}	40.8^{f}
Soil treatment as bio- enhanced compost								
S. rolfsii + *B. subtilis* + *Bradyrhizobium* sp	15^{cd}	10^{c}	75^{cd}	26^{de}	0.08^{de}	2.8^{dc}	2.2^{de}	61.6^{e}
R. solani + *T. longibrachiatum* + *Bradyrhizobium* sp	10^{de}	10^{c}	80^{bc}	28^{cd}	0.09^{cd}	3.2^{bc}	2.4^{d}	76.8^{bc}

Means followed by the same letter within a column do not differ significantly at $P < 0.05$, Duncan's multiple-range test.

Table 7. Effect of seed or soil treatment with *Bacillus subtilis* and *Trichoderma longibrachiatum* on disease incidence and symbiotic N_2- fixing parameters of cowpea inoculated with *Bradyrhizobium* sp. in the presence of *Fusarium solani*.

Treatments	Disease expression			N_2- fixation parameters after 90 days				
	% pre-emergence damping off	% post-emergence damping off	% Survival plants	No. of nodules / plant	Dry weight of nodules (g) / plant	Dry weight of shoots (g) / plant	% N_2 / plant	Total N_2 (mg) / plant
Seed treatment as peat-based culture								
Control (un-inoculated)	0	0	100^{a}	8^{fg}	0.001^{h}	2.3^{ef}	1.6^{fg}	36.8^{f}
Bradyrhizobium sp.	0	0	100^{a}	45^{a}	0.3^{a}	4.4^{a}	3.8^{a}	167.2^{a}
F. solani	30^{a}	45^{a}	25^{g}	4^{h}	0.001^{h}	1.4^{h}	1.2^{hi}	16.8^{h}
F. solani + *Bradyrhizobium* sp	25^{ab}	40^{ab}	35^{f}	9^{fg}	0.004^{fg}	1.7^{g}	1.4^{h}	23.8^{g}
F. solani + *B. subtilis* + *Bradyrhizobium* sp	10^{cd}	15^{c}	75^{d}	23^{bc}	0.09^{bc}	3^{bc}	2.1^{bc}	63^{c}
F. solani + *T. longibrachiatum* + *Bradyrhizobium* sp	5^{de}	10^{cd}	85^{bc}	25^{b}	0.11^{b}	3.1^{b}	2.4^{b}	74.4^{b}
F. solani + Rizolex-T 50 % dose (3 g/kg seeds) + *Bradyrhizobium* sp.	5^{de}	5^{de}	90^{b}	11^{f}	0.005^{f}	2.5^{e}	1.7^{f}	42.5^{e}
Soil treatment as bio-enhanced compost								
F. solani + *B. subtilis* + *Bradyrhizobium* sp	15^{c}	15^{c}	70^{de}	15^{d}	0.07^{d}	2.8^{d}	1.9^{e}	53.2^{d}
F. solani + *T. longibrachiatum* + *Bradyrhizobium* sp	15^{c}	15^{c}	70^{de}	18^{e}	0.06^{de}	2.8^{d}	2.2^{cd}	61.6^{c}

Means followed by the same letter within a column do not differ significantly at $P < 0.05$, Duncan's multiple-range test.

Table 8. Effect of seed or soil treatment with *Bacillus subtilis* and *Trichoderma longibrachiatum* on disease incidence and symbiotic N_2- fixing parameters of cowpea inoculated with *Bradyrhizobium* sp. in the presence of *Macrophomina phaseolina*.

Treatments	Disease expression			N_2- fixation parameters after 90 days				
	% pre-emergence damping off	% post-emergence damping off	% Survival plants	No. of nodules / plant	Dry. weight of nodules (g) / plant	Dry weight of shoots (g) / plant	% N_2 / plant	Total N_2 (mg) / plant
Seed treatment as peat-based culture								
Control (un-inoculated)	0	0	100^{a}	8^{ef}	0.00^{g}	2.3^{g}	1.6^{f}	36.8^{f}
Bradyrhizobium sp.	0	0	100^{a}	45^{a}	0.3^{a}	4.4^{a}	3.8^{a}	167.2^{a}
M. phaseolina	30^{a}	35^{a}	35^{f}	3^{h}	0.001^{g}	1.2^{j}	1.1^{h}	13.2^{h}
M. phaseolina + *Bradyrhizobium* sp	25^{ab}	30^{ab}	45^{e}	7^{g}	0.003^{ef}	1.6^{h}	1.4^{d}	22.4^{g}
M. phaseolina + *B. subtilis* + Bradyrhizobium sp	10^{cd}	5^{de}	80^{c}	25^{bc}	0.13^{bc}	3.4^{bc}	2.3^{bc}	78.2^{bc}
M. phaseolina + *T. longibrachiatum* + *Bradyrhizobium* sp	5^{de}	5^{de}	90^{b}	28^{b}	0.16^{b}	3.7^{b}	2.4^{b}	88.8^{b}
F. solani + Rizolex-T 50 % dose (3 g/kg seeds) + *Bradyrhizobium* sp.	5^{de}	5^{de}	90^{b}	11^{e}	0.004^{e}	2.4^{f}	1.5^{ef}	36^{f}
Soil treatment as bio-enhanced compost								
M. phaseolina + *B. subtilis* + *Bradyrhizobium* sp.	15^{c}	15^{c}	70^{d}	22^{d}	0.06^{cd}	2.7^{de}	2.2^{de}	52.5^{e}
M. phaseolina + *T. longibrachiatum* + *Bradyrhizobium* sp	10^{cd}	10^{cd}	60^{c}	26^{bc}	0.07^{c}	2.9^{d}	2.4^{d}	69.6^{d}

Means followed by the same letter within a column do not differ significantly at $P < 0.05$, Duncan's multiple-range test.

Biocontrol of damping-off of cowpea by antagonists under field conditions

Data presented in Table (9) reassure the efficiency of the *Bacillus subtilis* and *T. longibrachiatum* against the tested soil-borne fungal pathogens when applied as seed or soil treatment with peat based culture or bio-enhanced compost, respectively. The percentage of survival plants were increased from 15 to 90 % in soil infested with *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani* and *Macrophomina*

phasolina, respectively. Data concerning total N_2 -fixation parameters showed that the total N_2 dropped from 364.9 to 93.6, 86.4 and (129.6 - 238) mg/ plant in soil treated with pathogens, Rizolex-T 50%, antagonists, respectively (Tables 9).

Table 9. Effect of seed or soil treatment with *Bacillus subtilis* and *Trichoderma longibrachiatum* on disease incidence and symbiotic N_2- fixing parameters of cowpea inoculated with *Bradyrhizobium* sp. in the presence of certain root rot pathogens.

Treatments	Disease expression			N_2- fixation parameters after 90 days				
	% pre-emergence damping off	% post-emergence damping off	% Survival plants	No. of nodules / plant	Dry weight of nodules (g) / plant	Dry weight of shoots (g) / plant	% N_2 / plant	Total N_2 (mg) / plant
Seed treatment as peat-based culture								
Control (un-inoculated)	0	0	100^{a}	9e	0.002^{g}	3.5^{fg}	2.5^{f}	112.5^{f}
Bradyrhizobium sp.	0	0	100^{a}	55^{a}	0.43^{a}	8.9^{a}	4.1^{a}	364.9^{a}
"Infested soil	40^{a}	45^{a}	15^{g}	2f	0.001^{h}	2.2^{h}	1.2^{h}	26.4^{i}
Infested soil + *Bradyrhizobium* sp	35^{ab}	40^{ab}	25^{f}	15^{e}	0.002^{g}	3.6^{f}	2.5f	93.6^{h}
Infested soil + *B. subtilis* + *Bradyrhizobium* sp	15^{c}	20^{c}	65^{de}	35^{c}	0.21^{bc}	6.4^{bc}	3.3^{bc}	211.2^{bc}
Infested soil + *T. longibrachiatum* + *Bradyrhizobium* sp	10^{cd}	10^{de}	80^{c}	43^{b}	0.23^{b}	6.8^{b}	3.5^{b}	238^{b}
Infested soil + Rizolex-T 50 % dose (3 g/kg seeds) + *Bradyrhizobium* sp.	5^{de}	5^{ef}	90^{b}	14^{e}	0.003^{f}	3.6^{f}	2.4^{fg}	86.4^{g}
Soil treatment as bio-enhanced compost								
Infested soil + *B. subtilis* + *Bradyrhizobium* sp	15^{c}	15^{cd}	70^{d}	33^{cd}	0.09^{d}	4.8^{de}	2.7^{de}	129.6^{e}
Infested soil + *T. longibrachiatum* + *Bradyrhizobium* sp	10^{cd}	10^{de}	80^{c}	35^{c}	0.1^{de}	5.1^{d}	2.9^{d}	147.9^{d}

Means followed by the same letter within a column do not differ significantly at $P < 0.05$, Duncan's multiple-range test.

"Infested soil: naturally infested soil with *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani* and *Macrophomina phasolina*.

Although the total N_2 dropped with treatment with antagonists but it was still large than of unprotected plants and also protected plants by Rizolex-T 50%.

Application of chemical fungicides to protect seeds from the attack of soil borne pathogenic fungi was and still the primary means to control soil-borne diseases. However, the use of fungicides is becoming more and more controversial. Although fungicides are efficient against the pathogens, they harm the symbiotic N_2- fixation between bradyrhizobia and cowpea drastically leading to a significant decrease in quality and quantitiy of yield of seed cowpea. Results of the present study confirm the deleterious of fungicides on symbiotic nitrogen fixation. Application of Rizolex-T 50% at the recommended dose significantly reduced number and dry weight of nodules, dry weight of shoot, % N and total N_2 mg/plant of cowpea. The obtained results are in agreement with those reported by Basiony *et al.,* (1988) and Belal *et al.,* (1996).

One potential solution was the exploitation of nature biological potency to have sufficient biocontrol of soil-borne pathogenic fungi, with no toxic residues and in the same time biologically compatible to bradyrhizobia.

The preliminary screening resulted in the isolation of many bacterial and fungal isolates exhibiting marked antifungal activity against the tested pathogens. The predominant antifungal and bacterial strains were belonging to Bacilli and *Trichoderma*, respectively. This result is in agreement with numerous reports implicating member of those groups as microbial antagonists (Elad *et al.,* 1980; Cook and Baker, 1983; Belal *et al.,* 1996 and Loeffez *et al.,* 1986).

The antagonistic activity of *Bacillus* and *Trichoderma* was recognized by many investigators and several modes of action were proposed. Members of the genus Bacillus are known to be potent producers of many antibiotics which suppress both bacteria and fungal pathogens (Pusey, 1989). Antibiotics are low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. Regardless of the toxicity of some antibiotics probably by some *Bacillus* strains to the cells of mammals (*e.g.*, polymyxines, bacitracin, etc.) they continued to be in the focus of attention of scientists.

The amount of antibiotics produced by bacilli was approaching 167, being 66 derived from *B. subtilis*, 23 from *B. brevis* and the remaining peptide antibiotics are produced by other species of genus *Bacillus*. The main antibiotic producers of this genus are *B. subtilis* (*e.g.*, polymyxin, difficidin, subtilin, mycobacillin, bacitracin). As is generally assumed, these antibiotics are mainly polypeptides (Berdy, 1974; D'Aversa and Stern, 1997; Hancock and Chapple, 1999).

Most of the peptide antibiotics produced by *Bacillus* are active against Gram positive bacteria (Ming and Epperson, 2002). However, compounds such as polymyxin, colistin, and circulin exhibit activity almost exclusively upon Gram-negative forms, whereas bacillomycin, mycobacillin, and fungistatin are effective agents against molds and yeasts (Katz and Demain, 1977).

Several modes of action of *Trichoderma* were proposed to explain it antifungal activity. *Trichoderma* was reported to suppress the germination of *S. rolfsii* sIcerotia (Henis *et al.*, 1983), to attack many fungi by coiling around and pentrating into the hyphae and to lyse mycelia of fungi by producing β (1,3) glucanase and chitinase (Elad *et al.*, 1980; Elad *et al.*, 1983).

Attempts had been successfully carried out using antagonists to control soil – borne fungal pathogens on legumes (Harman, 1991; Belal *et al.*, 1996; El-Mohamedy *et al.*, 2006).

It is important to think about the practical aspects concerning the use of the antagonists as biocontrol agents. Bio-enhanced compost used for soil application face problems in transport and handling, whereas peat-based preparation used for seed treatment seems to be more appropriate. This form is known for preparing inocula of Rhizobium. Peat was also used also as a carrier for several antagonists (Belal *et al.*, 1996). Results reconfirmed the efficiency of the antifungal isolates when they were applied as seed or soil inoculation in form of peat based inocula or bioenhanced compost, rspectively. Signficantly high levels of protection could be achieved against *R. solani*, *S. rolfsii*, *F. solani* and *M. phaseolina* and in the meantime, nodulation and accumulation of nitrogen in cowpea plants were significantly higher than control treatment (unprotected plants).

Control of root rot pathogens through amended soil with organic materials formulated with bio-control agents may be attributed

to : 1) increasing the activity of indigenous micro flora resulting suppression of pathogens population on through competition or specific inhibition (Adams, 1990; Elad *et al.,* 1986; Ceuster *et al.,* 1991), 2) releasing degradation compounds such carbon dioxides, ammonia, nitrites, saponine or enzymes which are generally toxic to the pathogens (Lakshmanan and Nair, 1984; Liu and Huany, 2000), 3) inducing plant defense mechanisms (Windham *et al.,* 1986), 4) cellulose and glucanase are prevalent to high concentration in soil as a result of biodegradation of cellulose and lignin. From the foregoing results, it can be concluded that the % disease expression representing in pre- and post- emergence damping - off was reduced by the application of the isolated antagonists at the time of planting as seed treatment or soil treatment. Moreover, the N_2 – fixation parameters of cowpea plants were also significantly increased by inoculation with these antagonists strains.

The efficiency of the antifungal strains of *Bacillus subtilis* and *T. longibrachiatum* to protect cowpea plants against the major soil-borne pathogenic fungi without affecting the symbiotic N_2- fixation has been clearly proved throughout the present investigation. So, it could be suggested that such soil and seed treatments could represented an environmentally ecofriendly strategy for controlling soil borne pathogens as substitute of chemical fungicides.

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الملخص العربي

المكافحة الحيوية لبعض أمـراضا اللوبيا المحمولة بالتربة وعلاقتها بالبكتيريا المثبتة للنيتروجين (البراديرايزوبيوم)

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تتسبب أمراض تساقط البادرات لنباتات اللوبيا عن *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium solani*, and *Macrophomina phaseolina*. تم عزل سلالتين من الكائنات المضادة من عينات تربة الرايزوسفير لنباتات اللوبيا السليمة. أظهرت هذه الكائنات المضادات قدرة تضادية ضد كل الفطريات المختبرة المسببة للأمراض. تنتمى واحدة من هذه الكائنات المضادة إلى البكتيريا والتي وجد أنها تنتمي الى مجموعة الباسيلاى والتي تم تعريفها على أنها *Bacillus subtlis* , ووجد أن هذه السلالة تنتج أقصى كمية من نواتج الأيض التي تضاد الفطريات الممرضة عند نهاية مرحلة الطور اللوغاريتمي. ووجد أن السلالة الأخرى من الكائنات المضادة تنتمي إلى الترايكودرما وتم تعريفها على أنها *T. longibrachiatum* . ووجد أن *T. longibrachiatum* تثبط تماما انبات الأجسام الحجرية لفطر *S. rolfsii*.

أظهرت النتائج مدى توافق هذ الكائنات المضادة مع البراديرايزوبيوم سواء تحت ظروف المعمل أو الصوبة وعدم تأثير الكائنات المضادة على قدرة البراديرايزوبيوم في تثبيت النيتروجين, بينما أثرت الفطريات الممرضة على البراديرايزوبيوم وعلى مقاييس تثبيت النيتروجين. أدت معاملة تغليف البذور(الكائنات المضادة المحملة على البيت موس) أو معاملة التربة (االكمبوست المحسنة بالكائنات المضادة) بهذه الكائنات المضادة أثناء وقت الزراعة تحت ظروف الصوبة أو الحقل في تربة معداة (صناعيا أو طبيعيا) بأي من المسببات المرضية إلى تقليل حدوث المرض وزيادة نسبة نباتات اللوبيا الباقية وذلك بالاضافة إلى الحفاظ على عملية تثبيت النيتروجين. التأثير كان متشابه إلى حد ما فى حالات كثيرة بالتأثير الذى أحدثه مبيد Rizolex-T 50 وفي زيادة نسبة الانبات وتقليل الشدة المرضية لأعفان الجذور ولكن كان تأثير المبيد سلبيا على البراديرايزوبيوم ومقاييس تثبيت النيتروجين.