Trichoderma harzianum as a Seed Coat to Control *Botrytis cinerea* Leaf Spot on Faba Bean

Zahra Ibrahim El-Gali*(1)

(1). Department of Plant Protection, Faculty of Agriculture, Omer Al-Mukhtar University, El-Beida, Libya.
(*Corresponding author: Dr. Zahra Ibrahim El-Gali. E-Mail: Zahra.Ibrahim@omu.edu.ly).

Received: 01/05/2020 Accepted: 01/06/2020

Abstract

The present investigation was conducted to demonstrate and to exploit *T. harzianum* isolated from local soil by coated seeds to control *B. cinerea*, the causative agent of brown spot disease in faba bean cv. Minor in greenhouse. Faba bean leaves were collected on 14 days after inoculation. The results showed a pronounced and significant reduction in percent disease incidence. *B. cinerea* was applied to leaves of pretreated plants, spot area was less appeared (22.3%), and the protection percentage reached about 77.7%. Whereas, the protection percentage did not exceed 18.3% and the spot area was more appeared on non-treated plants (81.7%). Plant length and root extension measurements were enhanced in pretreated plants and inoculated with pathogen.

Keywords: *Botrytis cinerea*, *Trichoderma harzianum*, Leaf spot, Faba bean.

Introduction:

Faba bean (*Vicia faba* L.) is a species of (Fabaceae) and one of the economic important legumes grown in Libya and many parts of the world, which can be planted in the open field in two-year seasons, summer and winter. They may be consumed as seeds (green or dried, rehydrated), used as a source of protein in human diet for substitute of animal protein, animal feed and also as green manure (El-Saghier, 1986). The crop had high nutritional value due to its richness in vitamins and protein. In addition, helps to improve the soil fertility through to fix nitrogen. Therefore, improving the production of this crop is one of the objectives in agriculture in many countries.

Brown spot disease, caused by *Botrytis cinerea* Pers is the most serious disease affecting faba bean in Libya and in several countries in all the countries of North Africa (Abo-Ghniya, 1991). It occurs on all top growth of the plant. The leaves are usually the first infected organs are first brown circular spots 1 to 3 mm in diameter. When the temperature conditions are favorable, these spots enlarge, reaching from 10 to 15 mm are zoned merge and thus covering a large part of the leaf surface, causing defoliation of plants. Mode and the development of infection have been described by Mansfield and Deverall (1974).

Botrytis diseases on different crops are usually controlled by spraying with chemical fungicides are hazardous to human health. In addition, the repeated applications of chemicals have negative impact and their residues caused lot of problems such as environmental pollution and resistance in disease-causing organisms to fungicides (Stangarlin *et al*., 1999; Leroux *et al*., 2002). Biological control is a promising strategy for control of foliar diseases in several crops, and Alternatives of these fungicides. Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection.

Among beneficial microorganisms *Trichoderma* spp. are the most common bio-control agents of plant pathogenic fungi that caused soil-borne, air-borne and post-harvest diseases in several crops (Jegathambigai *et al*., 2009; Christopher *et al*., 2010; El-Gali *et al*., 2015). Several studies were investigated role of *Trichodermna* bio-control agents against diseases of leaves spots and plant growth enhancement (Prasad, 2013 ; El-Gali, 2015). The objective of the present research was determine (i) to study the disease severity in plant subjected to *Trichoderma* and (ii) to measure effect of *Trichoderma harzianum* on specific growth parameters in plant.

Materials and Methods:

Fungal material:

The bioagent fungus *T. harzianum* was isolated from soil samples taken from the field in El-Beida city in northeast of Libya, and The pathogenic fungus *B. cinerea* was isolated from a diseased plant, and all the fungi were maintained in the collection of Microbiology Laboratory of Plant Protection, Faculty of Agriculture, Omer Al-Mukhtar University. Both fungi used in experiments were grown on PSA plates for 7-14 days.

Seed selection:

Vicia faba, Variety-minor seeds were obtained from market of El-Beida and stored at room temperature. Healthy seeds without cracks and other visible deformations were selected and surface and sterilized for 4-5 minutes with 1% sodium hypochlorite solutions. Seeds were then rinsed twice with sterile distilled water and dried in air.

For coating with *T. harzianum*, seeds dipping into 1% Carboxy Methyl Cellulose (CMC) solution as a sticker for 2 min. The seeds were taken from CMC and rolled over *T. harzianum* 14 days old culture in Petri-plates (Ramezani, 2010), then allowed to dry overnight (Fig 1-a). The plastic pots (20 cm diam.) were filled with sterilized soil, 2-coated seeds were sown into pots. Each experiment was run in 10 replicates and repeated twice. There were 4 treatments in each assay including health control, pathogen control, bioagent alone and their mixture (Biofun. + Pathofun.). Plants were watered regularly (once a day) so that the soil was maintained near saturation (Fig. 1-b,c).

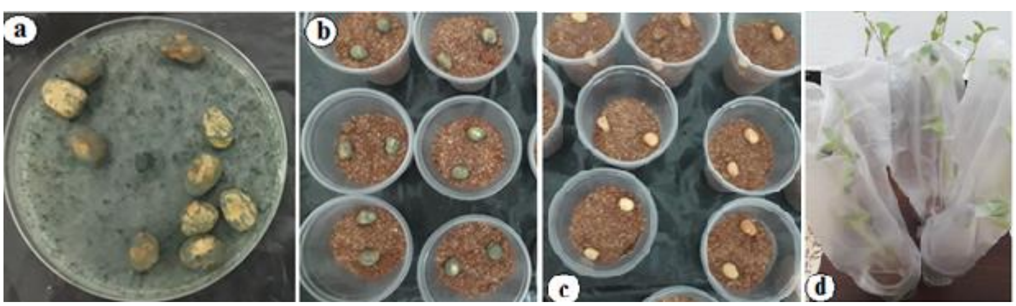

Fig. 1.a: Faba bean seeds coated with *T. harzianum*, b: seeds sown and c: seedling covered

Plant leaf inoculation with *B. cinerea:*

Plants were infected with *B. cinerea* on same day, at 20 days after germination. *Botrytis* mycelium disks of actively growing hyphae (0.5 cm in diameter) from 8-day-old cultures on PSA were placed on the upper surface of five leaves per plant seedling (one disk per leaf). Inoculation was performed on leaves that were carefully chosen (similar size and position of insertion). All the plants were covered with plastic bag (Fig. 1-d), then removed after 24h.

Data collection:

Data was collected from the 2week after inoculation. The development of *B. cinerea* was estimated by measuring the areas of the lesions around the mycelial disks. *B. cinerea* leaf invasion is expressed as the percentage of damaged tissue of the total leaf areas and protection against *B. cinerea* as the percentage of the difference between the necrosis areas of the damaged tissues of non-treated plants and that of the treated ones, divided by that of non-treated ones according to formula (1) and (2) that remind by Mohamed *et al*. (2007):

$$\% \, Infection = \frac{Spot\ area}{Leaf\ area} X\ 100 \ldots\ldots\ldots (1)$$

$$Protection\ percentage = \frac{\% \, inf.control - \% \, inf.treatment}{\% \, inf.control} X\ 100 \ldots\ldots\ldots\ldots\ldots (2)$$

for plant length and root extension, Five plants were examined, removed from soil, then washed under tap water to cleaning soil granules on roots. The measurements were recorded by used the ruler (Fig. 2).

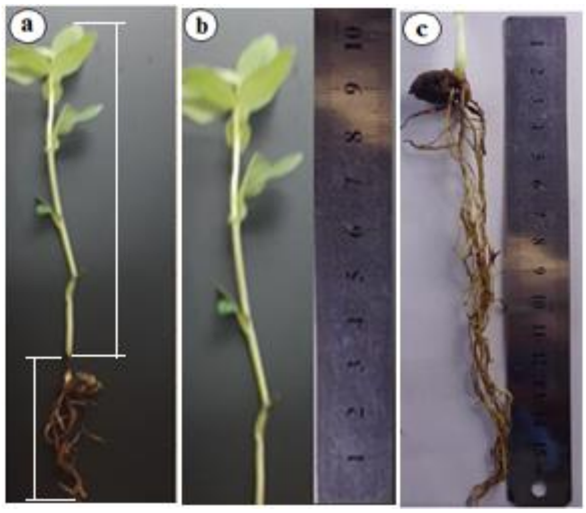

Fig.2.a: Measurements of Faba bean seedlings, b: Shoot length, c: Root length.

Statistical analyses:

The experimental design of the present study was a randomized complete design . Analysis of variance (ANOVA) of the data was performed with the Co-STAT statistical. Least significant difference (LSD) at 5% was used to compare treatment means by using Duncan's multiple range test (DMRT). Percentage date was transformed into arcsine angles before carrying out the ANOVA to produce approximately constant variance.

Results:

Observation in *vivo*, after 14 days of inoculation, results showed that the large spots or lesions on plants leaves were found in case the seeds non coated with *T. harzianum* and inoculation with *Botrytis* alone compared with small lesions on leaves from seed coated with biofungus and also inoculated with pathofungus (Fig.3).

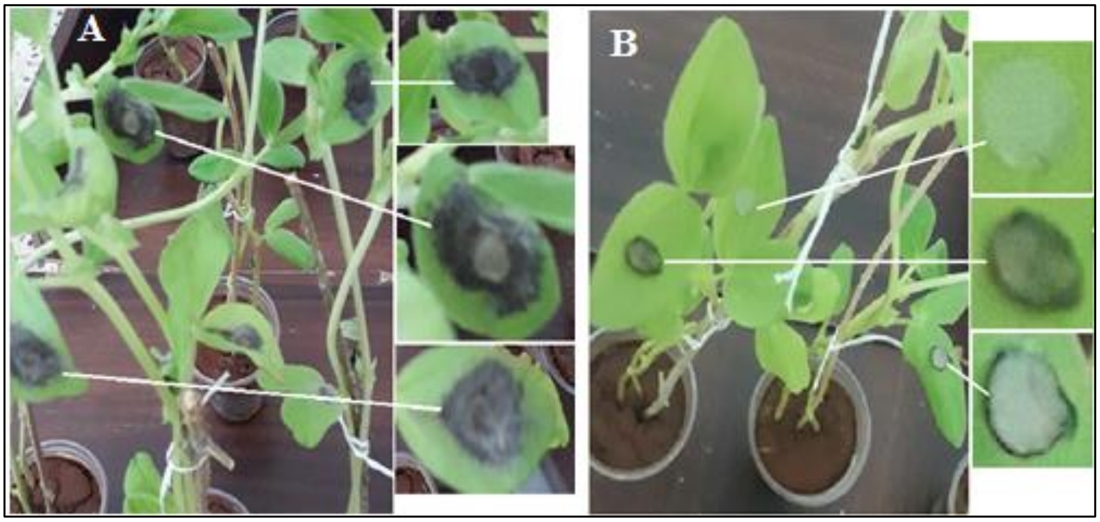

Fig.3. Efficacy of *T. harzianum* in controlling brown spot caused by *B. cinerea* on leaves. (L): Inoculation with pathogen without treated with Th; (R): Treated with Th and then inoculated with pathogen

The degree of protection obtained with *T. harzianum* could be estimated by recorded the dimensions (length and width) of leaf and spot or nicrosis on leaf (Bc. + Th), then, compared with the necrosis measured for plants infected by *B. cinerea* without pretreatment (Fig. 4-A).

After 14 days of *B. cinerea* inoculation, the phenomenon was even more obvious. For the final measurements, in the non-pretreated plants, the spread of *B. cinerea* infection resulted in the rotting of infected leaves that finally fell from the stem (Fig. 4-B), whereas in the other cases (Bc + Th), pathogen progression was slowed and protection could be estimated for *Trichoderma* pretreatment.

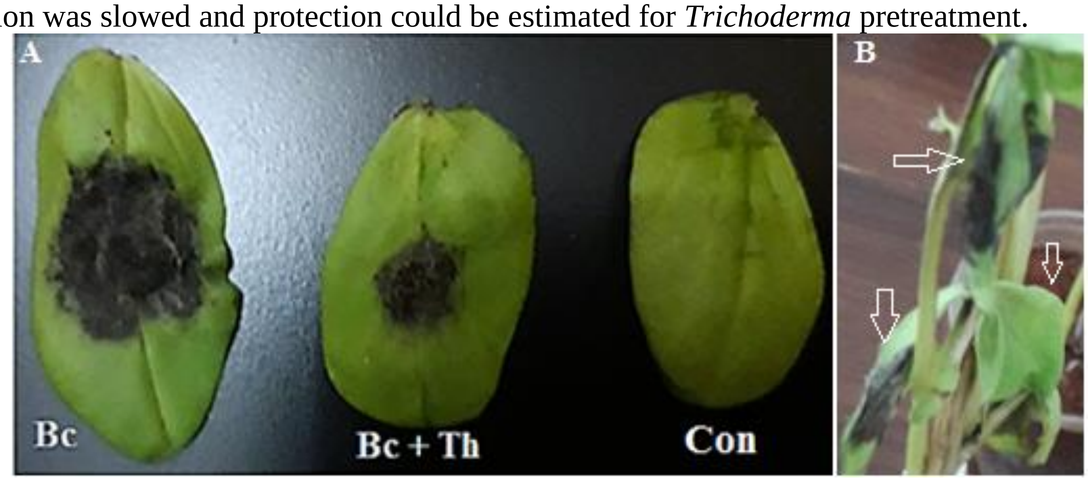

Fig.4. Botrytis spot symptoms at 14days after infection on faba bean leaves. A: (Bc: *Botrytis* alone, *Trichoderma* + *Botrytis*) and control, B: Wilt and died leaves

In pot culture under glasshouse condition spores of *T. harzianum* applied as seed coating caused highly significant reduction in disease incidence as compared to *Botrytis* treatment (Table 1), showed the results of the treatments on percentage disease severity over 2weeks. Plants from seeds that were coated with *Trichoderma* spores had less diseases (22.3%) compared to (81.7%) in plants which were also inoculated with *B. cinerea* but seeds were not coated with *Trichoderma* spores, however the level of disease severity varied significantly.

Concerning to % protection on plants, data in Table (2) shows that, the treatment with bioagent increased significantly the protection percentage. It recorded 77.7% in case Th. + Bc. higher than 18.3% in *Botrytis* alone.

Table 1. Disease incidence on faba bean seedlings subjected to *T. harzianum*

Treatments	% Infection	% Protection
Control	0.0 (0.0) c	100 (90.0) a
***Botrytis* alone**	81.7 (64.67) a	18.3 (25.33) c
***Trichoderma* alone**	0.0 (0.0) c	100 (90.0) a
Th. + Bc.	22.3. (23.03) b	77.7 (23.03) b
LSD at 5%	18.2***	0.55***

Control: seedlings from seed non coated and without inoculation.
Each value is a mean of 10 replicates
Values between brackets are angular transformed (arc sine angles √y) data
Values followed by the same letter(s) are not significantly different at P= 0.05

One set of the plants (3 pots; 2 plants/pot) were kept to 14 days to observe the effect of the treatment on growth parameters (plant length and root extension). Plants treated with *T. harzianum* followed by infected with pathogen *B. cinerea* showed stimulating vegetative growth and root development compared than the plant from the control *B. cinerea* alone and the others without infection respectively. Plants subjected to *T. harzianum* seed coat pretreatment exhibited increased shoots and roots measurements compared with no pretreated plants (Fig. 5).

Fig. 5. Enhanced roots exploration in faba bean seedlings. a and c: roots from seeds without coat by bioagent, b and d: roots from seeds coating with bioagent

The data presented in Table (2) indicates that the length of the plants infected by the pathogen was less among all types of studied treatments. It recorded 16.8 cm less than 26 cm in case of Th. + Bc. Concerning treatment with *Trichoderma* on roots extension, the results indicated that it increases the roots but non-significant differences were recorded between all treatments.

Table 2. Increased plant response in faba bean seedlings treated by *T. harzianum*

Treatments	Plant length (cm)	Root extension (cm)
Control	20 bc	12.8 b
***Botrytis* alone**	16.8 c	11.0 b
***Trichoderma* alone**	31 a	21.2 a
Th. + Bc.	26 ab	13.3 b
LSD at 5%	6.7**	4.3**

Control: seedlings from seed non coated and without inoculation.
Each value is a mean of (3 pots; 2 plants/pot)
Values followed by the same letter(s) are not significantly different at P= 0.05

Discussion:

The results presented in this paper describe the ability of *T. harzianum* to induce and reduce the severity of the diseases causing by *B cinerea* on faba bean leaves and stimulating vegetative growth and root development of the treated plants. Many bio control agents, such as fungi, bacteria and viruses, are not only able to control the pathogens that cause plant disease, but are also able to promote plant growth and development (Harman *et al*., 2004). *Trichoderma* species are well known as biocontrol agents for control of several crop diseases (Batta, 2001; Montealegre *et al*., 2010; El-Gali 2015).

Induction systemic resistance (ISR) has been reported against foliar diseases. In this case, Koike *et al.* (2001) found that *Trichoderma* GT3-2 was able to induce resistance in melon plants against *Colletotrichum orbiculare*, the causal agent of cucumber anthracnose. Also, Sawant (2014) mentioned several studies about *Trichoderma* used against foliar diseases. In previously study also, El-Gali (2015) demonstrated that treating carob (*Ceratonia siliaua* L.) seedlings with *T. harzianum* conidial suspension and culture filtrate before inoculation with *A. alternata* has significantly reduce disease incidence on leaves.

The ISR is attributed to an increase in the activities of the defense enzymes, peroxidase and chitinase in the leaves of plant (Sawant, 2014). Induction of this resistance was explained by a lignin deposition at the point of pathogen infection in the epidermal tissues of cucumber hypocotyls. In other cases, induction of resistance was accompanied by biochemical reactions such as salicylic acid, jasmonic acid and/or ethylene secretions (Pieterse, *et al*., 2001 ; Kloepper, *et al*., 2004)

Others studies have shown that it also induces local and systemic resistance in plants, and suppresses enzymes involved in pathogenesis. Some isolates of *Trichoderma*, including *T. harzianum* T39 have the ability to deactivate or minimize the activities of enzymes involved in pathogenesis by *B. cinerea* viz. endo-and exo-polygalacturonases, pectin methyl esterase, pectate lyase, and cutin esterase, etc (Zimand, *et al*., 1996; Kapat *et al*., 1998; Elad and Kapat, 1999).

In faba bean a significant increase of plant growth treated with *T. harzianum* was observed on the measured parameters (plant length, root extension), in treatment of *Trichoderma* compared to non-treated seedlings. Similarly results were obtained by El-Gali (2015) showed that the plants treated with *Trichoderma* suspension or culture filtrate followed by infected with pathogen *A. alternata* showed a good vegetative growth, root development and enhanced leaf area compared than the plant from the control *A. alternata* alone and the others without infection respectively.

The enhanced growth response of several plants following application of *Trichoderma* spp. has also been well documented (Yedidia *et al*., 2003; Abd-El-Kareem, 2007; Sriram *et al*., 2009; El-Gali, 2015). The compounds produced by the BCA in the fungal culture contained various secondary metabolites, like peptaibols, which may also act as elicitors of plant defense mechanisms against pathogens. In fact, the application of peptaibols was found to activate a defense response in tobacco plants (Benítez *et al*., 2004; Viterbo *et al*., 2007). Similarly, the peptaibol isolated and identified from the Lib1 culture could represent a molecular factor possibly involved in the induction of defense mechanisms in *Trichoderma*-treated plants. Vinale *et al*., (2008) have found that some *Trichoderma* compounds, such as 6-pentyl-α-pyrone (6PP) acted as effectors on plant growth, possibly by acting in an auxin-like manner or by stimulating the hormone production in the plant, thus enhancing growth of the root system and plant size. In Other study, Lo and Lin (2002) also reported that the fungus increased both root, shoot and leaf area growth of cucumber. The increased growth response induced by *T. harzianum* may be attributed to that the fungus could improve nitrogen use efficiency in plant and also could also solubilize a number of poorly soluble nutrients, such as Mn+4, Fe+3 and Cu+2 etc. (Altomare *et al*., 1999).

Conclusion:

The results showed that *T. harzianum* as a seed coating was able to reduce disease incidence of *B. cinerea* causative of brown spots in faba bean plants. Plants treated with *T. harzianum* followed by infected with pathogen *B. cinerea* showed a good growth compared to the plant in the control treated with *B. cinerea* alone and the other treatments without infection respectively.

References:

Abd-El-Kareem, F. (2007). Induced resistance in bean plants against root rot and *Alternaria* leaf spot diseases using biotic and abiotic inducers under field conditions. Res. J. of Agric. and Bio. Sci., 3(6): 767-774.

Abo-Ghniya, A. (1991). Horticulturist crops disease. Al-Fatah University publications. 272 pp. (*In Arabic*).

Altomare, C.; W.A. Norvell; T. Bjorkman; and G.E. Harman (1999). Solubilization of phosphate and micronutrients by the plant-growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol., 65: 2926-2933.

Batta, Y.A. (2001). Effect of fungicides and antagonistic microorganisms on the black fruit spot disease on persimmon", Dirasat: Agric. Sci., 28(2 and 3): 165-171.

Benítez, T.; A.M. Rincón; M.C. Limón; and A.C. Codón (2004). Bio control mechanisms of *Trichoderma* strains. Int. Microbiol., 7: 249-260.

Christopher, D.J.; T.S. Raj; S.U. Rani; and R. Udhayakumar (2010). Role of defense enzymes activity in tomato as induced by *Trichoderma virens* against Fusarium wilt caused by *Fusarium oxysporum* f sp. *lycopersici*. J. of Biopest., 3(1): 158 – 162.

Elad, Y.; and A. Kapat (1990). The role of *Trichoderma harzianum* protease in the bio control of *Botrytis cinerea*. Eur. J. Plant Pathol., 105: 177-89.

El-Gali, Z.H. (2015). *Trichoderma harzianum* as a root treatment to control *Alternaria alternata ceratoni* leaf spot on *Ceratonia siliqua* L. Sky Journal of Agriculture Research. 4(8): 161-166.

El-Saghier, Kh. (1986). Field crops. Al-Fatah University publications. 373 pp. (*In Arabic*).

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

Jegathambigai, V.; M. Wilson; R.S. Wijeratnam; R..L.C. Wijesundera (2009). *Trichoderma* as a seed treatment to control *Helminthosporium* leaf spot disease of *Chrysalidocarpus lutescens*. World J. of Agric. Sci., 5(6): 720-728.

Kapat, A.; G. Zimand; and Y. Elad (1998). Effect of two isolates of *Trichoderma harzianum* on the activity of hydrolytic enzymes produced by *Botrytis cinerea*. Physiol. Mol. Plant Path., 52:127-37.

Kloepper, J.W.; C.M. Ryu; and S. Zhang (2004). Induced systemic resistance and promotion of plant growth by *Bacillu*s spp. Phytopathology. 94: 1259-1266.

Koike, N.; M. Hyakumachi; K. Kageyama; S. Tsuyumu; and N. Doke, (2001). Induction of systemic resistance in cucumber against several diseases by plant growth-promoting fungi: lignification and superoxide generation. European Journal of Plant Pathology. 107: 523-533.

Leroux, P.; R. Fritz; D. Debieu; C. Albertini: C. Lanen; J. Bach; M. Gredt; and F. Chapeland (2002). Mechanisms of resistance to fungicides in field strains of *Botrytis cinerea*. Pest Manage. Sci., 58: 876-888.

Lo, C.T.; and C.Y. Lin (2002). Screening strains of *Trichoderma* spp for plant growth enhancement in Taiwan. Plant Path. Bull., 11: 215-220.

Mansfield, J.W.; and B.J. Deverall (1974). Changes in wyerone acid concentrations in leaves of *Vicia faba* after infection by *Botrytis cinerea* or *B. fabae*. Annals of Applied Biol., 77: 227-235.

Mohamed, N.; J. Lherminier; M.J. Farmer; J. Fromentin; N. Béno; V. Houto: M.I. Milat; and J.P. Belin (2007). Defense response in graperine leaves against *Botrytis cinerea* induced by applications of *Pythium oligandrum* strain or its elicitin, oligandrin, to roots. Phytopathol., 97: 611- 620.

Montealegre, J.; L. Valderrama: S. Sánchez; R. Herrera; X. Besoain; and L.M. Pérez (2010). Biological control of *Rhizoctonia solani* in tomatoes with *Trichoderma harzianum* mutants. Elec. J. of Biotechnol., 13.(2): 1-11.

Pieterse, C.M.J.; J.A. Van Pelt; S.C.M. Van Wees; J. Ton; K.M. Léon-Kloosterziel; J.J.B. Keurentjes; B.W.M. Verhagen; M. Knoester; I. Van der Sluis; P.A.H.M. Bakker; and L.C. Van Loon (2001). Rhizobacteria mediated induced systemic resistance: triggering, signaling and expression. European J. of Plant Path., 107: 51-61.

Prasad, B.S.; G. Kamala; and P.S. Ganesh (2013). Efficacy of *Trichoderma viride* to induce disease resistance and antioxidant responses in legume *Vigna mungo* infested by *Fusarium oxysporum* and *Alternaria alternata* Int. J. of Agric. Sci. and Res., 3(2): 285-294.

Ramezani, H. (2010). Antagonistic effects of *Trichoderma* spp. against *Fusarium oxsysporum* f.sp. *lycopersici* causal agent of tomato wilt. Plant Prot. J., 2(1): 167-173.

Sriram, S.; S.B. Manasa; and M.J. Savitha (2009). Potential use of elicitors from *Trichoderma* in induced systemic resistance for the management of *Phytophthora capsici* in red pepper J. Biol. Cont., 23(4): 449–456.

Stangarlin, J.; K.R.F. Schwan-Estrada; M.E.S. Cruz; and M.H. Nozaki (1999). Medicinal plants and alternative control of phytopathogens. Biotecnol. Ciê. and Desenvolv.11:16-21.

Vinale, F.; K. Sivasitham; E.I. Ghisalberti; R. Marra; M.J. Barbetti; H. Li; S.L. Woo; and M. Lorito (2008). A novel role for Trichoderma secondary metabolites in interactions with plant. Physiol. Mol. Plant Pathol., 72: 80–86.

Viterbo, A.; A. Wiest; Y. Brotman; I. Chet; and C. Kenerley (2007). The 18mer peptaibols from *Trichoderma virens* elicit plant defence responses. Mol. Plant Path., 8(6): 737–746.

Yedidia, I.; M. Shoresh; Z. Kerem; N. Benhamou; Y. Kapulnik; and I. Chet (2003). Concomitant Induction of Systemic Resistance to *Pseudomonas syringae* pv. *lachrymans* in Cucumber by *Trichoderma asperellum* (T-203) and Accumulation of Phytoalexins. Appl. and Env. Microbiol., 7343–7353.

Zimand, G.; Y. Elad; and I. Chet (1996). Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. Phytopath., 86: 1255-60.