Egyptian Journal of Biological Pest Control, 25(2), 2015, 489-497

The Inhibitory Effects of Free and Encapsulated Arbuscular Mycorrhizal fungi and *Trichoderma viride* Against Charcoal Rot (*Macrophom inaphaseolina*) on Common Bean (*Phaseolus vulgaris* L.)

Massoud*, O. N. and S. M.Kamel**

*Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt.
**Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt, said_kamel88@yahoo.com
(Received: September30, 2015 and Accepted: November29, 2015)

ABSTRACT

Efficacy of arbuscular mycorrhizal fungi (AMF) and *Trichoderma viride* was assessed biocontrol of *Macrophom inaphaseolina* that causes charcoal rot in common bean plants *(Phaseolus vulgaris* L.*)* cv. Bronco. Pot experiment was used to evaluate the inhibitory effect of A. mycorrhizal fungi and *T. viride* in alginated (encapsulated) and free forms (unencapsulated) against *M. phaseolina*. The *in vitro,* results revealed that alginated and free) *T. viride* showed the highest inhibition percentages (63.3 and 61.1%, respectively). Alginated AMF and *T. viride* significantly decreased the severity of the disease more than the free ones and slightly less than the chemical fungicide (Vitavax 200 40% FS). That positively affected dry weights of plant shoots and roots. Result- significantly recorded more mycorrhiza colonization percent (73.7%) with alginated AM fungi and the least total fungal count with the fungicide treatment ($0.56x10^5$ spore/g dry rhizosphere). The chitinase enzyme significantly increased with alginated *T. viride* more than all other treatments, whereas alginated AM fungi significantly increased the antioxidant peroxidase enzyme activity. The highly increase of shoot protein content was achieved by alginated AM fungi (0.193 mg/g fresh weight), while alginated *T. viride* significantly gave the highest values of shoot proline and total phenols contents (0.0173µ mole/g and 0.263 mg/gm, respectively). Alginated *T. viride* still the unique one as it obtained the highest percentages of macroelments N (1.10), P (0.83) and K (0.95) %, respectively. Thus, the biocontrol agents and alginatedAM fungi and *T. viride* are considered the most promising effective bioagents to control *M. phaseolina*.

Key words: Arbuscular mycorrhizal fungi, biocontrol, *T. viride* alginated formula, chitinase & peroxidase enzyme, uptake of NPK, plant growth parameters.

INTRODUCTION

Macrophomina phaseolina (Tassi.) Goid., is an important soil borne pathogen causes charcoal rot in 500 plant species worldwide, especially bean plants (Purkayastha *et al*., 2006). The fungus establishment in soil forced the farmers to control it with costly and harmful chemicals. Alternative control means such as integrated biological management has become a necessity for its eradication as well as for saving our ecosystem. Biological control has been considered as an alternative to synthetic fungicides to control various diseases (Nico *et al*., 2005). Numerous researches have been focused on searching and selecting antagonist microorganisms on diverse soil pathogens as bacteria such as *Bacillus*, *Pseudomonas,* and *Streptomyces* besides *Trichoderma*, *Penicillium*, *Gliocladium*, *Aspergillus* and *Rhizopus* fungal species (Singh *et al*., 2008). However, the challenge that faces biological control is the packing of microbial bioagent by capsule that saves the antagonistic and vital activity for as long as possible and enables to transfer and use it as a biocide. The biocontrol microorganisms are wet or dry as formulated pellets and alginate type pellets are used in formulation of microbial pesticides. The alginate pellets are, however, containing spores of various biocontrol fungi (Lewis and Papavizas, 1987). *Trichoderma* spp. are opportunistic virulent plant symbionts as well as being parasitic to somepathogenic fungi (Harman *et al*., 2004). The mycoparastism mechanisms of *Trichoderma* spp. including coiling around pathogen hyphae, penetration and subsequent dissolution of host cytoplasm can put them in an advanced rank as strong bioagents (Howell, 2003).

In recent past decades, arbuscular mycorrhizal fungi have shown encouraging results in biological control by many researchers. Several trials are being made with AM fungi continuously for biological control of several pathogens. The fungi (AMF) symbiosis is a well-known terrestrial symbiotic association formed between fungi and roots of vascular plants (Douds and Siedel, 2012). They also particularly relevant due to implications for plant fitness (Verbruggen *et al*., 2013) by increasing defense mechanisms against pathogens (Doley, 2012 and Arabi *et al*., 2013) leading to improvement in plants productivity. The potential role of AM fungi for biological control was evaluated with overall response of growth defense related enzymes and disease incurred upon by *M. phaseolina* in tomato plant (Douds and Siedel, 2012).

The aim of the current study was to evaluate the inhibitory action of arbuscular mycorrhizal fungi and *Trichoderma viride* against *M. phaseolina* and its disease (charcoal rot) of common bean under greenhouse conditions.

MATERIALS AND METHODS

Isolation and identification of the pathogenic fungi

The common bean plants showing typical symptoms of charcoal rot were collected from Kafr-Elshiekh governorate. The fresh collected diseased plant tissues were thoroughly washed under running tap water, cut to small pieces and sterilized with 0.05% sodium hypochlorite for 5-min. Then, they were washed briefly in sterile distilled water then dried on sterile filter paper, plated onto PDA and incubated at 28°C for 5 days. The growing colonies were purified by hyphal tip method (Kokalis-Burelle *et al.,* 1992) and maintained on PDA at 4°C. The isolated fungal colonies were identified based on the morphology and microscopic characteristics. Identity of the fungus was gentely confirmed by the staff of Department of Disease Survey and Fungal Taxonomy, Plant Pathology Research Institute, ARC, Giza, Egypt, as *Macrophomina phaseolina.*

Isolation and preparation of *T. viride* inoculum

Isolate of the *Trichoderma viride* was kindly obtained from Plant Pathology Research Institute (PPRI), ARC, Giza, Egypt. The fungus was grown separately on potato dextrose broth (PDB) in Erlenmeyer flasks containing 200 ml medium. One ml of the conidial suspension (10^6conidia/ml) was inoculated and the flasks were incubated at 28±1 °C for three weeks.

Preparation of Arbuscular Mycorrhizal (AM) inoculum

The mycorrhizal roots of stockpot culture of *sorghum bicolor* possessed the mixed genera of *Glomus*, *Gigaspora* and *Acaulospora* colonized between 80 – 90% were used as an inoculum (Giovanetti and Mosse 1980) , were obtained according to methods of in order to use throughout the present experiments.

Alginate preparation of *T. viride* as bioagent:

The preparation of *T. viride* capsule carrier using sodium alginate was carried out according to the method described by Essa *et al.* (2014).

Alginate preparation of AMF as bioagents

Fragments (0.1 mm) of colonized *Sorghum bicolor* were sterilized in a solution (40 mg Streptomycin, 2gm Chloramine – T Trihydrate and 0.1 ml Tween 80, all the contents were dissolved in 100 ml sterilized distilled water). Root fragments were mixed in 1.5% sodium alginate that were poured in sterile Petri dishes to form a thin 2.0 mm layer. The mixture was hardened by 0.5 M of $CaCl_2$ solution and after 30 min it was washed with sterile distilled water. Alginate material was then cut into 3x2 mm^2 pieces (Vassilev *et al.*, 2001).

Plant materials

Common bean seeds *(Phaseolus vulgaris* L.*)* of susceptible cultivar (Bronco) were gently obtained from the responsible staff of Horticulture Research Institute, Agriculture Research Center (ARC), Giza, Egypt. At the beginning of trials, seeds were surface sterilized with Sodium Hypochlorite (5%) for 5 min. and then washed several times with sterile distilled water.

Soil growing medium

The soil used was clay loam in texture having the following characteristics: according to Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt. pH 7.5; EC 2.10 dsm^{-1}; soluble cations Ca^{+2}, Mg^{+2}, Na^{+} and K^{+} (mg/l) 4.35, 4.6, 13.2 and 0.65, respectively. Soluble anions Co^{-3}, Hco3, Cl and So_4^{2-} (mg/l), traces, 12.11, 7.11 and 7.11, respectively. Total N, P, K (mg/kg) 39.14, 15.17 and 234.0, respectively. Also, Co_3^{-} 2.8 (%), organic matter 1.35 (%) and field capacity 36.5 (%), respectively.

Experimental design

Five bean seeds (cv. Bronco) were sown in plastic pots in greenhouse of Vegetable Dis. Dep., Plant Pathol. Res. Inst. ARC, Giza, Egypt. during March 2014. Before 7 days of sowing, pathogen inoculum (12 g) was used to infest inoculated pots (30-cm diam.) containing 4 kg soil of each. Complete randomized blocks design was adopted with 6 treatments in five replicates as following:

(1) alginated AM, (2) free AM,
(3) alginated *T. viride,* (4) free *T. viride*,
(5) fungicide (Vitavax 200 40% FS) and
(6) pathogen only.

Wet capsules (0.5 gm) of each bioagent (AMF and *T. viride* were placed onto the infested soil of each pot at 5 cm deep from the surface, whereas spore suspension of *T. viride* (1.8 x 10^6) that enriched on C·zapex·^{s} Dox Broth (Allen, 1950) was used individually at a rate of (5 ml/ pot). The root fragments of free unalginated mycorrhiza (0.5 gm) were also added as the same manner. The fungicide Vitavax 200 40% FS, (a) 5, 6-dihydro-2-methyl-1, 4 oxathine 3-carboxanilide. + (b) tetramethylthiuram disulfides] obtained from Chemtura Australia comp. Pty Ltd at the concentration of 75 ml/100 kg seeds was used.

In vitro, antagonistic action of alginated and unalginated *T. viride* against *M. phaseolina*

The fungal isolate was cultured onto PDA medium for 7 days, then plugs of (5 mm) was taken and re-cultured again onto PDA plate (9 mm) on one side and then one pellet or one disc of *T. viride* growth was cultured onto the opposite side of the same plate. Four plates were served as replicates for each treatment. Plugs of (5mm) of *M. phaseolina* grown onto PDA

were used as control. Mycelial growth samples, cut from the interaction region (pathogen hyphae) in dual-culture tests (14 days), were fixed on slide glass to observe the presence of coiling structures for wall disintegration under an inverted Binocular Light Microscope (Laica brand, 40x).

% Inhibition = {D1(colony diameter in the control) - D2(colony diameter in the treatment))/(D1(colony diameter in the control)} x 100

Sample plants were harvested after 60 days of sowing for various observations of the tested parameters.

Disease severity

Disease severity was evaluated according to scale of Shahzad and Ghaffar (1992), it was calculated as recommended by Liu *et al.* (1995).

$$\% \text{ Disease severity} = \frac{\sum nxr}{5N} x100$$

Where n= number of plants in each numerical rate, N= total number of plants multiplied by the maximum numerical rate, r = 5. Also, % efficacy for each bioagent was calculated as follows:

$$\% \text{ Efficacy} = \frac{\text{value of the control-value of the treatment}}{\text{value of the control}} \text{ X100}$$

On the other hand, shoots and roots dry weights (g)/ plant were recorded at 60 days after sowing.

Chitinase enzyme

The two tested bioagents; arbusclar mycorrhiza and *T. viride* were evaluated for their ability to excrete chitinase enzyme according to the methods described by Boller and Mauch (1988).

Soluble protein, proline and total phenol contents as well as peroxidase activity

Various parameters in fresh bean shoot were assayed as follows: Total Soluble protein (Lowry *et al.*, 1951), proline (Bates *et al.*, 1973), total phenols (Malick and Singh, 1980) and peroxidase enzyme activity (Putter, 1974)were determined according to the referred authors.

Determination of miscellaneous parameters

Total fungal count (- x 10^5 spore/gm rhizosphere) was recorded similar to methods of (Difco Manual, 1985) and the percentage of AM colonization in bean roots was also determined (Phillips and Hayman 1970). Also, the percentages of macro elements; nitrogen, phosphorus and potassium in shoot samples were determined according to Black, (1982).

Statistical Analysis

The randomized complete block design was used. The data were subjected to analysis of variance (ANOVA) using XLSTAT PRO statistical analysis software (Addinsoft). The treatments were repeated three times, and Treatment means were separated using a Fisher's least significant difference (LSD) test. All analyses were, however, conducted at a significance value of $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of *T. viride* on fungal growth

The antifungal potency of *T. viride* displayed high percentage reduction (inhibition) on the radial growth of *M. phaseolina* as show in (Table, 1 & Fig. 1). Both alginated and free *T. viride* preparations showed a remarkable reduction of the fungal growth compared to the control (*M. phaseolina* only). However, the alginated *Trichoderma* fungus showed a tendency of relatively higher effect (63.55 % inhibition) compared to the free 61.11. *Trichoderma* spp. has been the cynosure of many researchers, who have been contributing to biological pursuit to the use of fungi (Verma *et al.*, 2007). Species of the genus *Trichoderma* are important biocontrol agents of several soil borne pathogens (Benitez *et al.*, 2004). The antagonistic effect of *T. viride* against *M. phaseolina* relied on different mechanisms for the control of pathogens (Naseby *et al.*, 2000). Microscopic study showed that *T. viride* was capable to overgrow and degrading mycelia of *M. phaseolina* by coiling around the hypha and lysis of hyphae *M. phaseolina* (Fig. 1). *Trichoderma* spp. attach the host hyphae via coiling, and penetrate the cell wall by secreting cell wall-degrading enzymes (Viterbo *et al.*, 2002).

Effect of *T. viride* on disease severity

Data in table (2) shows significant decreases in disease severity when bean plants were inoculated with AMF and *T. viride* was used. The chemical fungicide Vitavax 200 40% FS revealed higher efficacy (70 %) and lower disease severity % (21 %). Alginated AM- fungi and *T. viride* showed lower disease severities 31 and 23 %, respectively and higher efficacies 55.7 and 67.1% more than the free ones. *M. phaseolina* only recorded the highest disease severity percentage (70%). Although the fungicide Vitavax had the highest effect on the pathogen, both A. mycorrhizal fungi and *T. viride* in alginated and free forms could reduce the severity of the disease and led to increasing in efficacy percentages. These positive results are in accordance with those found by (Al-Askar and Rashed, 2010) who stated that several AMF species have been found to control different species of soil borne pathogens, *i.e. Macrophomina, Pythium, Rhizoctonia, Sclerotinium* and *Verticillium* under greenhouse and field conditions. The present data show that *T. viride* still more efficient in decreasing the severity of charcoal

Table (1): *Invitro* antagonistic effect of *T. viride* against *M. phaseolina* mycelial growth

Treatment	Formula	Radial mycelial growth (cm)	% Inhibition
T. viride + *M. phaseolina*	alginated	3.3[b]	63.33
	free	3.5[b]	61.11
pathogen only	-	9.0[a]	-
LSD at 0.05	-	0.59	

Means followed by the same letter (s) within a column in each block are not significantly different ($P \leq 0.05$).

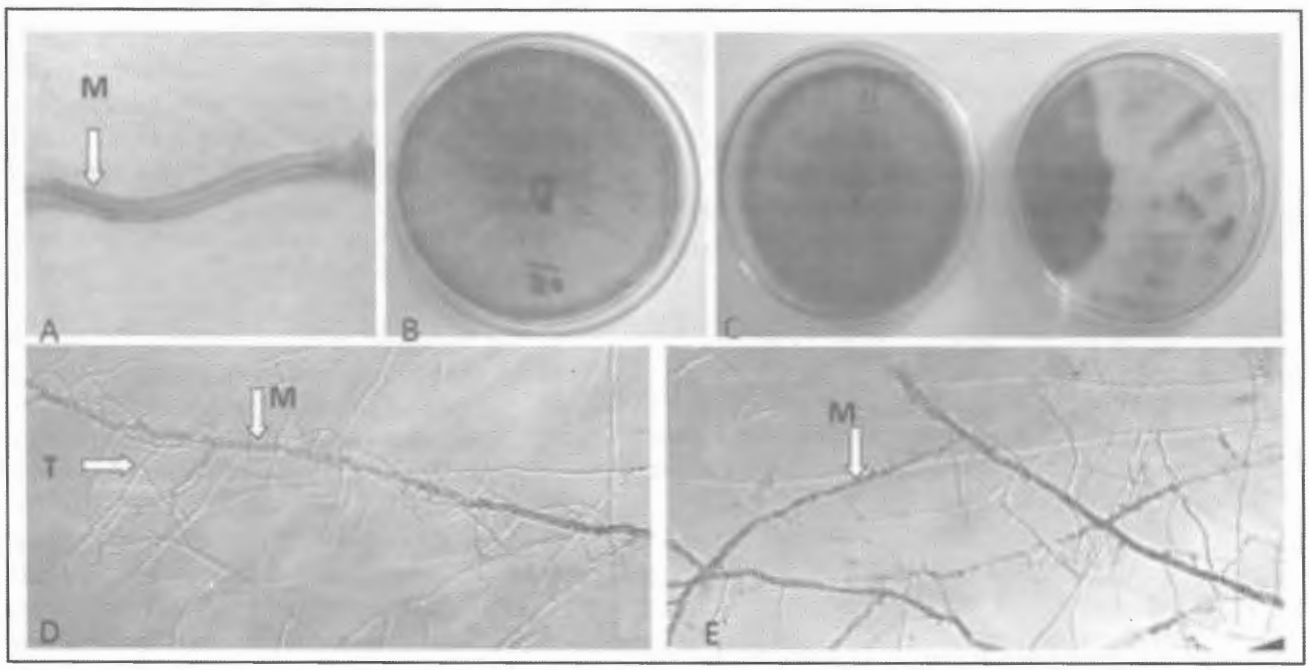

Fig. (1): (A) Symptoms on the root of bean plant, (B) Growth of *M. phaseolina* isolate, (C) Antagonism between *T. viride* and *M. phaseolina* after 7 days of incubation at 30 °C, (D) Lysis of the fungal hypha by *T. viride* and (E) Coiling hypha of *T. viride* on *M. phaseolina*.

Table (2): Effects of Arbuscular mycorrhizal fungi and *T. viride* as bioagents on decreasing percentages of disease severity

Treatment	Formula	% Disease severity	% Efficacy
A. mycorrhiza	alginated	31[b]	55.7
	free	34[b]	51.4
T. viride	alginated	23[cd]	67.1
	free	26[c]	62.8
Vitavax 200 40% FS		21[d]	70.0
Control (pathogen only)		70[a]	
LSD at 0.05		4.3	

Table (3): Shoot and root dry weights of bean plants (60 day old) grown in soil infested with *M. phaseolina* and inoculated with A. mycorrhiza and *T. viride* as bio-agents

Treatment	alginated	Shoot dry weight(g)	Root dry weight (g)
A. mycorrhiza	free	31.3[c]	5.1[bc]
	alginated	29.7[d]	4.2[bc]
T. viride	free	34.6[b]	6.1[ab]
	alginated	31.7[c]	5.1[bc]
Vitavax 200 40% FS	-	38.6[a]	7.3[a]
Control (pathogen only)	-	21.6[e]	3.0[c]
LSD at 0.05	-	1.51	1.87

Table (4): Effects of inoculation by A. mycorrhiza and *T. viride* as bioagents on colonization percentags of AMF and total fungal count (-x10^5 spore/g dry rhizosphere soil) in soil infested with *M. phaseolina*

Treatment	Formula	% Colonization of AMF	Total fungal count (-x10^5) spore/g dry rhizosphere
A. mycorrhiza	alginated	73.7[a]	0.87[e]
	free	60.1[b]	1.7[d]
T. viride	alginated	41.3[c]	3.7[c]
	free	35.3[d]	5.7[b]
Vitavax 200 40% FS	-	17.0[e]	0.56[e]
Control (pathogen only)	-	10.7[f]	13.7[a]
LSD 0.05	-	3.24	0.70

Means followed by the same letter (s) within a column in each block are not significantly different ($P \leq 0.05$).

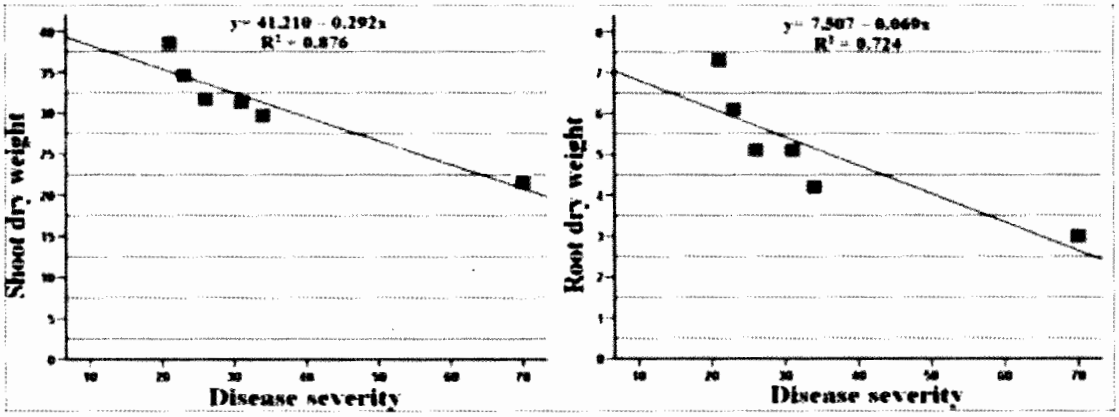

Fig. (2): Association between disease severity with shoot and root dry weight of common bean for 6 treatments during 2014/15 growing season.

rot than AMF particularly in alginated form. This might be attributed to the ability of *Trichoderma* to affect directly on mycelia or survival propagules of other fungi through production of toxic secondary metabolites (Sarrocco *et al.*, 2006). AMF have also been reported in combating the soil borne diseases by inducing plant defense proteins, *i.e.* Pathogenesis Related (PR) proteins (Agrawal *et al.*, 2002 and van Loon *et al.*, 2006) and physical barriers (Sharma *et al.*, 1992). Moreover, AMF are biotrophic in nature, surviving within the root system until crop maturity and hence may give mechanical strength to plant roots against soil borne pathogens (Sharma *et al.*, 1992).

Effect of *T. viride* on the growth of plant shoots and roots

Macrophomina phaseolina caused several deformities on bean plants that are being reflected on the remarkable reduction in shoots and roots dry weights (Table, 3). The existence of biocontrol agents in the rhizosphere compartment improved the plant growth and increased the nutrients uptake. Alginated mycorrhizal fungi and *T. viride* exhibited more shoot and root dry weights than the free ones. Although the fungicides still the superior one, the bioagents could satisfy the plant requirements where via improving soil structure along with to their great capability to increase plant growth and yield through efficient nutrient uptake. AMF also alleviate some nutrient deficiencies (Turkmen *et al.*, 2008). *Trichoderma* fungus had the potential to stimulate plant growth independent of any plant disease. The plant growth promoting fungi (PGPF) belong to Trichoderma genera have been shown to trigger systemic resistance and are beneficial to several crop plants not only by promoting their growth but also by protecting them from the disease infection (Shivanna *et al.*, 1996).

The association of the shoot and root dry weights with disease severity were determined through regression analysis during 2014/15 growing season (Fig., 2).

Effect of *T. viride* on roots conilization by AMF and total fungal count infested soil

Alginated mycorrhizal fungi (Table 4) gave the highest mean values of percentages mycorrhiza colonization in bean roots (73.7%). *M. phaseolina* alone exhibited the lowest mycorrhizal colonization (10.7%). Root- colonizing plant beneficial fungi like AMF are important in protecting plants from root pathogens (Sharma *et al.*, 2009) and yet helped in giving mechanical strength of the plant roots. AM fungi stimulate the activity of beneficial soil microorganisms and root exudation is modified both qualitatively and quantitatively by AM symbiosis and this led to the increase in AM colonization (Soliman *et al.*, 2015). Regarding the total fungal count (Table, 4), the pathogen only

(control) recorded the maximal fungal count over other treatments being (13.7 x 10^5 spore/g dry rhizosphere). The free treatments of AMF and *T. viride* gave more fungal count than the alginated ones. Freely applied *T. viride* exhibited more fungal count (5.7 x 10^5 spore/g dry rhizosphere) than the corresponding free one (1.7 x 10^5 spore/g dry rhizosphere). The least count, however, obtained with the fungicide Vitavax 200 (0.56x10^5 spore/g dry rhizosphere). The increases in total count in the free *A. mycorrhiza* and *T. viride* were higher than the alginated ones depended on the ability of *Trichoderma* to associate freely with soil around plant roots and debris and could have the potential to stimulate plant growth and besides its ability to suppress most of pathogenic fungi (Pandya and Saraf, 2010). AMF treatment could decrease plant diseases incidence and systemically reduce all diseases infection and thereby encourages the other beneficial organism to be multiplied rapidly particularly the inoculated ones (Al-Askar and Rashad, 2010).

Effect of *T. viride* on chitinase and peroxidase enzymes activity

Data in Table (5) reveal that both alginated AMF and *T. viride* have the ability to secrete chitinase enzyme more than the other treatments. Alginated *T. viride* yielded higher chitinase activity (0.95 mg/g dry soil) more than AMF that showed (0.71 mg). Fungal cell walls are all characterized by the presence of chitin, cellulose or both. The microbial bioagents produce chitinase enzyme in order to degrade or dissolving cell wall chitin of other pathogenic organisms. An increase of chitinase activity has been detected during early interaction between roots of host plant that being infected with pathogen and the bioagents particularly AMF and *T. viride* where new chitinase isomers have been reported by Maksimou *et al.*, (2014) that were specifically induced in several AM and *Trichoderma* associations.

Peroxidase activity in shoots of bean plants varies with the tested treatments (Table, 5). The activity was higher the interacted treatment of both bioagents and the pathogen. Alginated AM fungi and *M. phaseolina* treatment and that for (*T. viride* and *M. phaseolina)* recorded the highest increases in peroxidase enzyme activity over those for the free ones. They showed 0.0087 and 0.0083 mg activity, respectively. Peroxidase is involved in cell wall reinforcement during plant reactions to pathogens (Collinge *et al.*, 1994). Peroxidase activity associated with epidermal and hypodermal cells also increased in mycorrhiza roots. The presence of pathogen with the bioagent increased the level of peroxidase activity than the pathogen only (control). There are reports of increased antioxidant activities in response to infection in host plant, which provide defenses from reactive oxygen species (Anand and Mohan, 2014).

Table (5): Effects of Arbuscular mycorrhizal fungi and *T. viride* as bioagents on the enzymes activity (chitinase and peroxidase)

Treatment	Formula	Chitinase enzyme activity mg glucoseamine/g dry soil	Peroxidase enzyme activity mg/g/min
A. mycorrhiza	alginated	0.71^{b}	0.0087^{a}
	free	0.53^{c}	0.0071^{b}
T. viride	alginated	0.95^{a}	0.0083^{a}
	free	0.81^{ab}	0.0081^{a}
Vitavax 200 40% FS	-	0.32^{d}	0.0051^{c}
Control (pathogen only)	-	0.1^{e}	0.0056^{c}
LSD at 0.05		0.17	0.00064

Means followed by the same letter (s) within a column in each block are not significantly different ($P \leq 0.05$).

Table (6): Effect of AMF and *T. viride* as bioagents on bean shoot protein, proline and total phenols content

Treatment	Formula	Shoot soluble protein content (mg/gm fresh weight)	Shoot proline content µ mole/gm fresh weight	Shoot total phenols content mg/gm fresh weight
A. mycorrhiza	alginated	0.193^{a}	0.0123^{ab}	0.204^{c}
	free	0.176^{b}	0.0102^{ab}	0.196^{cd}
T. viride	alginated	0.164^{c}	0.0173^{a}	0.263^{a}
	free	0.143^{d}	0.0114^{ab}	0.239^{b}
Vitavax 200 40% FS	-	0.071^{e}	0.0080^{b}	0.161^{e}
Control (pathogen only)	-	0.113^{f}	0.0100^{ab}	0.193^{d}
LSD at 0.05		0.01	0.008	0.008

Means followed by the same letter (s) within a column in each block are not significantly different ($P \leq 0.05$).

Table (7): Effects of Arbuscular mycorrhizal fungi and *T. viride* as bioagents on the uptake of nitrogen, phosphorous and potassium (%) in bean shoots

Treatment	Formula	N%	P%	K%
A. mycorrhiza	alginated	0.85^{bc}	0.81^{ab}	0.85^{ab}
	free	0.77^{c}	0.71^{b}	0.76^{b}
T. viride	alginated	1.10^{a}	0.83^{ab}	0.95^{a}
	free	$1.0a^{b}$	0.75^{ab}	0.83^{ab}
Vitavax 200 40% FS	-	$1.0a^{b}$	0.86^{a}	0.90^{ab}
Control (pathogen only)	-	0.06^{d}	0.03^{c}	0.037^{c}
LSD at 0.05		0.17	0.13	0.13

Means followed by the same letter (s) within a column in each block are not significantly different ($P \leq 0.05$).

Effect of *T. viride* on total soluble protein, proline and total phenols content of plant shoots

The variability in biochemical activity in bean cultivar was observed after the growth period (60 days) as illustrated in Table (6). The shoot protein and proline contents were increased significantly after inoculation by alginated AMF and *T. viride* in soil infested with the pathogen. Alginated mycorrhizal fungi treatments recorded more soluble protein content (0.193 mg/g in fresh shoots) than alginated *T. viride* that showed (0.164 mg). The least value of increase in protein was obtained with Vitavax 200 40% FS that showed (0.071 mg), whereas control treatment (pathogen only) gave (0.113 mg). On the contrary, alginated *T. viride* and the pathogen produced more proline content than alginated AMF with the pathogen. They recorded values by (0.0173 and 0.0123 µmol. respectively). Moreover, the fungicide Vitavax 200 40% FS recorded the least proline content. The pronounced increase in soluble protein content has been suggested to induce fresh protein synthesis in host plants after infection (Doley *et al.,* 2014). The proline is considered to provide diseases resistance as they scavenge reactive oxygen species generated during pathogen attack and other kinds of biological stresses (Chen and Dickman, 2005). The total phenol content of bean plants, in the presence of pathogen, was higher when the plants inoculated with AMF and *T. viride* than uninoculated plants. Alginated *T. viride* exhibited the highest value of total phenols (0.263 mg/g in fresh shoot), whereas the fungicidal treatment gave the least content being (0.161 mg/g in fresh shoot). The increase in total phenol content may be attributed to high phenol levels which are known to be antimicrobial compounds and found to be higher in resistant cultivars (Singh *et al.,* 2010). The significant increase in shoot phenols content was also attributed to pathogen invasion to the host plant tissues. The first stage of defense mechanism in plants is the rapid accumulation of phenols at the infection site, which restricts or may slow down the growth of pathogen because of its antioxidant and antimicrobial properties (Lamba *et al.*, 2008).

Effect of *T. viride* on N, P & K contents of plant shoots

Table (7) reveals that plants infected by the pathogen and inoculated alginated *T. viride* recorded high values of N, P and K being (1.1, 0.83 and 0.90 %, respectively). Fungicide treatment also resulted on the highest values of N, P and K, whereas alginated AMF revealed moderate percentages of contents. While, the control treatment (*M. phaseolina* only) gave the least percentages. *T. viride* and AMF fungi besides their ability to inhibit the deleterious effect of *M. phaseolina* pathogen, they could enhance the growth of bean plants through mobilization of phosphorus and nitrogen into plants and could regulate K^+ transport into host plants. Therefore, they might be considered as plant growth promoting fungi where they enhance directly the growth of the plant as a symbiotic interaction (Pandya and Saraf, 2010).

The decrease of N, P and K uptake with the plants infected by the pathogen only was attributed to severe deformation and lysis of the host roots and consequently, it could inhibit all physiological processes and banned the translocation of nutrients from roots to shoots (Doley and Jite, 2012).

In conclusion, the management of charcoal rot of bean plants could be based on strategies that integrate several control measures. The present study showed remarkable results when bean plants were inoculated with each of AM fungi and *T. viride* in either alginated or free forms. The harmful effects of *M. phaseolina* can be lowered due to the role of these efficient bioagents through its bio-control characteristics apparently as improved growth of host plant, anatomical changes in roots, competition and mycoparasitism.

REFERENCES

Al-Askar, A. A. and Rashad, Y. M. 2010. Arbuscular mycorrhizal fungi: A biocontrol agent against common bean *Fusarium* root rots disease. Plant Pathol. J., 9(1): 31-38.

Allen, O. N. 1950. Experiments in soil bacteriology. Burgess Publishing, Co., USA.

Anand, S. P. and Mohan, G. 2014. Antioxidant activity, phenol and flavonoid contents of *Acacia sinuata* (Lourr) Merr. Biosci. Discov., 5:24-27.

Arabi, M. I. E., Kanacri, S., Ayoubi, Z. and Jawhar, M. 2013. Mycorrhizal application as a biocontrol agent against common root rots of barley. Research in Biotech., 4:07-12.

Bates, L. S., Waldren, R. P. and Teare, L. D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39:205-207.

Benitez, T., Rincon, A. M., Limon, M. C. and Codon, A. C. 2004. Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol., 7: 249–260.

Black, C. A., Evans, O. D., Ensminger, L. E., White, J. L. N., Clark, F. E. and Dinauer, R. C. 1982. Methods of soil analysis Part 2: Chemical and Microbiological Properties, Soc. of Am. Inch. Publ., Madison, Wisconsin, USA, 1572 pp.

Boller, T. and Mauch, F. 1988. Colorimetric assay for chitinase. Meth. Enzymol., 161:430-435.

Chen, C. and Dickman, M. B. 2005. Proline suppresses apoptosis in the fungal pathogen *Colletetric humtrifolli.* Proceeding of the National Academy of Sci. USA, 102: 3459- 3464.

Collinge, D. B., Gregersen, P. L. and Thordal, Christenen H. 1994. The induction of gene expression in response to pathogenic microbes, In Mechanism of Plant Growth and Improved Productivity, Modern Approaches and Prespectives, A. S. Basea ed., Marcel Dekker, New York, pp. 391-433.

Difco, Manual, 1985. Dehydrated culture media and reagents for microbiology 10th ed. Difco Laboratories Defro it Michigan, USA, 4823: 487-623.

Doley, K. 2012. Biological control of soil-borne root diseases in Ground-nut by inoculating microbial inoculants (AM fungi and *Trichoderma*). Ph. D. Thesis, University of Pune, (M. S.) India.

Doley, K. and Jite, P. K. 2012. Effect of arbuscular mycorrhizal fungi on growth of groundnut and disease caused by *Macrophom inaphaseolina*. J. Exp. Sci., 3(9): 46-50.

Doley, K., Borde, M., Dudhane M. and Jite, P. K. 2014. Efficiency of *Glomus fasciculatum* and *Trichoderma viride* in bio-control of soil-borne pathogen (*Macrophom inaphaseolina*) on different groundnut cultivars. Biosci. Disc., 5(2):163-169.

Douds, D. D. and Seidel, R. 2012. The contribution of arbuscular mycorrhizal fungi to the success or failure of agricultural practices. In: Edwards, C.A., Cheeke, T., Coleman, D., Wall, D., eds. Microbial ecology in sustainable Agroecosystems. Boca Raton, FL: Taylor Francis Group, pp.133-152.

Essa, T. A., Mohanna, Naglaa, A. and Mansour, A. S. 2014. Longevity of *Trichoderma harzinum* and *Trichoderma viride* in alginate preparation in relation to the stock propagation media. Egypt. J. Biol. Pest Cont., 24(2): 471-476.

Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol., 84: 489-500.

Harman, G. E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol., 2: 43-56.

Howell, C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis., 87: 4–10.

Kokalis-Burelle, N., Backman, P. A., Rodriguez-Kabana, R. and Ploper, L. D. 1992. Potential for biological control of early leaf spot of peanut using *Bacillus cereus* and chitin as foliar amendments. Biol. Cont. 2:321-328.

Lamba, P., Sharma, S., Munshi, G. D. and Munshi, S. K. 2008. Biochemical changes in sunflower plants due to seed treatment/ spray application with bio-control agents. Phytoparasitica, 36(4): 388-399.

Lewis, J. A. and Papavizas, G. C. 1987. Application of *Trichoderma* and *Gliocladium* in alginate pellets for control of *Rhizoctonia* damping-off. Plant Pathol., 36(4): 438-446.

Liu, L. Kloepper, J. W. and Tuzun, S. 1995. Introduction of systemic resistance in cucumber against *Fusarium* wilt by plant growth-promoting rhizobacteria. Phytopathol., 85: 695-698.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurements with Folin-Phenol reagent. J. Biol. Chem., 193:265-275.

Maksimou, I., Troshina, N., Surina, O. and Cherepanova, E. 2014. Salicylic acid increases the defense reaction against bunt and smut pathogens in wheat calli, J. Plant Interact., 9:306-314.

Malick, C. P. and Singh M. B. 1980. Plant enzymology and histo-enzymology. Kalyani publishers, New Delhi, 286 pp.

Naseby, D. C., Pascual, J. A. and Lynch, J. M. 2000. Effect of biocontrol srtauns of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. J. Appl. Microbiol., 88(1): 161-169.

Pandya, U. and Saraf, M. 2010. Application of fungi as a biocontrol agent and their biofertilizer potential in agriculture. J. Adv. Dev. Res., 1(1) 90-99.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedure for cleaning roots and staining parasitic

and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc., 55:158-160.

Nico, A. I., Mónaco, C., Dal Bello, G. Alippi, Y. H. 2005. Efectos de la adición de enmiendasorgánicas al suelosobre la capacidad patogénica de *Rhizoctonia solana.* II. Microflora asociada y antagonismo in vitro de los aisladosmás frecuentes. RIA 34:29-44.

Purkayastha, S., Kaur, B., Dilbaghi, N. and Chaudthury, A. 2006. Characterization of *Macrophom inaphaseolina*, the charcoal rot pathogen of cluster bean, using conventional techniques and PCR based molecular markers. Plant. Pathol., 55:106–16.

Putter, J. 1974. Peroxidase. In: Bergmeyer HU, ed. Methods of Enzymatic Analysis. New York, USA: Academic Press, pp 567-1124.

Sarrocco, S. N., Miikkelsen, L., Vergara, M., Jensen, D. F., Lubeak, M. and Vannacci, G. 2006. Histopathological studies of sclerotia of phytopathogenic fungi parasitized by GFP transformed *Trichoderma virens* antagonistic strain. Mycol. Res., 110: 179-182.

Shahzad, S. and A. Ghaffar. 1992. Root rot and root knot disease complex of mungbean and its biological control. pp. 349-256. In: Status of Plant Pathology in Pakistan. Proc. National Symp., (Eds.) A. Ghaf Far & S. Shahzad. Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Sharma, A. K., Johri, B. N., Gianinazzi, S., 1992. Vesicular arbuscular mycorrhiza in relation to plant diseases. World J. of Microbiol. And Biotechnol., 8: 559–563.

Sharma, D., Kapoor, R. and Bhatnagar, A. K. 2009. Differential growth response of *Curculigo orchioides* to native arbusqular mycorrhizal fungi (AMF) communities varying in number and fungal components. Eur. J. of Soil Biol. 45:328-333.

Shivanna, M. B., Meera, M. S. and Hyakumachi, M. 1996. Role of root colonization ability of plant growth promoting fungi in the suppression of talke-all and common root rot of wheat. Crop Prot., 15: 497-504.

Singh, N., Pandey. P., Dubey, R. and Maheshwari, D. 2008. Biological control of root rot fungus *Macrophom inaphaseolina* and growth enhancement of *Pinusrox burghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. World J. Microbiol. Biotechnol.24:1669-1679.

Singh, A., Singh, K. P., Rajwarand, G. S. and Singh, U. P. 2010. Phenolic acid content-a criterion for selection of resistance apple cultivars against *Podosphaera leucotricha* (EII. and Ev.) Salmon. J. Arch. Phytopathol. Plant Prot., 43:1138-1143.

Soliman, Amira, Sh., Morsy, Ebtsam, M. and Massoud, O. N. 2015. Tolerance of bio-fertilized *Delonixregia* seedling to irrigation intervals. J. Hortic. For. 7(3): 73-83.

Turkmen, O., Sensoy, S., Demir, S. and Erdinc, C. 2008. Effects of two different AMF species on growth and nutrient content of pepper seedlings grown under moderate salt stress. African J. Biotechnol., 7(4):392-396.

Van Loon, L. C., Rep, M., and Pieterse, C. M. J. 2006. Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol., 44, 7:1–7.

Vassilev, N., Vassileva, M., Fenice, M. and Federici, F. 2001. Immobilized cell technology applied in solubilization of insoluble inorganic rock phosphates and plant acquisition. Bioresource Technol., 79: 263-271.

Verbruggen, E., Marcel, G. A., van der Heijden, Rillig, M. C. and Kiers, E. T. 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol., 197:1104-1109.

Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y. and Valero, J. R. 2007. Antagonisic fungi, *Trichoderma* spp.: Panoply of biological control. Biochem. Eng. J., 37: 1-20.

Vincent, J. M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 150: 850-853.

Viterbo, A., Ramot, O., Chemin, L., Chet, I. 2002. Significance of lytic enzymes from *Trichoderma* spp. in the biocontrol of fungal plant pathogens. Antonie van Leeuwenhoek 81: 549-556.