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ORIGINAL PAPER

Collaborative Potentialities of *Trichoderma* spp. and *Saccharomyces cerevisiae* Against Damping-off and Root Rot Diseases of Faba Bean

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ABSTRACT

Faba bean (*Vicia faba* L.) is growing mainly for green pods and dried seeds. It is an essential source for proteins, carbohydrates, iron, zinc, calcium, vitamin B group, health gaining antioxidants, plant sterols and dietary fibers. Faba bean also supplies an important grant to crop, soil texture and fertility by fixing atmospheric nitrogen. In Egypt, root rot diseases caused by many complex soil-borne fungi, which destroy the productivity of faba bean. Wherein, our study has been attained the novel procedure for management of root rot diseases by mutual potentiality of *Trichoderma* spp. and *Saccharomyces cerevisiae* culture filtrates. *Rhizoctonia solani* isolate No. 4 and *Fusarium solani* isolate No. 7 were chosen as the most aggressive pathogens in the pathogenicity test causing the highest incidence of damping-off and root rot diseases of faba bean. Anatomically, the cross sections in the primary root examined by light microscope showed a comprehensive destruction of several parts of epidermal root cells, the degradation of cortical cells followed by plasmolysis of cell components during infection with *R. solani*, as well. On other words, under scanning microscope, *F. solani* has the more virulence, with a broad disruption in some xylem vessels, closes many xylem vessels by fungal hyphae, whereas the dimensions of some xylem vessels were larger than like it in healthy root. *Trichoderma* spp. showed particular variety of antagonistic activity towards pathogenic fungi, *T. harzianum* isolate No. 2, *T. viride* No. 4 and *T. album* No. 1were the most powerful towards pathogens. Further, the dual combination of *T. viride* and Yeast filtrates suppressed entirely the growth of *R. solani*. On the other side, *T. album* + Yeast culture filtrate suppressed the growth of *F. solani*. Results of Pot experiment indicated that the highest protection of faba bean against damping-off and root rot diseases was occurred when treated with Topsin M 70 % wp and a dual combination of *T. viride* + Yeast culture filtrates ranked the second treatment. The application of both *Trichoderma* spp. and Yeast culture filtrates each alone or in combination picture, stimulated the activity of plant defense enzymes, enhanced nodules status and nitrogenase activity as well as photosynthetic pigments. *T. viride* + Yeast culture free cells gave the highest values of peroxidase, polyphenoloxidase, nodules status, nitrogenase and photosynthetic pigments. Moreover, *T. album* + Yeast showed the highest chitinase activity. Hopefully, the mixture of culture filtrates of *T. viride* + Yeast is recommended for alleviation the injurious effects of root rot fungal pathogens in faba bean as substitutive for fungicides.

Keywords: Faba bean, *Vicia faba*, Damping-off, Root rot, *Trichoderma* spp., Yeast, culture filtrate

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INTRODUCTION

Faba bean *(Vicia faba* L.) is the major fabaceous crop in Egypt, with several names being faba bean, broad bean, fava bean, horse bean and field bean. It is one of the original house-trained food legumes in the world (Metayer, 2004). Faba bean is growing mainly for green pods and dried seeds, with nutritional value of protein (about 28%), carbohydrates (about 58%), vitamins and other compounds, so it is an important crop for human nutrition and animal feeding (Crepon *et al.*, 2010). Moreover, Rubiales (2010) pointed out that faba bean is an excellent source of iron, zinc, calcium, vitamin B group, health benefiting antioxidants, plant sterols and dietary fibers. Also, faba bean supplies an important benefit to crop, soil texture and fertility by fixing atmospheric nitrogen with *Rhizobium leguminosarum* (Bendehmane *et al.*, 2012 and Mohsen *et al.*, 2013), where the efficiency of Faba bean to establish 20 – 60 kg N ha^{-1} under tropical environmental conditions (Tolera *et al.*, 2015). For these reasons, faba bean is a remarkable plant and the crop protection strategy is one of the major scopes of the agriculture policy in many countries, especially Egypt.

However, Faba bean crop is liable to attack by numerous fungal diseases, which interfere during germination, growth, and development stages. Damping-off and root rot diseases are serious diseases associated with fungal infections, attacking faba bean seeds, seedlings, roots and other features of growth (El-Sayed, 2017 and Hassan *et al.*, 2017). Damping-off is caused by a number of fungal pathogens including *Rhizoctonia solani* and *Fusarium* spp., which lead to poor emergence that is often the first sign

of damping-off, with clinical symptoms that occurre before plant emergence (pre- emergence damping-off) or in the young seedlings (post-emergence damping-off). Further, the symptoms could be included poor stand, yellow seedlings with no secondary roots or a brown/black tap root and plant death (Abdel-Monaim, 2013 and Abd El-Hai and El-Saidy, 2016). The symptoms of root rot include stunted, yellow plants and the roots are discolored and become much thinner than a healthy plant or there may be no secondary roots at all. Rhizoctonia root rot is first diagnosed by poor or declining stands, root development is poor, and roots are generally black and soft. Moreover, Fusarium root rot symptoms include brown to radish-brown discoloration and lack of secondary roots (Abdel-Razik *et al.,* 2012). On other words, root rot infection occurs the injurious anatomical changes such as complete destruction of epidermis and separation of some area in cortex tissue followed by degradation and dissolution of cell components (Abd El-Hai and Ali, 2018).

Management strategy of soil-borne diseases is a difficult mission because the most pathogens live near the roots and the rhizosphere and their persistency in the soil, as well as each pathogen has a wide host range (Rauf, 2000). Wherein, employing fungicides has been intricated by development of fungicidal resistance and adverse effects on germination, growth, and productivity of faba bean and also on the accompanied microflora. Moreover, the long-term fungicides application is not economic, and their residues have a harmful impact on environment and human health (Vinale *et al.,* 2008). Whereby, there is an urgent demand to find out substitutive efficient strategies towards soil- borne fungal diseases.

Among the management procedures of plant diseases, the most have been applied as a single bio-control agent which may not be functioning in all soil's environment. Consequently, the application of a mixture bio-control agents is more closely emulate the natural situation, broaden the spectrum of the bio-control activity and enhance the efficacy and reliability of the control (Gnanamanickam, 2002 and Abd El-Hai and Ali, 2019). So, in developing countries, the formulation of bio-control agents' vestiges a vigorous disease management, where the costs of chemical treatments could be unaffordable.

Several *Trichoderma* spp. could be mycoparasitic pathogenic fungi, due to restore the beneficial balance of natural ecosystem, which is often lost in the crop situation (Harman *et al.,* 2004). Seed dealing with *Trichoderma harzianum* compacted significantly the symptoms of damping-off that instigated by *Fusarium* spp. and *R. solani* in faba bean, lentil and chickpea (Abou-Zeid *et al.,* 2003 and Abdel-Monaim, 2013). Moreover, seed soaking followed by foliar spraying of peanut plants with individual and/or combination of *T. harzianum*, *Saccharomyces cerevisiae* or *Rhizobium japonicum* culture filtrates minimize the incidence of damping-off and root rot diseases caused by *F. solani*, *R. solani* and *S. rolfsii* (Hassan *et al*., 2017 and Abd El-Hai and Ali, 2019). Likewise, bread yeast was operative for monitoring seed borne fungi of faba bean (Elwakil *et al.,* 2009).

A novel strategical procedure was conducted for management of root rot fungi, as well as increment nodulation and physiological activities of faba bean by collaborative effect between *Trichoderma* spp. and *Saccharomyces cerevisiae* culture filtrates.

MATERIALS AND METHODS

Source of faba bean seeds:

Faba bean (*Vicia faba* L.) seeds cv. Giza 843 were purchased from Legume Crop Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Isolation, purification and identification of Fungal pathogens:

Faba bean plant samples infected with root rot were gathered from diverse localities of Dakahliya governorate, Egypt. The infected roots were cut into minor pieces and surface sterilized with 2% sodium hypochlorite for two min. The sterilized pieces then were rewashed with sterilized water and dried, after that they were placed on potato dextrose agar (PDA) medium supplemented with streptomycin-sulfate (100 µg ml^{-1}) and incubated at 25^{o}c. The isolated fungi were purified, and identified based on their cultural, morphological, and microscopic characters according to Booth (1977); Sneh *et al*. (1991) and Barnett and Hunter (1998). The isolated fungi were sub-cultured in PDA slants and kept at 6°C in a refrigerator.

Preparation of fungal inoculum:

Fungal isolates of both *R. solani* and *F. solani* were prepared using medium consisted of (sorghum: coarse sand: water at rate of 2: 1: 2 (v/v)). The well mixed media were bottled in 500mL bottles, autoclaved under 1.5 air pressure for two hours. Sterilized media were inoculated with agar discs of 6-days old colony from the periphery of each isolated fungus. The inoculated bottles were incubated at 25°C for 15 days then

used for soil infestation in pots containing sterilized soil at the rate of 3%.

Pathogenicity test:

This experiment was carried out in the greenhouse at Plant Pathol. Res. Inst., Agric. Res. Cen., Giza, Egypt. The tested fungi were used to infest sterilized pots (containing clay soil) at the rate of 3% w/w soil. The infested pots were left for 7 days before sowing to enhance the growth and distribution of fungal inoculum. Faba bean seeds cv. Giza 843 were surface sterilized and sown at the rate of 10 seeds/pot. Five pots were used as replicates for each isolate as well as a check (un-infested soil) using three replicates. To determine the virulence of fungal isolates; % pre- and post-emergence damping-off were recorded at 15 and 30 days after sowing, respectively. Each of the emerged fungi was represented by 8 isolates of *R. solani* and 10 isolates of *F. solani*. As the isolates were tested for their pathogenic capabilities. According to their virulence, the most aggressive *R. solani* No. 4 and *F. solani* isolate No 7 were selected for further studies.

Anatomical studies:

a- Light microscope:

Tissue blocks from healthy and artificially infected primary roots were washed in distilled water and dried between folds of sterilized paper towels and processed. The tissues were cut into small portions (5 mm in length), dipped in FAA (Formalin: ethyl alcohol: glacial acetic acid "1:18:1"). The samples were left in this solution till processing further. Dehydration of specimens was processed by using series of ethyl alcohol *i.e*., alcohol 30, 50, 70, 80 and 95%, then absolute alcohol. The specimens were transferred in a series of ethyl alcohol and xylene as following: absolute alcohol 75% + xylene 25%, alcohol 50% + xylene 50%, absolute alcohol 25%+xylene 75% and pure xylene consequently each for 30 minutes. Then waxy blocks were prepared by embedding in specimens melted paraffin wax at 54 to 56°C for 4-8 h in order to completely replace the xylene with paraffin wax (Bennet *et al*., 1976**).** A thin section of 12-15µ thickness were cut with the help of a microtome (Microm, Germany). Stained in crystal violet and erythrosine (double staining) then cleared in xylol and mounted in Canada balsam (Gerlach, 1977). The cross sections were examined using light microscope (100×) for knowledge, description and determining the anatomical changes occurred due to infection by the pathogenic fungi.

b- Scanning electron microscope:

The anatomical changes in faba bean root responded to the tested pathogens were checked up using Paraffin Scanning Electron Microscope (PSEM) according to Denise and Anjali (2019).

Trichoderma species isolation:

Twenty isolates belonging to the genus *Trichoderma* were cultured on selective medium according to Elad *et al.* (1981). Trichoderma isolates were preserved into PDA slants and identified on the basis of their cultural and microscopic morphological characters according to Rifai (1969) and Bissett (1991). Trichoderma isolates were identified as *Trichoderma harzianum* (9 isolates), *T. viride* (8isolates) and *T. album* (3 isolates).

Antagonistic activity of *Trichoderma* spp. against the most aggressive pathogenic isolates:

The direct antagonism of *Trichoderma* spp. against *R. solani* isolate No. 4 and *F. solani* isolate No. 7 was carried out using the dual test technique (Chet, 1987). Each of the pathogenic fungi and the antagonist Trichoderma were cultured on PDA medium for 7 days at 25°C. For growth rate, PDA plates were inoculated in the center with a 5 mm disc of individual Trichoderma. The colony diameter of both pathogenic fungi and Trichoderma was measured after 5 days. For dual cultural test, sterilized plates of PDA (90 mm in diameter) were inoculated with a 5 mm disc of each pathogenic fungus alone at 10 mm from the edge of petri dish. Another 5mm of the tested Trichoderma was placed after 24h from the pathogen inoculated on the opposite side at 70 mm from the pathogen disc. Plates inoculated with each of pathogen only served as control. The individual and dual culture growth rates were recorded after 5 days from incubation at 25°C. The interaction between dual culture mycelia was scored for degree of antagonism using the scale of 1 to 5 according to Bell *et al.* (1982). Where, 1= *Trichoderma* overgrowing pathogen (where 2,3 and 4) and 5= Pathogen overgrowing Trichoderma. The most potent antagonist to the pathogen from each of *Trichoderma* species was selected for further studies. In this investigation *T. harzianum* isolate No. 2, *T. viride* isolate No. 4 and *T. album* isolate No. 1) were selected.

Preparation of Trichoderma filtrates:

Three species of Trichoderma were selected, on the base of antagonism test, and were grown in PD liquid medium at 25°C on the shaker at 140 rpm in the dark for 8 days. The culture of each *Trichoderma* species was filtered through filter paper followed by centrifugation and sterilized using membrane filter (0.22 Mm in pore size). The culture filtrate was kept in the refrigerator in a dark bottle under cooling until use.

Preparation of Yeast filtrate:

Yeast strain of *Saccharomyces cerevisiae* was kindly obtained from Microbial Activity Unit, Microbiology Department, Soils, Water and Environment Research Institute, ARC, Giza, Egypt. It was grown on mannitol broth medium (100mL) in Erlenmeyer Flask. The inoculated flask was incubated for 36h on the shaker at 24°C then frozen for 24 hr. to allow partial breaking of the cell wall and obtained on materials of yeast metabolism. The resulted culture was filtered through filter paper then centrifuged and sterilized by filter membrane (0.22 Mm in pore size). The filtrate was kept in refrigerator at 5°C in dark bottles till use.

Bio-filtrates vis pathogens fungal growth:

Antagonism test was carried out between *Trichoderma* spp. and Yeast filtrates to ensure that there is no any antagonism between the tested metabolites, before the beginning this experiment. One ml of the aforementioned preparations of the Trichoderma, Yeast culture filtrates individually and their combinations (0.5 and 0.5ml from each) as well as 0.03g for Topsin M70% wp fungicide (fungal disinfectant) was added to 10 ml of PDA medium representing each treatment then poured in petri dishes (9cm in diameter) and shacked gently to mix well then left to solidify. The plates were inoculated with mycelia disc of the tested pathogenic fungus in the center and incubated at 25°C. Three replicates were also used for each treatment and three replicates were prepared to serve as control for each fungus (medium free culture filtrates). Fungal growth diameter was measured when particular control filled of petri dishes with fungal mycelia by reaching the plate's edge. The inhibition degree of pathogen growth under each used treatments compared to control was calculated using the following formula:

$$\textbf{Inhibition \%} = (C-T)/C \times 100$$

Where:

C= Growth of the pathogen (check)

T= Growth of the pathogen with each treatment.

Pot experiment:

This experiment was carried out in the greenhouse at Plant Pathol. Res. Inst., Agric. Res. Cen., Giza, Egypt. Before the beginning of this experiment, an antagonistic test was undertaken between both pathogens to ensure that there is no any antagonism between them. An equal amount of each pathogenic fungal inoculum was mixed with soil surface in plastic pots at the rate of 3% (w/w) under greenhouse conditions. The infested pots were irrigated and kept for 7 days before sowing to enhance growth and distribution of fungal inoculum. Faba bean seeds were soaked in culture filtrates of the tested bio agent before sowing for 3 hours as well as the fungicide Topsin M 70% wp as seed coating at 3g kg^{-1} seeds and sowing was in November 2021. The same treatments were used as activation dose by foliar spraying at 38 days from sowing. This experiment lasted for 65 days. Five pots were used as a replicate "10 seeds/pot", five replicates were used to determine the percentage of damping-off and the other five replicates were used to determine the percentage of infection by root rot as well as physiological aspects.

Disease assessment:

Pre- and post-emergence percentages of damping-off were recorded at 15 and 30 days from sowing, respectively. While the percentages of root rot, and survival of plants were calculated at 65 days from sowing according to the following formulas:

$$\textbf{Pre-emergence damping-off \%} = \frac{\text{No. of non-germinated seeds after 15 d.}}{\text{Total no. of planted seeds}} \times 100$$

$$\textbf{Post-emergence damping-off \%} = \frac{\text{No. of dead seedlings after 30 d.}}{\text{Total no. of planted seeds}} \times 100$$

$$\textbf{Root rot \%} = \frac{\text{No. of plants infected with root rot after 65 d.}}{\text{Total no. of planted seeds}} \times 100$$

Estimation of some plant defense enzymes activity:

The activity of definite enzymes *i.e.*, peroxidase, polyphenoloxidase and chitinase, which are related to plant defense against pathogen infection, were determined at 40 days from sowing (48h after foliar application). Peroxidase, polyphenoloxidase and chitinase activities were estimated according to Monreal and Reese (1969); Esterbaner *et al.* (1977) and Kato and Shimizu (1997), respectively. Peroxidase activity was calculated as mg equivalent of pyrogallol, the polyphenoloxidase activity was calculated as mg equivalent of catechol and chitinase activity was calculated as mg equivalent of glucose /g fresh weight.

Determination of nodulation status and nitrogenases activity:

The roots of faba bean were gathered at 65 days from sowing for determination of nodules number and nodules dry weight, as well as nitrogenase enzyme activity was estimated according to Lethbridage *et al.* (1982).

Determination of photosynthetic pigments:

The chlorophyll a, b and total chlorophyll and carotenoids contents were extracted with methanol 90% after adding traces of calcium

carbonate according to Robinson and Britz (2000) and determined according to the formulas of Mackinney (1941).

Statistical procedure:

The collected data were subjected to statistical analysis using analysis of variance and LSD at 5% level with MSTAT computer program according to Gomez and Gomez (1984).

RESULTS

Pathogenicity test of the isolated fungi:

Fungi belonging to two genera were isolated and identified as *R. solani* (8 isolates) and *F. solani* (10 isolates). All the isolates were tested for their pathogenic capabilities on faba bean cv Giza 843. Data in Table (1) show that all isolates of both fungi were pathogenic and caused pre- and post-emergence damping-off as well as typical root rot symptoms of faba bean seedlings. *R. solani* isolate number 4 was the most aggressive followed by *F. solani* isolate No. 7 then *F. solani* isolate No. 2 and *R. solani* isolate No. 5 based on the percentage of plant survival (58.66, 60.00. 62.60 and 62.66%) healthy plants, respectively. Among the isolates of *R. solani*, isolates Nos. 4, 5, 1, 2 and 6 were the most aggressive. The corresponding values of the diseased seed lines were 41.33, 37.32, 36.66, 35.32 and 34.66%, respectively. Meanwhile, the most aggressive isolates of *F. solani* were Nos. 7, 2, 1, 8 and 6. The corresponding values recorded 39.99, 37.99, 33.99, 31.33 and 30.66%, respectively. Generally, *R. solani* isolates showed the highest values of damping-off, being 34-16 on average. On the other hand, *F. solani* isolates caused a 30.59% damping-off on average.

Table (1): Pathogenicity test of the obtained isolates showing their capabilities to cause damping-off on faba bean cv Giza 843 plant under greenhouse conditions.

Isolate	Damping-off		Total	Plant survival %
	Pre-emergence %	Post-emergence %		
R. solani-No. 1	27.33 ab	9.33 defg	36.66	63.33 hij
R. solani-No. 2	24.66 cd	10.66 bcde	35.32	64.66 hij
R. solani- No. 3	24.00 de	7.33 g	31.33	68.66 fg
R. solani- No. 4	29.33 a	12.00 abc	41.33	58.66 i
R. solani- No. 5	26.66 bc	10.66 bcde	37.32	62.66 ijk
R. solani- No. 6	23.33 de	11.33abcd	34.66	65.33 hi
R. solani- No. 7	19.33 gh	7.33 g	26.66	73.33 bcd
R. solani- No. 8	22.00 ef	8.00 fg	30.00	70.00 ef
Means	24.58	9.58	34.16	65.84
F. solani- No. 1	23.33 de	10.66 cdef	33.99	66.00 gh
F. solani- No. 2	25.33 bcd	12.66 ab	37.99	62.00 jk
F. solani- No. 3	18.00 hij	11.33 abcd	29.33	70.66 def
F. solani- No. 4	18.66 ghi	9.33d efg	27.99	72.00 cde
F. solani- No. 5	18.00 hij	7.33 g	25.33	74.66 bc
F. solani- No. 6	20.66 bc	10.00 cdef	30.66	69.33 ef
F. solani- No. 7	26.66 bc	13.33 a	39.99	60.00 ki
F. solani- No. 8	19.33 gh	12.00 abc	31.33	68.66 fg
F. solani- No. 9	16.00 j	8.66 efg	24.66	75.33 b
F. solani- No. 10	16.66 ij	8.00 fg	24.66	75.33 b
Means	20.26	10.33	30.59	69.41
Check	1.33 k	0.66 h	1.99	98.01a

Different letters within a column indicate significant difference at $p \leq 0.01$ (Tuckey test)

Anatomical studies of root structure:

The anatomical changes in faba bean root structure due to both infection by the pathogenic fungi as compared to healthy root were carefully investigated. Cross-sections of healthy and infected plants were examined using a light

microscope (Fig. 1) and a scanning microscope (Fig. 2). The root structure of a healthy normal faba bean plant showed a well-formed structure of the epidermis (E) consisting of closely packed elongated cells. with thin walls and typically uniseriate, the cortex may be homogeneous and simple in structure; the cortical cells may be arranged in orderly radial rows and vascular bundles which are a central part of the root that is composed of the vascular system (xylem(x) and phloem(p) surrounded by pericycle and the associated parenchyma cells. The xylem forms discrete stands alternating with the phloem strands. Xylem is the principal water-conducting tissue and phloem is the food conducting tissue. The xylem is a complex tissue consisting of several different types of cells, living and nonliving. The most characteristic components are the tracery elements, which conduct water and plant support. Two fundamental types of tracheary elements occur in the xylem, tracheid and vessel members. The vessel members are joined into long continuous tubes which called tracheae.

As illustrated in Fig (1) there are clear differences in cross-sections of faba bean root structure of both healthy and infected plants with each of *R. solani* or *F. solani*. The infected roots with pathogenic fungi showed deformation in the anatomical structure that occurred mainly in epidermis, cortex, and littleness in vascular bundles compared with healthy plants. Infection with *R. solani* led to complete destruction in many parts of the epidermis that exhibited a dark brown epidermal surface. The cross-section with infected roots also shows a degradation of the cortex cell wall followed by hydrolysis of cell components. Generally, *R. solani* was the most aggressive because it caused injurious effects on anatomical root structure more than *F. solani* as observed in cross-sections under the light microscope, *i.e*., control -a, infected by *R. solani* – b, infected by *F. solani* -c

Concerning examination of the cross sections under scanning electron microscope, some differences are observed such as, hydrolysis, separation and eventually complete disruption in some xylem vessels and also presence of black areas in cross sections of infected plants due to degradation and dissolution of some cell components leading to cell death. *F. solani* was more effective and there are some fungal growth hyphae in the intracellular spaces as well as in the infected cells.

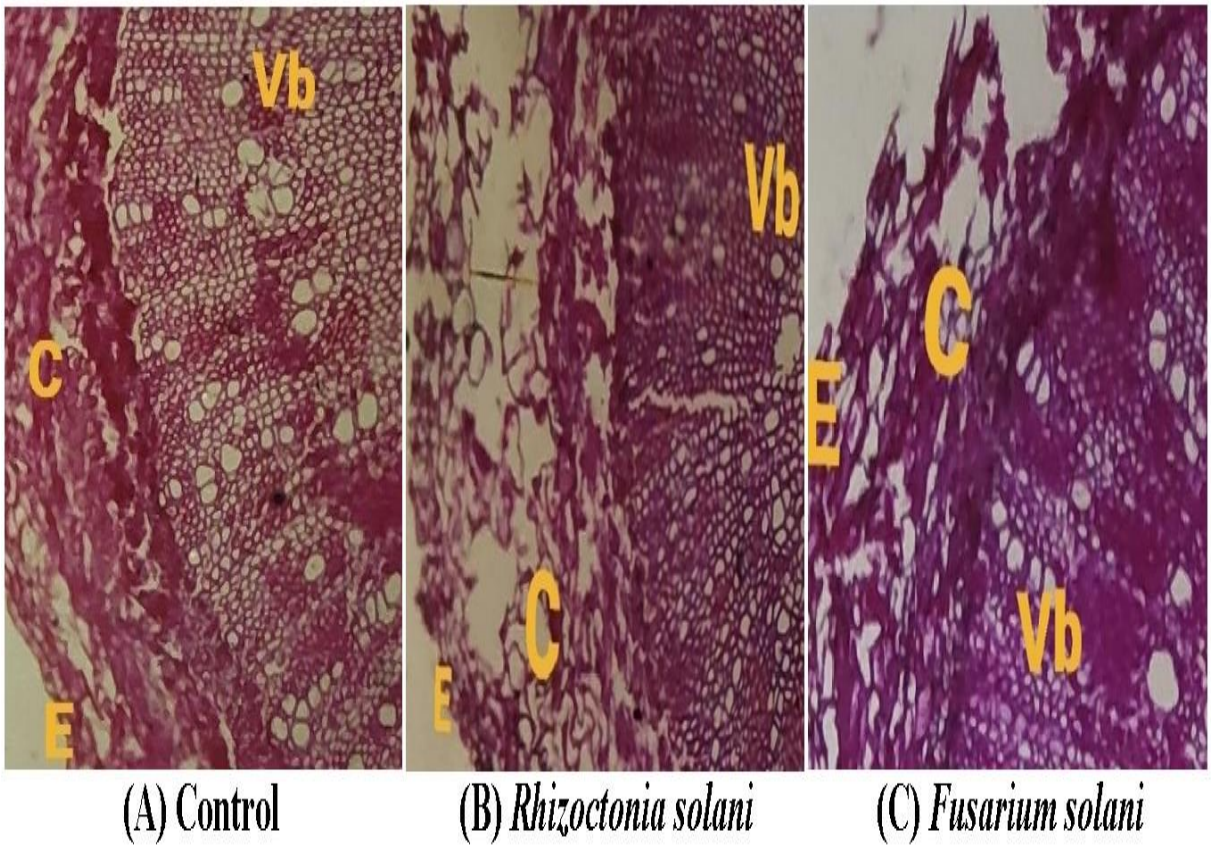

Fig. (1): Cross sections of faba bean primary roots show the anatomical structure changes with root rot fungi (×100), E =Epidermis; C = Cortex and Vb =vascular bundles.

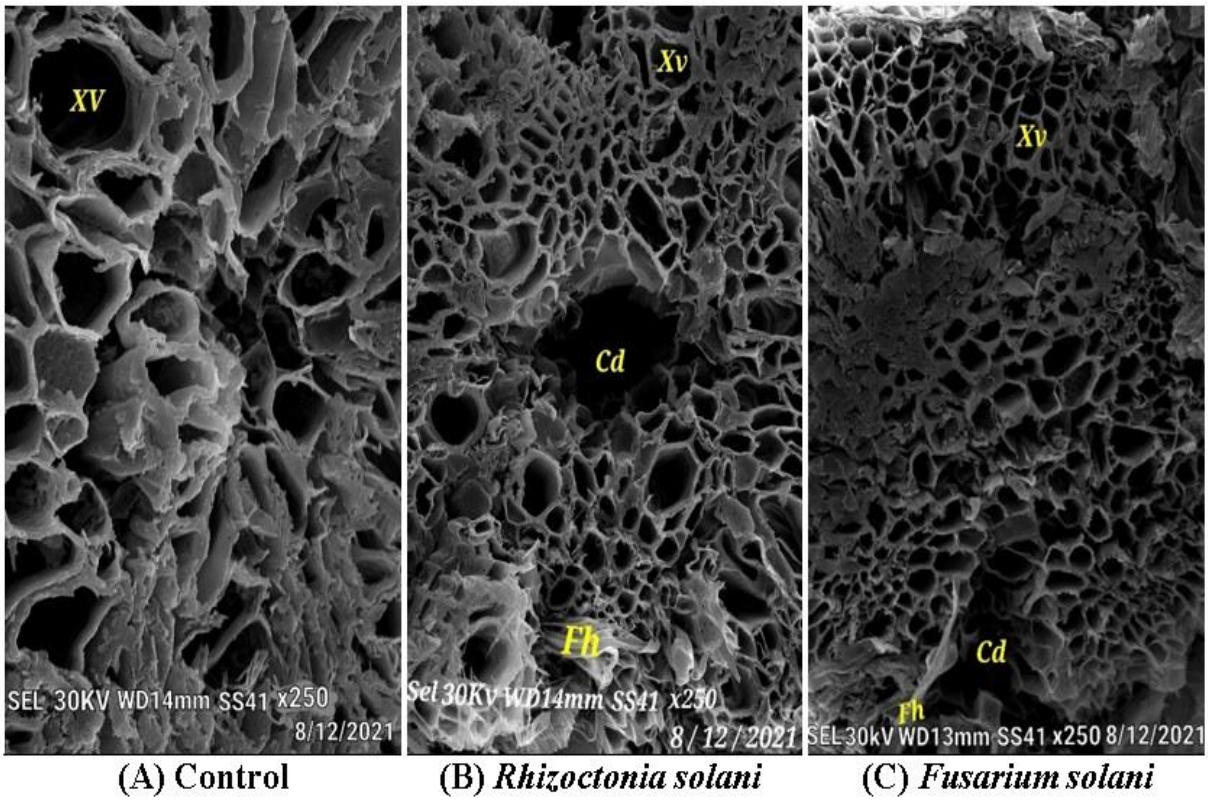

Fig. (2): Scanning electron microscope (SEM) micrographs of faba bean root cross sections under infested soil showing the anatomical structure changes due to infection by root rot fungi. Xv = Xylem vessels; Cd = cells death; Fh = Fungal hyphae.

Direct antagonism of isolated *Trichoderma* spp. against the most aggressive isolates of the pathogens:

Twenty isolates of *Trichoderma* spp. isolated from the phyllosphere of faba bean representing three species (*T. harzianum*, *T. viride* and *T. album*) were screened for their antagonistic activity in direct confrontation against the most aggressive isolates of the pathogens. Data in Table (2) show that all Trichoderma isolates showed different levels of antagonistic activity in dual culture test against both pathogenic fungi. *T. viride* isolate No. 4 was the most potent antagonist to *R. solani* followed by *T. harzianum* No. 2 then *T. album* No. 1 which reduced the fungal growth over 75%. On the other side, *T. album* No. 1 showed the highest degree of antagonism to *F. solani* followed by *T. viride* No. 4 then *T. harzianum* No. 2. The lowest growth percentage of *R. solani*, being (18%) was occurred under *T. viride* No. 4, while the lowest growth % of *F. solani*, being (17.5%) was recorded by *T. album* No. 1, at the same time, the best antagonism reaction (1) was recorded by the same applications which means the occurrence of strong mycoparasitism. Accordingly, *T. harzianum* isolate No. 2, *T. viride* No. 4 and *T. album* No. 1 were used in further studies.

Trichoderma, Yeast and fungicide vis linear growth:

The antifungal (inhibitory) effects of individual culture filtrate of three tested *Trichoderma* spp. and Yeast and their combination as well as the fungicide Topsin M-70 against pathogens linear growth are presented in Table (3). Before this experiment, the antagonism test was carried out among the filtrates of *Trichoderma* spp. and Yeast to ensure the presence or absence of any chemical antagonism among them. It was observed that all treatments used showed antifungal activity and have high potentiality in controlling both pathogens with different degrees. Topsin M-70 wp fungicide at 0.03g completely inhibited the growth of both tested pathogenic fungi. Moreover, the dual combination of *T. viride* + Yeast prevented the growth of *R. solani*. On the other side, a dual combination between *T. album* and yeast completely inhibited the linear growth of *F. solani*. It is worthy to mention that; Yeast treatment gave the highest reduction of linear growth of both fungi as compared to the individual treatments. At the same time, *T. viride* came next for *R. solani* while *T. album* came next for *F. solani*.

Table (2): Effect of Trichoderma isolates on the most aggressive isolates of both pathogenic fungi (direct confrontation).

isolates	*R. solani* No. 4 "cm"	*Pathogen growth%	** Antagonism reaction	*F. solani* No. 7 "cm"	*Pathogen growth, %	** Antagonism reaction
check	8.50a	-	-	7.60 a	-	-
T. harzianum- No. 1	3.97 c	46.71	3	3.37 cd	44.34	3
T. harzianum- No. 2	1.80 i	21.18	2	2.13 hi	28.03	2
T. harzianum- No. 3	2.17 h	25.53	2	2.37 gh	31.18	2
T. harzianum- No. 4	4.4 b	51.76	3	3.50 bcd	46.05	3
T. harzianum- No. 5	3.13 e	36.82	2	3.37 cd	44.34	3
T. harzianum- No. 6	2.50 f	29.41	2	2.20 ghi	28.95	2
T. harzianum- No. 7	2.63 f	30.94	2	2.30 gh	30.26	2
T. harzianum- No. 8	3.30 e	38.82	2	2.90 e	38.18	2
T. harzianum- No. 9	3.83 c	45.06	3	3.57 fg	46.97	3
T. viride- No. 1	3.00 e	35.29	2	2.70 ef	35.53	3
T. viride- No. 2	3.27 e	38.47	2	3.93 e	51.71	3
T. viride- No. 3	2.57 f	30.24	2	2.47 fgh	32.50	2
T. viride- No. 4	1.53 j	18.00	1	1.97 i	25.92	2
T. viride- No. 5	2.27 gh	26.71	2	2.40 fgh	31.56	2
T. viride- No. 6	3.53 d	41.53	3	3.20 d	42.11	3
T. viride- No. 7	2.00 hi	23.53	2	2.30 gh	30.26	2
T. viride- No. 8	2.56 fg	30.21	2	2.53 fg	33.29	2
T. album- No. 1	2.03 hi	23.88	2	1.33 j	17.50	1
T. album- No. 2	3.87 c	45.53	3	3.73 b	49.08	2
T. album- No. 3	387 c	45.53	3	3.90 b	51.32	3

Different letters within a column indicate significant difference at $p \leq 0.01$ (Tuckey test)
*Pathogen growth %= Radius growth of the pathogen in the direction of Trichoderma radius growth in the absence of Trichoderma × 100; **Numbers refer to antagonism reactions of Trichoderma with the pathogen based on the antagonism scale of Bell *et al.* (1982) after 6 days of dual growth.

Table (3): Effect of bio filtrates and their combinations on fungal linear growth of *R. solani* and *F. solani* in comparison with the fungicide Topsin M-70, when particular control filled the Petri-dishes.

Treatments	*R. solani*		*F. solani*	
	*Linear growth	Inhibition%	**Linear growth	Inhibition %
T. harzianum (T. h)	1.80 c	80.00	2.17 b	75.89
T. viride (T. v)	1.57 d	82.56	1.92 c	78.11
T. album (T. a)	2.07 b	77.00	1.40 d	84.44
Yeast (Y)	1.27 e	85.89	0.87 e	90.33
T.h + y	0.67 g	92.56	0.63 f	93.00
T.v + y	0.00 h	100	0.53 f	94.11
T.a + y	1.03 f	88.56	0.00 g	100
Topsin M-70	0.00 h	100	0.00	100
Control	9.00 a	-	9.00 a	-

Different letters within a column indicate significant difference at $p \leq 0.01$ (Tuckey test)
* After five days incubation at 25°C, ** after eight days incubation at 25°C.

Pot experiment:

Disease assessment:

The efficacy of bio-filtrates of *Trichoderma*. spp. and Yeast as well as their combinations compared with Topsin M 70% wp and non-treated check for controlling damping-off and root rot diseases of faba bean had evaluated in infested soil with a mixture of both pathogenic fungi under greenhouse conditions. Data in Table (4) show that, any of the tested bio filtrates each alone and their combinations significantly reduced the incidence of pre- and post-emergence

damping-off as well as root rot severity percentage. The fungicide was more effective in this respect followed by dual combination, *i.e*., *T. viride* + Y then. *T. harzianum* + Y and *T. album* + Y, Concerning the effects of individual treatments, irrespective fungicide, the best protection against these diseases was recorded under Yeast application followed by *T. viride* - without significant differences between them. Moreover, T. *harzianum* came the third followed by *T. album*.

Table (4): Effect of bio filtrates on damping-off and root rot diseases of faba bean under artificially infested soil with mixture of the two pathogenic fungi.

Treatments	Damping-off		Root rot %
	Pre-emergence %	Post-emergence %	
T. harzianum (*T. h*)	12.00 bc	8.00 f	8.00 b
T. viride (*T. v*)	10.66 bc	6.66 bcd	8.00 b
T. album (*T. a*)	12.66 b	8.66 b	8.66 b
Yeast (Y)	10.00 bc	6.66 bcd	8.00 b
T. h + y	6.66 d	4.00 def	5.33 cd
T. v + y	5.33 d	3.33 ef	4.00 d
T. a + y	9.33 c	5.33 cde	6.66 bc
Topsin M- 70	2.66 e	1.33 f	3.33 d
Control	30.66 a	14.00 a	14.66 a

Different letters within a column indicate significant difference at $p \leq 0.01$ (Tuckey test)

Plant defense enzyme activity:

As shown in Table (5), the activity of peroxidase, polyphenoloxidase and chitinase enzymes was stimulated by any of bio filtrates treatment as compared with control. While the tested fungicide (Topsin M 70% wp) had no significant effect on the activity of these enzymes. The combination of *T. viride* + Y gave the highest activity of peroxidase and polyphenoloxidase followed by *T. harzianum* +Y treatment. Moreover, the dual combination of *T. album* +Y showed the highest chitinase activity followed by the individual treatments (*T. album*) then the combination of *T. harzianum* + Y. Regarding the effects of individual treatments, in order to peroxidase and polyphenoloxidase; Yeast filtrates came first followed by *T. viride* then *T. harzianum*. On the other side, *T. album* came first in increasing chitinase activity followed by *T. harzianum* then *T. viride* while, Yeast filtrate came late.

Table (5): Effect of bio filtrates and their combinations on enzymatic activity (mg per gm f.w.) in faba bean plants grown in infested soil under greenhouse conditions.

Treatments	Peroxidase	Polyphenoloxidase	Chitinase
T. harzianum (*T. h*)	0.618 e	0.397 f	0.554 b
T. viride (*T. v*)	0.694 d	0.487 e	0.435 d
T. album (*T. a*)	0.595 e	0.342 g	0.674 a
Yeast (Y)	0.786 bc	0.507 d	0.39o e
T. h + y	0.843 ab	0.653 b	0.564 b
T. v + y	0.896 a	0.676 a	0.473 c
T. a + y	0.777 bc	0.593 c	0.686 a
Topsin M- 70	0.403 f	0.215 h	0.183 f
Control	0.404 f	0.216 h	0.184 f

Different letters within a column indicate significant difference at $p \leq 0.01$ (Tuckey test)

Nodulation status and nitrogenase activity:

Data presented in Table (6) show nodulation status (number and dry weight) of nodules as well as nitrogenase activity as a response to bio-filtrate treatments. Soaking faba-bean seeds in the bio-filtrates of the tested bioagents and in their combinations significantly enhanced the No. of nodules/ plant. dry weight of the nodules/ plant and nitrogenase activity compared with control treatment (pathogenic fungi mixture). Generally, dual combinations were more effective than individual treatments. The highest values were recorded under the combination of T *viride* + Y followed by T. *harzianum* + Y then T. *album* + Y, being 56.0, 53.56 and 49.33 nodule/plant; 1.410, 1.377, 1.253 g nodule dry

weight/plant and 98.00, 86.67 and 78.33 nitrogenase activity, respectively. Regarding the individual treatments, yeast filtrate recorded the highest values of the tested parameters followed by T. *viride* then T. *harzianum* treatments. Meanwhile, the fungicide Topsin M-70 resulted in the lowest figures of the assessed No. of nodules and their dry weight / plant and nitrogenase activity, being 19.67 nodule, 0.493 g and 42.33 nitrogenase activity. Control treatment recorded 14.33 nodule /plant, 0.433 g nodule dry weight/plant and 40.33 nitrogenase activity.

Table (6): Effect of bio filtrates and their combinations on nodulation status and nitrogen activity in faba bean plants grown in soil infested by a mixture of the two pathogenic fungi.

Treatments	Nodule No./plant	Nodule dry w/plant	Nitrogenase
T. harzianum (*T. h*)	33.67 e	0.947 e	66.66 f
T. viride (*T. v*)	40.67 d	1.073 d	70.66 e
T. album (*T. a*)	30.33 f	0.833 f	58.67 g
Yeast (Y)	46.33 c	1.127 c	76.00 d
T. h + y	53.56 a	1.377 a	86.67 b
T. v + y	56.00 a	1.410 a	98.00 a
T. a + y	49.33 b	1.253 b	78.33 c
Topsin M- 70	19.67 g	0.493 g	42.33 h
Control	14.33 h	0.433 h	40.33 i

Different letters within a column indicate significant difference at $p \leq 0.01$ (Tuckey test)

Photosynthetic pigments

Chlorophyll content in plant leaves is considered a good parameter reflecting the health condition of the plant. Data shown in Table (7) reveal that the individual bio-filtrates and their combinations increased significantly photosynthetic pigment contents (chlorophyll a, b and carotenoids) in faba bean leaves. The highest concentration occurred due to using *T. viride* + Y combination followed by *T. harzianum* + Y then *T. album* +Y filtrate. Yeast filtrate was the most effective as compared with other individual treatments, *T. viride* came the second followed by *T. harzianum*.

Table (7): Effect of bio filtrates and their combinations on photosynthetic pigments (mg./g.f.w.) in faba bean leaves grown in infested soil under greenhouse conditions.

Treatments	Chl. A	Chl. B	Total chl.	Carotenoids
T. harzianum (*T. h*)	0.913 d	0.633 c	1.527 e	0.357 d
T. viride (*T. v*)	0.940 d	0.647 b	1.587 d	0.383 c
T. album (*T. a*)	0.877 e	0593 c	1.470 f	0.340 e
Yeast (Y)	0.983 c	0.670 b	1.623 c	0.407 b
T. h + y	1.150 a	0.723 a	1.873 a	0.440 ab
T. v + y	1.190 a	0.780 a	1.970 a	0.450 a
T. a + y	1.060 b	0.707 a	1.767 b	0.410 b
Topsin M- 70	0.817 f	0.500 d	1.317 g	0.300 f
Control	0.820 f	0.497 d	1.317 g	0.293 f

Different letters within a column indicate significant difference at $p \leq 0.01$ (Tuckey test)

DISCUSSION

Root rot of faba bean is caused by some complex fungi such as *R. solani*, *Fusarium* spp., *Macrophomina phaseolina* and *Sclerotium rolfsii* (Abd El-Hai and El-Saidy, 2016; El-Sayed, 2017; Hassan *et al*., 2017 and Mahmoud, 2017). Infection of seeds and roots by fungal diseases cause damping-off due to seed rot and seedlings death before emergence or seedlings death after emergence. In this investigation, all the obtained isolates of *Rhizoctonia solani* and *Fusarium solani* were pathogenic and caused damping-off and root rot symptoms.

The harmful anatomical changes of root structure due to fungal infection may be due to exposure of cell walls to cellulolytic and pectolytic enzymes produced by more pathogens. Hence, the parenchyma tissues were rapidly degraded by microorganisms more than lignified vascular tissues (Fouda and Abdalla, 2000). In addition, an increase in ethylene production after fungal infection promoted the activity of exo and endo cellular hydrolytic enzymes. Similar findings were obtained by Abd El-Hai (2001). In this context, Mahmoud *et al*. (2013) reported that *Fusarium solani* and *Rhizoctonia solani* produced cellulolytic and pectolytic enzymes, in turn occurs degradation of the cell wall and

hydrolysis of cell components (Abd El-Hai and El-Saidy, 2016).

The present results indicated that all the tested Trichoderma isolates (20) showed different levels of antagonistic activity against both tested pathogenic fungi. *T. harzianum* No. 2, *T. viride* isolate No. 4 and *T. album* isolate No. 1 were the most potent antagonists to the root rot pathogens. The direct antagonism of *Trichoderma* spp. against plant pathogens might be due to competition, parasitism and antibiosis (Harman, 2006) which inhibit the fungal growth. Moreover, Abd-El-Khair *et al.* (2010) and Hassan *et al.* (2014) summarized the different mechanisms actions of Trichoderma in competition on nutrient, antagonism, antibiosis, inhibition of pathogen or plant enzymes; processes of biodegradation, nitrogen and carbon cycling; complex interactions with plant in the root zone of the rhizosphere, which involve plant growth stimulation, biocontrol of diverse plant pathogens and decomposition of organic matter.

Our results showed that the use of both *Trichoderma* spp., Yeast culture filtrate and their combinations reduced the mycelial growth of both tested pathogenic fungi. Moreover, faba bean seed soaking followed by spraying the foliar after 38 days from sowing with the same treatments has been reported as the best protection against for damping-off, root rot under artificial infection of soil with a mixture of pathogenic fungi. Antifungal activity of Trichoderma culture filtrates may be due to production high quality fungal toxic metabolites which include non-volatile metabolites such as Trichoderma amide, anthraquinones and steroidal antibiotic (Reino *et al.,* 2008). Moreover, Gajera *et al.* (2013) stated that the antifungal activity of Trichoderma is due to its ability to produce lytic enzymes, antifungal antibiosis and can also be competitors. On the other side, the inhibitory effects of Yeast filtrate against fungal pathogens might be due to the production of soluble antifungal metabolites diffused into the medium and their production of hydrolytic enzymes capable of degrading the cell wall of the pathogens (Urquhart and Penja, 2002). In addition, the use of Yeasts in controlling plant pathogens may be explained based on the production of proteinaceous killer toxins (Santos *et al.,* 2004).

In this study, the application of both *Trichoderma* spp. and Yeast filtrates as well as their combinations stimulated the activity of plant defense enzymes, enhancement of nodules status and nitrogenase activity as well as photosynthetic pigments in turn enhancement of the plant healthy. Plant defense enzymes play an important role in plant protection from pathogen infection. Hence, there is a positive relationship between polyphenoloxidase and chitinase production and the ability to control plant diseases by degrading cell walls of the pathogen in turn, inhibitory the growth (Harman *et al.,* 2004). Nawar and Kuti (2003) explained that the role of peroxidase in activities of plant resistance includes oxidative cross-linking of pre-existing hydroxyl proline-rich structural proteins in the cell wall which making it more resistant to degradation by microbial enzymes and it increases lignin production. In this investigation, fungal infection gave the lowest values of No. of nodules/ plant. dry weight of the nodules/ plant and nitrogenase activity. The injurious effects of both pathogenic fungi on nodulation status and nitrogenase activity might be due to it causes root rot leading to decrease in root nodules followed by decrease the number and biomass of nodules, in turn decrease in nitrogenase activity. These effects are in agreement with El-Hersh *et al*. (2011) who found that there is significant correlation among nodules number, nodules weight and nitrogenase activity. On the other side, the enhancement of nodulation status and stimulated nitrogenase activity; under both bio-filtrates treatments; may be due to the activation of enzymes responsible for the reduction of dinitrogen from atmosphere in turn, enhancement the plant health. Also, it contains chitinase and cellulase enzymes which may contribute to promotion of nodule formation. *Trichoderma* spp. also secrete auxin like substances which lead to enlargement in root size and rooting depth followed by increase the number of nodules and improving nitrogenase activity (Adams *et al*., 2007; Elwakil *et al*., 2009 and Abdel-Monaim, 2013).

Photosynthetic process (carbon assimilation) consists in the synthesis of certain carbohydrates from CO_2 and H_2O by green cells in the presence of light. Hence, the infection with root rot pathogens causing a reduction in photosynthetic capacity and not photosynthesis by killing and damage of root system which leads to reduce absorption surface of water, subsequently disturbance in metabolic processes (Saleh, 1997). On the other side, both bio filtrates induced photosynthetic pigments due to the increase in cytokinin content that causes an increase in the number of chloroplasts in leaves with increasing intensity of cytoplasm ribosomes, followed by stimulation of chlorophyll synthesis (Robert-Seilanniantz *et al*., 2007 and Choi *et al*., 2010). Moreover, in the present study the role of both culture filtrates in enhancing photosynthetic

pigments may be due to its effects on fungal linear growth and nullifies the deleterious effects of fungal infection on root system.

Moreover, increasing of photosynthetic pigments leads to increasing carbohydrate content that is considered a main repository of energy and comprises structural polysaccharides of plant cell walls and principally cellulose, hemicelluloses and pectin that consider the first barrier against plant pathogen invasion (Hamideh *et al.*, 2013). Generally, the mixture of *T. viride* and yeast was more effective in decreasing the harmful effects of the two pathogens on most character studies in this investigation than each treatment of them alone. This effect may be due to the application of a mixture of bio-control agents, which is more closely imitate the natural situation, broaden the spectrum of bio-control activity and enhances the efficacy and the reliability of the control (Gananamanickam, 2002).

Therefore, bi-combination between *T. viride* and yeast culture filtrate (culture free cell) becomes a highly recommended for reducing damping- off and root rot diseases of faba bean.

REFERENCES

Abd El-Hai, K.M. 2001. Physiological and pathological studies on soybean plants under different levels of salinity. Ph. D. Thesis, Fac. Of Agric., Mansoura Univ., Egypt.

Abd El-Hai, K.M. and Ali, A.A. 2018. Amelioration of the structural and biochemical features of kidney bean against root rot and rust diseases. J. Plant Prot. And Path., Mansoura Univ., 9(3): 237-245.

Abd El-Hai, K.M. and Ali, A.A. 2019. Formulation of Trichoderma, Saccharomyces and Rhizobium metabolites against damping-off and root rot pathogens in peanut plant. Asian J. Biol. Sci., 12(2): 114-121.

Abd El-Hai, K.M. and El-Saidy, A.E.A. 2016. Pre-sowing seed treatment with some organic compounds for controlling root rot disease and improving faba bean productivity under salinity affected soil conditions. J. Plant Production, Mansoura Univ., 7(10): 1053-1061.

Abd-El-Khair, H.; Khalifa, R.K.M. and Haggag, K.H. 2010. Effect of *Trichoderma* species on damping off disease incidence, some plant enzymes activity and nutritional status of bean plants. J. Am. Sci., 6, 486-497.

Abdel-Monaim, M.F. 2013. Improving biocontrol of damping-off, root rot and/or wilt diseases of faba bean by salicylic acid and hydrogen peroxide. Egypt. J. Phytopathol., 41(2): 45-60.

Abdel-Razik, S.A.; Sallam, N.M.A.; Eraky, A.M.I. and Hassan, M.H.A. 2012. Integrated control of root rot and wilt disease of faba bean by soil amendment with suppressive compost in combination with seed coating with an antagonistic yeast. Archives of Phytopathology and Plant Science, 45(14): 1692-1704.

Abou-Zeid, N.M.; Arafa, M.K. and Attia, S. 2003. Biological control of pre-and post-emergence damping-off diseases on faba bean, lentil and chickpea in Egypt. Egypt. J. Agric Res., 81: 1491-1503.

Adams, P.; De-Leij, F.A.A. M. and Lynch, J.M. 2007. *Trichoderma harzianum* Rifai 1295-22 mediates growth promotion of crack willow (*Salix fragilis*) saplings in both clean and metal-contaminated soil. Microbial Ecol., 54: 306-313.

Barnett, H. and Hunter, B. 1998. Illustrated Genera of Imperfect Fungi. Mac Millan Publ. Co. ISBN: 0-89054-192-2. New York.

Bell, D.; Wells, H. and Markham, C. 1982. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, 72, 379-382.

Bendahmane, B.S.; Mahiout, D.; Benzohra, I.E. and Benkada, M.Y. 2012. Antagonism of three *Trichoderma* species against *Botrytis fabae* and *B. cinerea*, the causal agents of chocolate spot of faba bean (*Vicia fa*ba L.) in Algeria. World Applied Sci. J., 17: 278-283.

Bennet, H.S.; Wirinck, A.D.; Lee, S.W. and McNeil, J.H. 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. Stain Tech. 55: 71-97.

Bissett, J., 1991. A revision of the genus Trichoderma. II. Infrageneric classification. Canadian J. Botany, 69: 2357-2372.

Booth, C. 1977. Fusarium Laboratory Guide to the Identification of the Major Species. 1st Ed., Commonwealth Mycological Institute, England, 58 pp.

Chet, I. 1987. Trichoderma: application, mode of action and potential as biocontrol agent of soil borne plant pathogenic fungi. Innovative approaches to plant disease control. John Wiley and Sons, New York, PP.137-160.

Choi, K.; Huh, S.U.; Kojima, M.; Sakakibara, H.; Pack, K.H. and Hwang, I. 2010. The cytokinin-activated transcription factor ARR2 promtes plant immunity via TGA3/NRR 1-

dependent salicylic acid signaling in Arabidopsis. Div. Cell, 19: 284-295.

Crepon, K.; Marget, P.; Peyronnet, C.; Carrouee, B.; Arese, P. and Duc, G. 2010. Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crops Res., 3, 329-339.

Denise, C. and Anjali, S.L. 2019. A scanning microscopy for viewing plant-microbe interactions at tissue and cell-type resolution. Phytopathology, 109: 1302-1311.

Elad, Y.; Hadar, Y.; Hadar, E.; Chet, I. and Henis, Y. 1981. Biological control of *Rhizoctonia solani* by *Trichoderma harzianum* in carnation [fungi]. Plant Diseases, 65: 675-677.

El-Hersh, M.S.; Abd El-Hai, K.M. and Ghanem, K.M. 2011. Efficiency of molybdenum and cobalt elements on lentil pathogens and nitrogen fixation. Asian J. Plant Pathol., 5(3): 102-114.

El-Sayed, A.S. 2017. Management of damping-off and root-rot diseases of faba bean by bioproducts and inducer resistance chemicals. Egypt. J. Phytopathol., 45(1): 135-156.

Elwakil, M.A.; Awadallah, O.A.; El-Refai, I.M.; El-Metwally, M.A. and Mohammed, M.S. 2009. The use of bread yeast as a biocontrol agent for controlling seed-borne fungi of faba bean. Plant Pathology J. 8(4) 133-143.

Esterbaner, H.; Schwarzal, E. and Hayn, M. 1977. A rapid assay for catechol oxide and laccase using 2-nitro-5-thiobenzoic acid. Anal. Biochem., 77: 486-494.

Fouda, R.A. and Abdalla, M.E. 2000. Effect of paclobutrazol on growth, yield and anatomical structure of peanut plant infected with pod and root rot pathogens. J. Agric Sci. Mansoura Univ., 25(1): 169-183.

Gajera, H.; Domadiya, R.; Patel, S.; Kapopara, M. and Golakiya, B. 2013. Molecular mechanism of Trichoderma as bio-control agents against phytopathogen system–a review. Current Research in Microbiology and Biotechnology 1: 133-142

Gerlach, D. 1977. Botansche Mikrotecknik. Eineeinfuhrung Thieme Verlag, Stullgart, BRD.

Ghoniem, A.A.; Abd El-Hai, K.M.; El-Khateeb, A.Y.; Eldadamony, N.M.; Mahmoud, S.F. and Elsayed, A. 2021. Enhancing the potentiality of *Trichoderma harzianum* against Pythium pathogen of beans using chamomile (*Matricaria chamomilla*, L.) flower extract. Molecules, 26(4): 1178.

Gnanamanickam, S.S. 2002. Biological Control of Crop Diseases. CRC Press, NY., 480 pp.

Gomez, K.A. and Gomez, A.A. 1984. Statistics for Agriculture Research. 2nd Ed. John Willey and sons, New York, USA. 680 pp.

Hamideh, G.; Samavat, S. and Ardebli, Z.O. 2013. The alleviating effects of humic substances on photosynthesis and yield of *Plantaga ovate* in salinity conditions. International Res. J. of Applied and Basic Sci, 4(7): 1663-1686.

Harman, G. E. 2006. Overview of Mechanisms and Uses of *Trichoderma* spp. Phytopathology, 96, 190-194.

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

Hassan, M.; Khalil, M.S.M.; Mahmoud, A. and Morsy, K.M.M. 2017. Effect of water and seeds magnetization on root rot and wilt diseases of faba bean. Egypt. J. Phytopathol., 45(2): 199-217.

Hassan, N.; Elsharkawy, M.M.; Shimizu, M. and Hyakumachi, M. 2014. Control of root rot and wilt diseases of Roselle under field conditions. Microbiology, 42, 376-384.

Kato, M and Shimizu, S. 1987. Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic-dependent peroxidase degradation. Can. J. Bot., 65: 729-735.

Lethbridage, G.; Davidson, M.S. and Sparling, G.P. 1982. Critical evaluation of the acetylene reduction test for estimating the nitrogenase activity of nitrogen fixing bacteria associated with the roots of wheat and barley. Soil Biol. Biochem., 14: 27-35.

Mackinney, G. 1941. Absorption of light by chlorophyll solution. J. Biol. Chem., 140(2): 315 -322.

Mahmoud, N.A. 2017. Comparative study on the activity of *Trichoderma asperellum* and *T. album* and their role in controlling faba bean root rot and wilt diseases. Egypt. J. Phytopathol., 45(2).183-198.

Mahmoud, S.Y.M.; Hosseny, M.H.; Shaikh, K.A.A.; Obiadalla, A.H.A. and Mohamed, Y.A. 2013. Seed borne fungal pathogens associated with common bean (*Phaseolus vulgaris* L.) seeds and their impact on germination. J. Enviro. Studies, 11:19-26.

Metayer, 2004. *Vicia faba* breeding for sustainable agriculture in Europe. Gie feverole. D7: WP 1- EUFABA.

Mohsen, A.A.; Ebrahim, M.K.H. and Ghoraba, W.F.S. 2013. Effect of salinity stress on *Vicia faba* productivity with respect to ascorbic acid

treatment. Iran J. Plant Physiol., 3(3): 725-736.

Monreal, J. and Reese, E.T. 1969. The chitinase of *Serratia marcescens*. Can. J. Microbiol., 15: 689-696.

Nawar, H.F. and Kuti, J.D. 2003. Wyerone acid phytoalexin synthesis and peroxidase activity as markers for resistance of broad bean to chocolate spot disease. J. Phytopathol., 151: 564-570.

Rauf, B.A. 2000. Seed-borne disease problem of legume crops in Pakistan. Pak. J. Sci. Ind. Res. 43: 249-254.

Reino, J.L.; Guerrero, R.F.; Hernandez-Galan, R. and Collado, I.G. 2008. Secondary metabolites from species of the biocontrol agent Trichoderma. Phytochem. Rev., 7: 89-123.

Rifai, A. 1969. A revision of the genus Trichoderma. Mycological Papers. 116: 1-56.

Robert-Seilanniantz, A.; Navarro, L.; Bari, R. and Jones, J.D. 2007. Pathological hormone imbalances. Curr. Opin. Plant Biol., 10: 372-379.

Robinson, J.M. and Britz, S.J. 2000. Tolerance of field grown soybean cultivars to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydrogenase redox status and long-term photosynthetic productivity. Photosynthesis Res., 64: 77-87.

Rubiales, D. 2010. Faba beans in sustainable agriculture. Field Crops Research, 115: 201-202.

Saleh, O., 1997. Wilt, root rot and seeds diseases of groundnut in El-Minia Governorate, Egypt. Egypt. J. Phytopathol., 25: 1-18.

Santos, A.; Sanchez, A. and Marquina, D. 2004. Yeasts as biocontrol agents to control *Botrytis cinerea*. Microbiol. Res., 159: 331-338.

Sneh, B.; Burpee, L. and Ogoshi, A. 1991. Identification of *Rhizoctonia* species. APS Press. USA, PP. 133.

Tolera, A.; Semu, E.; Deble, T.; Wegary, D. and Kim, H. 2015. Determination soil Rhizobium populations, intrinsic antibiotic resistance, nodulation and seed yield of faba bean and soybean in western Ethiopia. World J. of Agric. Sci., 11(5): 311-342.

Urquhart, E.J. and Penja, Z.K. 2002. Hydrolytic enzymes and antifungal compounds produced by *Tilletiopsis* species, phyllosphere Yeasts that are antagonists of powdery mildew fungi. Can. J. Microbiol., 48: 219-229.

Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Marra, R.; Woo, S.L. and Lorito, M. 2008. Trichoderma a plant pathogen interactions. Soil Biol. Biochem., 40: 1-10.