Efficiency of *Trichoderma* spp. against of some pathogenic fungi causing of broad bean root rot disease

IOP Conf. Series: Journal of Physics: Conf. Series **1294** (2019) 092003 doi:10.1088/1742-6596/1294/9/092003

Efficiency of *Trichoderma* spp. against of some pathogenic fungi causing of broad bean root rot disease

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Abstract. This study aims to a survey broad bean root rot disease in Babylon governorate (80 km south Baghdad-Iraq), and evaluation the antagonistic activity of the biological control agents *Trichoderma harzianium* and *T. viride* against pathogens. The result showed the distribution of the disease in all fields in percentage of disease incidence 65-100% and severity 22.50-68.75%. Results of isolation and identification showed the presence of 6 species of fungi associated with infected plants with variable percentage of presence. *Fusarium solani* and *Rhizoctonia solani* were present in most samples, whereas other fungi *Macrophomina phasealina* , *Aspergillus niger*, *Trichoderma* sp. and *Penecillium* sp. were found with a low rate. *T. harzianums* and *T. viride* fungi had highly antagonistic ability against of pathogenic isolates, and protect broad bean plant from infection by pathogenic fungi by reduced diseases incidence and severity and increased plant growth promoting compared to control (with pathogen). The bio-control agents *T. viride*, *T. harzianium* alone increased plant length to٣٨.٦٧, ٣٩.٧٩ cm and fresh weight to٢٤.٣٣,٢٤.٧٦ g and dry weight to 2.00, 2.27g respectively compared to control.

Key words: Broad bean, Root rot, Biological control, fungi, Pathogen.

1. Introduction.

Vicia faba L. are important economic crops as an important food source for humans and the residue or seed is used as a source of animal feed and is involved in increasing nitrogen content in the soil by root contract bacteria [1,2]. In 2017, the total cultivated area was 32569 donums, while production was 60466 ton [3]. Root rot disease is the major disease affecting the legume family crops. Root rot disease causing by many pathogens found in the soil, mainly *Rhizoctonia solani*, *Fusarium solani* and *Pythium* spp. [4,5,6,7]. These fungi attack seeds, seedling and roots, leading to large losses in production both qualitatively and qualitatively [8,9]. Several methods have been used to control the disease, including the use of chemical control However, chemical pesticides have some negative effects on the environment, human health and non-target organisms due to their extensive and incorrect use [10,11]. Recent trends in the world include the use of the principle of clean agriculture by moving away from the use of chemicals and the use of biotechnologies to introduce microorganisms that reduce the harm of plant pathogens while increasing production. Biological control is a specialized method for its effect, safe and does not cause pollution of the environment. As well as the most important and most advanced methods of controlling agricultural pests today [9, 12, 13, 14]. The most successful organisms in controlling plant pathogens are *Trichoderma harzianum*, which has developed a number of mechanisms to attack pathogens and improve root and plant growth.

One of the most important of these mechanisms is the state of innate parasitism Mycoparasitism [15, 16, 17, 18]. And the production of many pathogenic inhibitors of plant pathogens [19, 20]. (Howell, 2006 and Ramos et al., 2008). The secretion of enzymes that assist in the analysis of cell walls of a host [21]. As well as that *T. harzianum* inhibit the activity of pathogens enzymes [17]. *T. harzianum* is a fierce competitor because it is characterized by rapid growth and colonization of the base material, leading to the elimination of pathogenic fungi [22]. It was also known that *T. harzainum* stimulates induced Systemic Resistance (ISR) and the production of Pathogenicity-Related Proteins (P-RP) in the plant and stimulates the formation of cellulose cell walls in front of the progression of the pathogen [23]. On the other hand, the fungus *Trichoderma viride* is one of the most important fungi used to control many diseases that cause fungi that attack seeds and seedlings, and both root and foliage [24, 25]. The importance of rot root disease the rest of the Broad bean plant and to control the disease biologically objective of the research to Isolation and diagnosis of some fungi causing root rot disease and Evaluation of the efficiency of fungi *Trichoderma harzanium* and *T. viride* aganist the types of fungi causing the disease.

2. Materials and methods.

2.1. Field survey.

A field survey was carried out for some fields in Babylon governorate for the period 6/10 - 25/11/2017 to determine the disease incidence of root rot disease. Six fields were selected within six areas (Table 1), ranging between 1-5 donums.

The severity of infection was calculated using the disease index of 5 degrees as follows:

• = Normal plant, 1 = discoloration or decay of roots more than 0 - 25%. 2 = root rot more than 25 - 50%. 3 = root rot more than 50 - 75%. 4 = root rot more than 75 - 100% without plant death. 5 = death of Plant. The severity of the disease was calculated according to the Mckinney equation [25]. as follows:

% Inhibition=(Number of plants in the class 0 × 0) + ... (number of plants in class 5 × 5) / total number of plants examined x 5)x 100.

2.2. Isolation and diagnosis of fungi from the roots of the diseased plant.

Samples were taken from the roots of the infected plants with root rot and sterilized with sodium hypochlorite solution (1% free chlorine) for 2 minutes and Distilled sterile for 1 minute and then dried with sterilized filter paper. Transfer 4 pieces to 9 cm diameter Petri dishes contained on the Potato Dextruse Agar (PDA). The *Rhizoctonia*, *Fusarium* and other fungus species, was identified depending on the them characteristics [26, 27, 28].

2.3. Preparation of fungal inoculums.

R. solani and *F. solani* were prepared in accordance with Dewan [29]. Local millet seeds were used for the preparation of fungal inoculum. Millet seeds were washed with water to remove dust and impurities, then soaked for 6 hours with water. Left for half an hour to remove excess water. Distributed in 250 mL glass flasks with 50 g of seed / flask. The flasks sterilized in the autoclave for one hour, each flask inoculated with 1 cm disk from the PDA medium containing the colony of of *R. solani* and *F. solani* isolates separately. The flask were incubated at 25 ± 1°C for 15 days with shaking every 3 days to ensure ventilation and distribution on all seeds. This method was also used to prepare the *Trichoderma harzianum* and *T. viride* inoculum (obtained from the Biological control Laboratory).

2.4. Effect of isolates of Rhizoctonia solani and Fusarium solani in the eggplant.

The experiment was carried out in a pots of 12.5 cm diameter and 1 kg capacity, filled with 1 kg of sterile soil at 121°C and pressure of 1.5 kg / cm 2 for 1 hour. The sterilization process was repeated the following day to ensure sterilization and soil left for 7 days before use, the soil was inoculated with *R. solani* inoculum for isolates R1...R6, and *F. solani* ((F1, F2, F4, F5, F6) by 1% (weight / weight). the eggplant seeds were sown with 5 seeds per pot. After planting, the disease incidence and severity were calculated after one month of planting and as described in paragraph

2.5. Test the antagonistic potential of Trichoderma harzianum and Trichoderma viride against Rhizoctonia solani and Fusarium solani on the PDA medium.

The *T. harzianum* and *T. viride* fungi were tested with *R. solani* (R3) and *F. solani* (F2) isolates in a double culture method, on PDA medium [30].

3. Results and discussion.

3.1. Field survey of root rot disease on broad bean in some fields of Babylon province for the agricultural season 2016-2017.

The results (Table 1) showed the presence and spread of root rot disease in all areas covered by the survey with varying rates of infection ranging from 65-100% and a severity of 22.50-68.75%. The highest severity was found in the samples of the Gilawia, Al-Sada and Haswa respectively with disease incidence100%. The reason for the increase in infection in these areas is due to the fact that they are specialized in the cultivation of the others. This crop is grown annually, leading to the accumulation of the pathogenic fungus inoculum, especially the Sclerotia, which remain in the soil for a long period of up to five years [31, 32]. The results also showed that the lowest disease incidence was in the Al- Badaa district and the lowest severity of infection appeared in the sample areas of the Mashrooa Al-Mussaib, and this is probably the result of the field planted with the crop for the first time.

Table 1. Field survey of root rot disease for some fields in Babylon province for the agricultural season 2016-2017.

No. sample	Field site Babylon	Area Donum	Date\2016	Disease Incidence (%)	Disease Severity (%)
١	Gilawia	٢	١٠-٦	١٠٠	٤٣.٧٥
٢	Al-Sada	٣	١٠-٢٦	١٠٠	٦٢.٥٠
٣	Mashrooa Al-Mussaib	١	١٠-٣١	٧٠	٢٢.٥٠
٤	Al- Badaa	٥	١١-١٧	٦٥	٤٤.٧٦
٥	Haswa	٤	١١-٢٤	١٠٠	٦٨.٧٥
٦	Alexandria	٣	١١-٢٥	٧٦	٥١.٨٧

3.2. Isolation and diagnosis of fungi associated with the roots and crown of the infected plants.

It was found that microscopic identification of the fungal growths of the infected plant parts on the PDA had 6 fungi associated with the roots and crown of the infected plants, including *Rhizoctonia solani* The most common fungus was found in all samples with 92% (Table 2). It was followed by *Fusarium solani* with an appearance rate of 87%. *Trichoderma* isolates were isolated from the sample of the Gilawia area,Al- Mussaib and Alexandria with 53%. The presence of *Trichoderma* may have been due to the fact that this fungus is a soil borne parasite on many soil borne fungi and has recently

increased the production of this fungus as a biological control agent and widely used and increased the turnout of farmers, especially in areas that are famous for growing vegetables, The fungus is also characterized by its ability to parasite on the pathogenic fungi within the plant tissues, making it safe from the surface sterilizer used during isolation [16]. *Aspergillus niger, Macrophomina phaseolina* and *Penecillium* spp. With recurrence rates ranging between 13-64%.

Table 2. The fungi associated of infected broad bean roots by root rot disease in Babylon province.

No.	Fungi	District of appearance*	appearance %
١	*Rhizoctonia solani* Kuhn	٦-١	%٩٢
٢	*Fusarium solani* (Mart.) Sacc.	٦-٥-٤-٢-١	%٨٧
٣	*Aspergillus niger* VanTieghem	٦-٥-٢- ١	%٦٤
٤	*Trichoderma* spp.	٦-٣-١	%٥٣
٥	*Macrophomina phaseolina* (Tassi) Goid.	٥-٤-٣-٢	%٣٤
٦	*Penicillium* spp.	١	%١٣

- The present site of sample collection.

3.3. The pathogenisity of R. solani and F. solani isolates.

The results indicated that all tested isolates of *R. solani* and *F. solani* were pathogenic to broad bean plants showed a significant increase in the disease incidence and severity of root rot disease compared to the treatment of the control, which had disease incidence rate of 0%(Figure 1). It was found that the R3 isolate was one of the most isolates were influential in the broad bean plant has the incidence rate of this isolate to 100% and the severity of the injury 80.0%, followed without significant differences with isolate R6, which caused the disease incidence and severity reached 100 and 74.67% respectively, while The infection rates by other isolates ranged between 80-100% and its severity 36.0-53.33%. The superiority of the R3 isolate on the other isolates in the cause of the disease may be due to the difference in the ability of these isolates on the secretion of enzymes analyst, such as an Cellulase, Pectinase that contribute to the analysis of the components of the host cells walls, in addition to the production of some materials which have a toxic effect on plant cells such as phenyl acetic acid or its hydroxyl derivatives, causing rotting and browning of the tissues [33]. Helmy *et al* [34] found 131 isolates of *Rhizoctonia solani* from the roots of the broad bean and cause root rot. The results of the experiment showed that the isolates of *F. solani* tested showed a high percentage and severity of the broad bean plant. The F2 isolate showed a significant superiority, causing 100% infection and 77.33% severity, and did not differ significantly from isolate F6. While the other four isolates ranged between 66.0-86.7% and the severity of the injury was 30.67 -61.33%. The fungus *F. solani* is an economically important pathogenic fungi cause of its presence in agricultural soils and is more dangerous when cultivating soils with sensitive crops [5]. The infection of plant by this fungi causeing Brown, dark brown or even black, or in the form of dark brown spots, in the root area. On the air parts, yellowish, wilt [35]. The appearance of the symptoms of infection on the plant due to the secretion of the fungus *F. solani*. some toxins such as polypeptide Anhydro, Fusariun, Javanicin, Fusaric acid [36, 37]. The results agree with Habtegebriel and Boydom [9] that showed *F. solani* is one of the most common root rot causes in the Faba bean plant and causes economic losses of over 70% in Ethiopia.

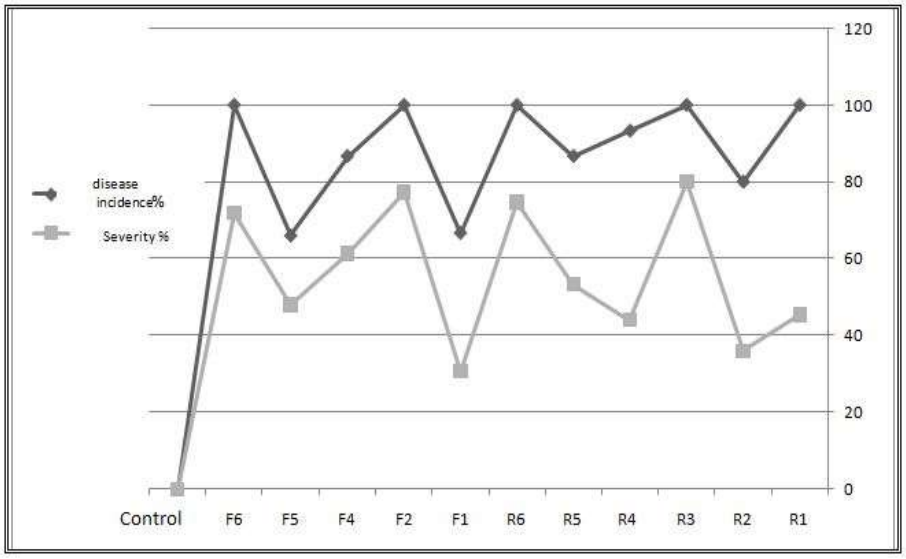

Figure 1. The pathogenisity of some fungal isolates associated with the roots infected by the root rot disease. R = *Rizoctonia solani*, F = *Fusarium solani*, the number beside the symbol represents the isolate number, L.S.D of the disease incidence (*p*: 0.05) = 11.23, L.S.D of the severity = 6.74.

3.4. Efficiency of Trichoderma harizianum and T. viride against the pathogenic fungi on the PDA.

The results (Figure 2) showed that *T. harzianum* (Th) was effective against the of *R. solani* (R3). It reduced the fungus growth rate to 2.67 cm with an inhibition rate of 70.37%. The growth of pathogenic fungi was 68% and 68.52%. The results also showed the effectiveness of biocontrol agents *T. harzianum* and *T. viride* in reducing the growth of pathogen *F. solani* and inhibition ratio were 75.92 and 74.07%, respectively. This result was agreement with Abd al-Kareem [38], Sallam *et al*. [39], Akrami *et al* [40], Siameto *et al* [41] found that *T. harzianum* have a high degree of antagonistic against pathogenic fungi that cause root rot disease on many of host Plant. The activities of *T. harzianum* may be due to several reasons that have made this fungus an anti-biotic agent against many pathogenic fungi of the plant. These are include the direct parasitism on the fungal mycelium by twisting around its hyphae and analyzing its walls by enzymes produced by it, and the production of antibiotics that negatively affect the growth of pathogenic fungi [42, 16]. And the competitive activity on place and food is rapidly growing [15, 22, 43].

3.5. Effect of Biological control Agents and Beltanol in the disease incidence and severity of Broad bean root rot disease under the lath house conditions.

All biological control treatments showed a significant increase in the germination rate of the broad bean seeds and reduced the disease incidence of the root rot and severity with significant difference from the control treatment (Table 3). *T. harzianum* significantly reduced the incidence and severity of R3 to 33.3 and 14.6%, respectively, and significantly reduced the incidence and severity of F2 without significant difference between treatment of different fungus, compared to the treatment of pathogenic fungus R3 and F 2 alone, which had a 100% infection rate and severity of infection was 73.3 and 80.0%, respectively, which did not differ significantly from the treatment of the chemical pesticide Beltanol in the against pathogenic fungi. In addition, *T. viride* significantly increased the percentage of

seed germination to 86.7 and 93.3% with the fungus R3 and F2, respectively, compared to the single pathogenic fungi with a low germination rate of 33.3 and 40.0% respectively. Provide good protection for the plant from the pathogen infection R3 by reducing the incidence rate to 46.7% and its severity to 24.0%.

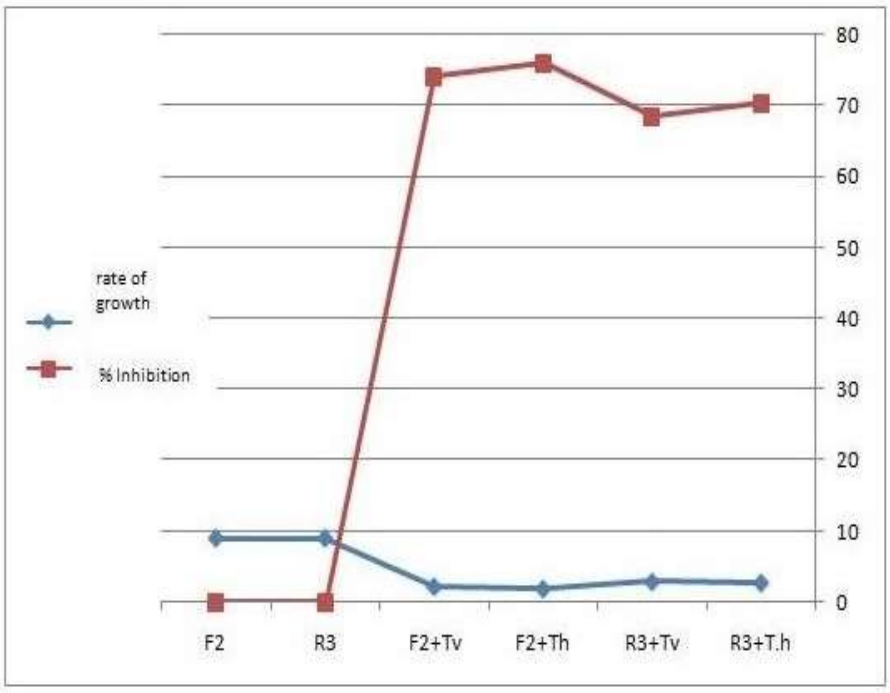

Figure2. Effect of *T. harzianum* (Th) and *T. viride* (Tv) in the growth of pathogenic fungi in the PDA medium. R = *Rizoctonia solani*, F = *Fusarium solani*, L.S.D of the rate of fungal growth (*p*: 0.05) = ٠.٤٢, L.S.D of the Inhibition = ٦.١٦.

The efficacy of the Tv inoculum did not differ significantly from the efficacy of the in control to pathogens. The contribution of biotic factors in reducing the disease incidence and severity of root rot disease was positively reflected on the studied plant growth parameters, which resulted in a significant increase in plant length and wet and dry weight, compared to plant treatment with pathogenic fungi in which the plant parameters were at the lowest levels. R3, 21.7 cm and wet weight 11.06 g, dry weight 1.03 g, which did not differ significantly from the treatment of fungus F2 pathogen separately. The results showed that the addition of fungus Th and Tv alone increased the significant increase in the length of the plant to 39.79 cm and raise The wet and dry weight reached 24.76 and 2.27g respectively, which did not differ significantly (p0.05) from Tv fungi except dry weight (Fig. 4). The results are consistent with Sallam *et al* [39] that *T. harzianum* and *T. viride* showed the highest adverse effects in the direction of *R. solani* and *F. oxysporum* f. sp. *phaseoli*, which caused the Damping off disease and wilt of beans and added to the soil before planting to provide protection for plants, significantly reduced the percentage of infection, which reflected positively on the yield of green bean plant. The effect of *R. solani* on plants may be due to the fact that fungi infect all parts of the plant under the ground such as seeds, roots before and after emergence [44, 45]. While the pathogenic fungus *F.solani* is characterized by its facultative parasitism on plants, causing a variety of pathological conditions, notably rotting and damping off disease on seedling [46]. This result was agreement with Srivastava *et al* [47] The *T. harzianum* significantly increased the wet and dry weight and length of the roots of the chickpea plant and reduced the infection by *M. phaseolina*. The results

are consistent with the findings of Khandelwal *et al* [48] of *T. viride* It is a promising solution to control pathogens that attack the plant which causes annual losses in the global agricultural economic crops and as an alternative to chemical pesticides to reduce their risks and contribute to increasing agricultural production. *T.viride* is one of the most biologically used agents in the control of plant pathogens and an effective substance for more than 250 biocides used in India [49].

Table 3. Effect of biological control factors on the disease incidence and severity of root rot disease and some plant growth parameters under the lath house conditions.

Treatments*	Seed germination (%)	Disease (%)		Plant Wight (g)		Plant high (cm)
		Disease Incidence	Disease Severity	Fresh	Dry	
R3	٣٣.٣	١٠٠.٠	٨٠.٠	١١.٠٦	١.٠٣	٢١.٧٠
F2	٤٠.٠	١٠٠.٠	٧٣.٣	١٢.١٣	١.١٧	٢٢.٨١
R3+Th	١٠٠.٠	٣٣.٣	١٤.٦	٢٠.١١	١.٥٢	٣٤.٦٧
R3+Tv	٨٦.٧	٤٦.٧	٢٤.٠	١٩.١٧	١.٣٢	٣٣.٢٠
F2+Th	٩٣.٣	٣٠.٠	١٦.٠	١٩.٩٤	١.٥٧	٣٢.٨٣
F2+Tv	٩٣.٣	٢٦.٧	١٤.٣	٢٠.٧٨	١.٦٤	٣٤.٣٣
R3+Bel	١٠٠.٠	١٣.٣	٤.٠	٢١.٥٩	١.٦٧	٣٦.٠٠
F2+ Bel	١٠٠.٠	٦.٧	٢.٦	٢١.٧٠	١.٧٢	٢٥.٦٧
T.v	٩٣.٣	٠.٠	٠.٠	٢٤.٣٣	٢.٠٠	٣٨.٦٧
T.h	١٠٠.٠	٠.٠	٠.٠	٢٤.٧٦	٢.٢٧	٣٩.٧٩
Control	١٠٠.٠	٠.٠	٠.٠	٢٢.٩٩	١.٧٧	٣٦.٧٠
P) L.S.D. (<0.05	١٣.١	١٣.٩	٧.٩	١.٢١	٠.١١	١.٣٣

*****Each number represents the rate of 3 replicates and each replicator of three plants, R3 = *Rhizoctonia solani* isolate 3, F2 = *Fusarium solani* isolate 2, *T. harzianum* = Th, Tv = *T. virede*, Bel= Beltanol.

The efficacy of *Trichoderma* may be due to the various mechanisms required to attack pathogens and improve the growth of roots and plants. Including the state of mycoparasitism [15, 17]. The production of many antibiotics [19, 20]. Secretion of the enzymes analyzed for cell walls of their pathogenic fungi [21]. It also competition and speed growth to colonization of space and materials [22]. It was also known to stimulate induced Systemic Resistance (ISR) and the production of pathogenicity-related proteins (P-RP) in plants [18].

4. Conclusion.

The conclusion of this study was The presence of broad bean root rot disease caused by fungus *Rhizoctonia solani* and *Fusarium solani* in the all districts in the province of Babylon, the possession of the biochemists *Trichoderma harzianum* and *T. viride*, which is highly antagonistic against to pathogenic fungi *F. solani* and *R. solani* and protection to the plants from infection by pathogenic fungi and increased the parameters of plant growth under the lath house conditions of.

5. References.

[1] Graham, PH, and C P Vance 2003 Legumes : Importance and constraints to greater use .*plant physiological*. **131**: 872-877.
[2] Prabhu,S D and D V Rajeswari 2018 Nutritional and Biological properties of *Vicia faba* L.: *A perspective review. IFRJ*. **25**(4): 1332-1340.
[3] Central statistical origination 2018 Production of vegetable production for 2017 Ministry of Planning. Republic of Iraq.
[4] El-Mosallamy, H M, M L Elain, S Yonis and M S Shalaby 1990 Histopathological studies on

root rot of broad bean plant induced by *Rhizoctonia solani* Kuhn, *Fusarium oxysporium* Shlech and *Macrophomina phaseolaina* Taub Zagazig Journal of Agriculture rese Garrett, S D 1977 Pathogenic root-infecting Cambridge Univ Press, London 293 pp.

[5] Brasileiro, B T R V , M R M Coimbra , M A M Jr and N T Oliveira 2004 Genetic variability within *Fusarium solani* species as revealed by PCR-Finger-Printing based on PCR markers. *Brazillian J. Microbiol*.**35** : 205-210.

[6] Larousse, M, C Rancurel, C Syska, F Palero1,2, C Etienne, B Industri,X Nesme, M Bardin and E Galiana 2017 Tomato root microbiota and *Phytophthora parasitica*-associated disease. *Microbiome*, **5**(56):3-11.

[7] Ahlem,N A A Rania, J Hayfa, A. Nawaim, S Lamia, H Walid, H Rabiaa and D Mejda 2018 Biostimulation of Tomato Growth and Suppression of Fusarium Crown and Root Rot Disease Using Fungi Naturally Associated to Lycium arabicum. *J.Agric. Sci. Food Res*. **9**(1):1-15.

[8] Van Leur, J A G, R J South well, and J M Mackie 2008 Aphanomyces root rot on faba bean in northern NSW. J. *Australasian Plant Disease Notes*, **3**: 8-9.

[9] Habtegebriel, B and A Boydom 2016 Integrated Management of Faba Bean Black Root Rot (*Fusarium solani*) through Varietal Resistance, Drainage and Adjustment of Planting Time. J Plant Pathol Microbiol 7: 363. doi:10.4172/2157- 7471.1000363.

[10] Compant, S, B Duffy, J Nowak, C Clement and E Actbarka 2005 Use of plant growth promoting bacteria for bio control of plant diseases: principles, mechanism of action and future prospects. *Applied Environ. Microbiol*. **71**(9): 4951 – 4959.

[11] Lorenz, E S 2009 Potential health effect of pesticides. Pesticide Safety Fact sheet, #uo 198. The Pennsylrania state Univ.8pp.

[12] Johansson, P M, L Johansson and B Gerhardson 2003 Suppression of wheat – seedling disease caused by *Fusarium culmorum* and *Microdochium nivale* using bacterial seed treatment. *Plant Pathology*, **52**: 2, 219-227.

[13] Montealegre, J R, R Herrera, J C Velasquez, P Silva, X Besoain, and L M Perez 2005 *Environ. Biotechnol*., *8*(3): 15-24

[14] Agrios, GN 2005 Plant Pathology. 5th Ed. Elsevier Inc. USA.998 pp.

[15] Elad, Y and Hadar 1981 Biological control of *Rhizoctonia solani* by *Trichoderma harzianum* in carnation . *Plant Dis*.**65** :675 – 677.

[16] Harman, G E 1996 *Trichoderma* for biocontrol of plant pathogens : from basic research to commercialized products. Cornell community , conference on biological Control, Cornell Univ. 7pp.

[17] Harman, G E 2006 Overview of Mechanisms and uses of *Trichoderma* spp. *Phytopathology*. **96**:190-194.

[18] Kamal, RK, V Athisayam, Y S Gusain and V Kumar 2018 Trichoderma: a Most Common Biofertilizer with Multiple Roles in Agriculture. *Biomed J Sci & Tech Res*, **4**(5) 1-3.

[19] Howell, C R 2006 Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. *Phytopathology*. **96**: 178-180.

[20] Ramos, A D S, S B Fiaux and S G Fleite 2008 Production of 6-Pentyl-x-Pyrone by *Trichoderma harzianum* in solid-State fermintation. *Brazilian J. of Microbiol*. **39**:712-717.

[21] Limon, M C, J A Pintro-Toro and T Benitez 1999 Increased antifungal activity of *Trichoderma harzianum* transformants that overexpress a 33-KDa chitinase. *Phtopathology*. **89**:254-261.

[22] Nederhoff, E 2001 Biological control of root disease-especially with *Trichoderma*. *Grower*. **56**:24-25.

[23] Hiber, K, M Daami-Remadi, and M EL-Mahjoub 2007 Induction of resistance in tomato plant against *Fusarium oxysporum* f. sp. *radicis-lycopersici* by *Trichoderma* spp. *Tunisian J. of Plant Prot*. **2**:47-58.

[24] Chakraborty B N, U Chakraborty, A Saha and P L Dey and K Sunar 2010 Molecular Characterization of *Trichoderma viride* and *Trichoderma harzianum* Isolated from Soils of

North Bengal Based. *Global J.Biotechnology & Biochemistry*. **5** (1): 55-61
[25] Mckinney, H H 1923. Biological control of nematode pests by natural enemies. *Annual Rev. Phytopathology* **18**:415-440.
[26] Parmeter, J R and H S Whitney 1970 Taxonomy and nomencleature of the imperfect stage In: *Rhizoctonia solani* Biology and pathology. Parmeter, J. R. Univ. of California . 7–19.
[27] Booth, C 1977 Fusarium laboratory guide to the identification of the major species.Commonwealth Mycological Institute Kew, Surrey, England.58 pp.
[28] Sneh, B, S Jabaji- Hare, S Neate and G Dijst 1996 *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, London. 578pp.
[29] Dewan, M. M 1989 Identify and frequency of occurrence of fungi in root of wheat and ryegrass and their effect on take – all and host growth. Ph.D. Thesis. Univ. West Australia. 210pp.
[30] Bell, D K, H D Well and G R Markham 1982 In vitro antagonism of *Trichoderma* species against six fungal plant Pathogens. *Phytopathology* . **72** : 379 – 382 .
[31] Lucas, G B, C L Campbell, and L T Lucas 1985 Introduction to plant disease, identification and management. The AVI Publishing Company, Inc. USA. 313 pp .
[32] Weinhold , R W and B S Sinclair 1996 *Rhizoctonia solani*: Penetration, colonization, and host response. In *Rhizoctonia* species taxonomy, molecular, biology, ecology, pathology, and disease control. (eds) Sneh, B., S. J. Hare , S. Neate , and J. Dijist. Kluwer acad. Publishers, Dordrecht the Nether Land. 163–174 .
[33] Roman-Aviles, B R, S S Snapp, and J D Kelly 2003 Fusarium root rot of common beans. Extension Bulletin E 2876, Michigan St. Univ. USA. 2pp.
[34] Helmy M M, E Gado, S, El-Deeb and H M Mostafa 2015 Phenotypic Diversity and Molecular Identification of the Most Prevalent Anastomosis Group of Rhizoctonia solani Isolated from Diseased Faba Bean Plants. *American J. Life Sci*. **3**(1):47-55.
[35] Vidhyasekaran, P1997 Fungal pathogenesis in plants and crops. Molecular Biology and host defence mechanism Marcel Dekkr ,INC pp 552.
[36] Nelson, B D, J M Hansen, C E Windels and T C Helms 1997 Reaction of Soybean cultivars to isolates of *Fusarium solani* from the Red River valley. *Plant Dis*. **81** : 664 – 668.
[37] Li, S X , G L Hartman and J M Witholm1999 Reproduction of crown rot of wheat caused by *Fusarium graminerarum* in the greenhouse. *Plant Dis*. **70** : 632 – 635.
[38] Abd-EL-Kareem, F 2007 Induced resistance in bean plants againts root rot and Alternaria leaf spot diseases using biotic and abiotic inducers under field conditions. *Res. J. of Agric. and Biol. Sci.* **3**(6): 767-774.
[39] Sallam, N M A, K A M Abo- Elyousr and M A E Hassan 2008 Evaluation of *Trichoderma* species as biocontrol agents for damping-off and wilt disease of *Phoaseolus vulgaris* L. and efficacy of suggested formula. Egypt. J. *Phtopathology*. **36**: 81-93.
[40] Akrami, M, A S Ibrahimov, D M Zafari and E Valizadeh 2009 Control fusarium rot of bean by combination of *Trichoderma harzianum* and *Trichoderma asperellum* in greenhouse condition. *Agric. J*. **4**:121-123.
[41] Siameto, E N, S Okoth, N O Amugune, and N C Chege 2011 Molecular characterization and identification of biocontrol isolates of *Trichoderma harzianum* from Embu District, Kenya. *Tropical and Subtropical Agroecosystems*. **13**:81-90.
[42] Chet, I, and R Baker 1981 Isolation and biocontrol of *Trichoderma hamatum* from soil naturally suppressive of *Rhizoctonia solani*. *Phytopathology*. **71**: 286–290.
[43] Adekunle, A T, T Ikotun, D A Florini and K F Cardwell 2006 Field evaluation of selected formulations of *Trichoderma* species as seed treatment to control damping-off of cowpea caused by *Macrophominaphaseolina*. *African J. of Biotechnol*. **5**:419-424.
[44] Ogoshi, A 1987 Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Ann. Rev. *Phytopathology*. **25**:125-143.
[45] Anne, E D, E L Patrik and R M Dennis 2002 *Rhizoctonia* damping – off and stem rot of

soybean. Ohio State Univ. Extension fact sheet plant Pathology. 2pp. Booth, C 1971 The genusfusarium. Commonwealth Mycological Institute, Kew, Surrey, England. 237 pp.

[46] Al-Hamdany, M A and M M Salih 1986 Wilt causing Fungi on broad bean. Indian *Phytopathology* .**39** : 620-622.

[47] Srivastava, J A R P Singh, A K Srivastava, A K Saxena and D K Arora 2008 Growth promotion and charcoal rot management in chickpea by *Trichoderma harzianum*. *J. Plant Protection Res*. **48**: 81-92.

[48] Khandelwal, S, J Mehta, R K Makhijani, G Sharma, R Kumar and S Chandra 2012 Isolation, characterization & biomass production of *Trichoderma viride* using various agro products- A biocontrol agent.Pelagia Res. *Library Advances in Applied Sci. Res*., **3** (6):3950-3955

[49] Mukherjee, P K, Arup K Mukherjee and Sandhya Kranthi2013 Reclassification of *Trichoderma viride* (TNAU), the Most Widely Used Commercial Biofungicide in India, as*Trichoderma asperelloides* .*The Open Biotechnol. J.,* **7**, 7-9.