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Effect of Some Microorganisms and Chemical Stimulants on Resistance to Fusarium Roots Rot and on Growth Characteristics of Beans

M. A. Khalil [1] and B. A. Hassouna [2]

[1]Plant Pathology Res. Institute, Agric. Research Center, Giza, Egypt.
[2]Soils, Water and Environ. Res. Institute., Agric. Research Center, Giza, Egypt.

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ABSTRACT

In the last few years, *Fusarium solani* caused root rot and among different important diseases attacked common beans (*Phaseolus vulgaris* L.) under the Egyptian climate conditions. The type of Fusarium that causes common bean roots rot has been identified through morphological characteristics on environmental media. Five isolates of *Fusarium solani* f. sp. *phaseoli* were isolated from naturally infected bean roots representing different localities of some governorates, was tested pathogenicity to common beans (cv. Nebraska), during summer growing season 2018. Results indicated that isolate which obtained from Derwa (Minia Gov.) was the virulent that induced the disease. Under laboratory conditions, the effect of some microorganisms (*Trichoderma harzianum*, *Trichoderma hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescence* and *Rhizobium leguminosarum* biovar *phaseoli*) and some induceding materials (Salicylic acid, Ascorbic acid and Boric acid) on mycelial growth of the pathogenic isolate of *F. solani* f. sp. *phaseoli*. The results showed that treatment *T. harzianum* compared with other treatments and control plates addition (PDA) medium as sterilized caused the highest decrease in the mycelial growth of the virulent isolate by about (63.62%).Under greenhouse conditions, previous treatments was evaluated in percentage incidence and disease severity Fusarium root rot disease. The results indicate that the previous treatments, whether singly or in combination with rhizobium, reduced the disease by varying degrees. On the other hand, the results indicated that the fungus *T. harzianum* was the most effective in reducing the percentage incidence and severity of disease by about (13.33 and 11.11%, respectively), while *T. harzianum* combination treatment with rhizobium gave better and more effective by about (8.89 and 6.67%, respectively) compared with other treatments and control. As well as, such treatments, whether singly or in combination with rhizobium, gave, significant increase growth characteristics by various degrees. While, *T. harzianum* mixed with rhizobium proved more effective in comparison with *T. harzianum* singly by about: in Plant height (25 and 34 cm.), number of nodules/plant (15.33 and 22.33), dry weight of nodules/plant (0.015 and 0.028 g), dry weight of root (1.68 and 2.47 g), dry weight of shoot (2.48 and 4.20 g) and nitrogen (N) content (2.14 and 3.18), respectively.

Keywords: Isolation-pathogenicity test-common beans-microorganisms and chemical stimulants growth characteristics.

1. Introduction

Common beans are one of the most widely cultivated food legume species in the world (Baudoin *et al*., 2001). Broughton *et al.* (2003) mentioned that common beans are leguminous plants with economic, nutritional, and cultural importance around the world. On the other hand, Broughton *et al.* (2003) and Vital *et al.* (2014) reported that common beans are one of the most important legumes for human consumption due to its nutritional value and high levels of protein, iron, carbohydrates, and bioactive compounds in many countries including Egypt. Fusarium root rot disease caused by *F. solani* f. sp. *phaseoli,* is one of the major concern in many bean growing areas in Egypt leading to enormous yield losses, especially when adverse environmental conditions such as soil moisture and soil compaction persist through planting and flowering. Unlike other root rotting diseases, does not cause,

Corresponding Author: M. A. Khalil, Plant Pathology Res. Institute, Agric. Research Center, Giza, Egypt.
E-mail:- mohamedalikhalel@gmail.com

seed rots and damping-off seedlings. The symptoms do not appear until a week or more after the seedlings emerges. Fusarium root rot is characterized by reddish-brown color along the tap roots and lower hypocotyls. Areas diseased of the plant enlarge with age and gradually turn brown. Longitudinal may develop cracks in older lesions and the cortical tissues are discolored and decayed. Root rot are particularly severe under water-stress condition (Burke and Hall, 1991). Also, El-Mougy *et al.* (2007) mentioned that the main pathogens responsible for root rot diseases are *F.solani* and *R. solani*; it is a serious disease for bean plants.

The objective of this study was to evaluate antagonism of *Trichoderma* and bacterial biological control agents against *F. solani* f. sp. *phaseoli in vitro* and evaluate their efficacy under greenhouse conditions. Mishra (1996) isolated, *Trichoderma* species including *T.viride*, *T.harzianum*, *T.hamatum*, *T. koningi*, *T.pseudokoningi*, etc, are common residents of rhizosphere (soil adhering to root surface) and rhizoplane (root surface) of plants. Application biological control using antagonistic microorganisms against seed and root rot pathogens proved to be successfully and its efficiency in controlling root rot pathogens, improving growth and yield quality of many crops (Rao *et al*., 2009). Atmospheric nitrogen is fixed into a form useful for plant growth with *R. leguminosarum* bv. *phaseoli* while, that establishes a symbiotic relationship with common bean within the root nodules (Paul and Clark, 1989 and Long, 2001) a relationship which often improves crop yield. Plant Growth Promoting Rhizobacteria (PGPR) group is a recognized that establishes symbiotic relationships with leguminous species and creates specialized structures called nodules on the plant root (Mortier *et al*., 2012). (PGPR) is a group of non-pathogenic bacteria that includes both free living (e.g. *Azospirillum brasilense*) and symbiotic (e.g. *Rhizobium* spp. and *Bradyrhizobium* spp.) nitrogen fixing microorganisms and known with rhizobia leguminous symbiosis has inhibitory effects against development of fungal diseases in plants (Das *et al*., 2017).

Induction of plant resistance to overcome pathogen infection is an effective way for controlling plant diseases and several reports indicated the efficiency of inducer resistance chemicals in reducing some plant diseases and use of such chemicals inducers would permit a reduction in the use of agrochemicals such fungicides (El-Mohamedy and Ahmed, 2009 and Bakeer, 2014). As well as, fungicidal treatments cause hazards to human health and increase environmental pollution; therefore there is need to alternatives. Fungicides were essential trial as seed treatment for controlling root rot diseases (El-Mougy, 2001).

2. Materials and Methods

2.1. Isolation, purification, identification and preparation of pathogen and bioagents associated with rhizosphere common beans

2.1.1. Source of the fungal isolates

Fusarium was isolated originally from naturally diseased bean plant exhibiting typical symptoms of root rot disease different regions of five Governorates in Egypt, *i.e.* Salehia (Sharkia Gov.), Wadi-El Mollak (Ismailia Gov.), Seds (Beni-suief Gov.), Derwa (Minia Gov.) and Minfaloat (Assiut Gov.). Parts infected of the plants were excised with a sterile scalpel and surface sterilized with 3% (w/w) NaOCl/ for 2 min. Sterilized pieces were washed twice with sterile water for 60 sec and cut into small pieces (1cm length) and transferred on PDA plates and added antibiotic amended. Plates were incubated at room temperature for 48h and transferred mycelium growth from the infected stem pieces to new PDA plates. After incubation 5 days, a single spore was isolated and cultured on new PDA plates. Pathogen was identified as *F. solani* f. sp. *phaseoli*, based on the characteristics according to Booth (1977). Koch's postulates were demonstrated for the pathogen and confirmed as the causal agent of root rot of *P. vulgaris* and were kept on the PDA environment for future studies. The pathogen inoculum was produced on PDA plates. They were inoculated with an agar pluge (5mm in diameter) containing actively growing *F. solani* mycelium and incubated under fluorescence for 10days at room temperature. Spores were washed from the plates with sterile distilled water and concentration was adjusted to and 10^{16} spore mL^{-1} with a haemocytometer. The inoculum of the *F. solani* isolate was prepared from pure colonies of isolates grown on PDA medium and inoculated sorghum grains were mixed with sterile soil and placed in bottles that were sterilized by pressurized steam (121°C) for a period of 2 hours.

2.1.2. Isolation of the bioagents

Different bioagent isolates isolated from the native microflora in the rhizosphere naturally infections with green bean roots rot and isolated pure and preserved on PDA slants for further use *Trichoderma* isolate isolated from rhizoplane by using standard method (Aneja, 1993). In which roots were washed in running tap water and 1cm long root pieces were cut from lateral roots and washed in sterilized distilled water. Then root pieces were transferred on plates containing potato dextrose agar (PDA) mixed with penicillin (100,000 unit/liter) and streptomycin (0.2g/liter) to inhibit the growth of gram-positive and negative bacteria. The Petri plates were incubated for 5 days at 28°C. *Trichoderma* isolate was made separated and identify as *T. harazianum* and *T .hamatum* according to morphological and microscopic characteristic described by Domsch, *et al.* (1980). Bean seeds were soaked for (15 min) in spore suspension of each *T. harazianum* and *T. hamatum* (3×10^6spore/ml). Besides that, 2 g soils added to 200 ml sterile distilled water in conical flask. Contents of flasks were then stirred vigorously for 5 min and Basic dilution were prepared to obtain10^{-3}; $10^{-4}$and$10^{-5}$and isolation of the bioagents was performed as described by Saleh (1997).

Also, Bacterial isolates of *B. subtilis* and *P. fluorescence* consisted of aqueous solutions prepared from 3day old cultures of bacteria was grown on PD broth media and inoculated individually into conical flasks 250 ml containing on 100 ml PD broth, and incubated at 27°C for 2 days and used for inoculation and selected for greenhouse pot experiments. Seeds of transplants were dipped in bacterial suspension (2.5×10^7cfu/ml) for half hour and they were transplanted directly in wet pot. The previously bioagents were added to infested soil with the most virulent isolate *F. solani* f. sp. *phaseoli*, (at the rate of 1 and 2% (w/w) after which they were transplanted directly in wet pot Chang and Kommedahl (1968).Whereas bacteria was identified as *B. subtilis* and *P. fluorescence* according to Burbage *et al.,* (1982). Pots (25cm in diameter) filled with disinfested soil at the rate of 2.5 kg clay: sand (2:1, v/v) and were sown with bean seeds (5seed/pot) in artificially infested soil.

2.2. Microorganisms and inoculants preparation

2.2.1. Rhizobia and rhizobacteria strains

Rhizobium leguminosarum bv. *phaseoli* of local isolates was used as a basic inoculant for common bean seeds. *Bacillus subtilis, Pseudomonas fluorescence* were used as separately co-inoculants with rhizobia. All previous types of bacteria used were kindly provided by Biofertilizer Production Unit, Agric. Microbiology Dept., Soils, Water and Environment Research Institute (SWERI) ARC, Giza, Egypt.

2.2.2. Preparation of inoculum

Rhizobium leguminosarum biovar *phaseoli* was growth on yeast mannitol extract broth (YEM) (Vincent, 1970) media containing (10g mannitol, 1g yeast extract, 1g KH_2PO_4, 0.1g NaCl, and 0.2g $MgSO_4$, $7PH_2O$ per liter, pH 6.8), incubated at 28ºC for three days until early log phase ($5x10^9$CFU/ml culture).Vermiculite supplemented at 10%Iresh peat packed in polyethylene bags (300g per bag),then sealed and sterilized by gamma irradiation (5.0 $x10^6$ rads). *Rhizobium* culture was injected into the carrier to satisfy 60%of water holding capacity. *Bacillus subtilis* used was grown on nutrient broth medium (Difco Laboratories, 1984) and incubated at 28ºC for 48 hr where that attained populations of $3.2x10^9$CFU/ml culture for *B. subtilis. Pseudomonas* was grown on king's medium (Atlas, 1995), incubated at 28ºC for 48 hr to maintain population of $3x10^9$CFU/ml for *Pseudomonas.* Afterwards, each culture of rhizobacteria was injected into the sterilized carrier as mentioned before in preparation of rhizobium inoculants. Inoculation of common beans seeds with *Rhizobium leguminosarum* bv. *phaseoli* and or rhizobacteria at rate of 300g each inoculants/40kg seeds their coating with inoculants using 16 %Arabic gum solution as adhesive agent.

2.3. Measurement of nitrogen content

The percentage of nitrogen (N) content of dried legume shoots and yield were determined per treatment according to Jackson (1958).

2.4. Preparation of organic acids and fungicides

Three organic acids, *i.e.* Salicylic acid (SA) C_6H_2 (OH) COOH, Ascorbic acid (AA) $C_6H_8O_6$, Boric acid (BA) H_3BO_3 and two fungicide, *i.e.* Topsin M70 (Thiophanate-methyl) and

RizolexT50%WP (Tolchlophos-methyl). Poured of mixture with PDA in sterilized petri plates *in vitro* and tested against the virulent isolate *F. solani* f. sp. *phaseoli*, on beans seed (cv. Nebraska) under greenhouse condition. Organic acids were added at concentration, 5.0mM for 24h before sowing to common bean seed and dressed then sown in artificially infested soil comparison with control treatment. As well as, fungicides Topsin M70 (1g/kg seeds) and Rizolex-T50%WP (3g/kg seeds) was added at the recommended dose. Five beans seeds sowing in each pot and three pots were used as replicates for each particular treatment.

2.5. Pathogenicity test of the causal pathogen *F. solani* f. sp. *phaseoli* on common beans (cv. Nebraska), at the different surveyed governorates during season 2018

Pathogenicity test of the isolated fungi was carried out to determine the pathogenic potentialities (virulence) of the different isolates of *F. solani* f. sp. *phaseoli*; the most aggressive isolate was used for further investigations. Pots (25cm in diameter) were sterilized by immersing in 5%formalin solution for 15 min and left for one week until complete formalin evaporation. Pots were filled with disinfested soil at the rate of 2.5 kg clay: sand (2:1, v/v). Five healthy common bean seeds were sown in each pot. Three pots were used as replicates for each isolate and three uninfested pots were used as control. Plants were irrigated when necessary and all pots were kept at greenhouse under natural conditions (day temperature 35°C, night temperature30°C, 16h photo period). Ten days after the seedlings emerge; five milliliters of spore suspension of each isolate around the hypocotyls of each plant in their testes pot. Percentage Incidence (PI) and Disease Severity (DS) of Fusarium root rot disease were assessed 21 days after inoculation for each treatment. Disease severity was estimated by assessing necrotic lesion on the roots and hypocotyls using a rating scale of 0-5 described according to Filion *et al.* (2003) as following:

Disease Severity (DS) =Sum (a×b) ×100÷ (A×K)……...….. (1)

a=No. of diseased plants having the same degree of infection. b=Degree of infection. A=Total no.of examined plants. K=Highest degree of infection.

2.6. Antagonistic effect of some microorganisms and chemical stimulants on virulent isolates of *F. solani* f. sp. *phaseoli in vitro* (Calculated as% of inhibition mycelial growth) compared with fungicides

2.6.1. Using, some bioagents

Studying antagonistic effect of some microorganisms *i.e. T.harzianum*, *T.hamatum*, *B. subtilis*, *P. fluorescence* and *Rhizobium* on growth of the virulent isolate of *F. solani* f. sp. *phaseoli in vitro* was an examined Petri plate (9cm. in diameter) containing Potato Dextrose Agar (PDA). A disc (5cm) from3days old culture of each the antagonists was transferred to one side of Petri plates containing solidified (PDA)medium and other side was inoculated with mycelia disc from edge of a three day-old of *F. solani* f. sp. *phaseoli*. Inoculated plates were incubated at 25±2°C., for five days. A disc of *T. harzianum*, *T. hamatum*, isolate, 0.5 cm in diameter, was placed on (PDA) medium on both sides of Petri dishes. Plates were streaked with bacterial, growth obtained from 3 days old culture at opposite sides at the periphery of plate by using needle. At the same time, one disk of the pathogen was placed at the center each plate. Three replicates to calculate the clear zones when growth of the pathogen covered the plate's surface (9.0cm in diameter) of control treatment. Inhibition zones were measured when the fungal growth was completely covered any plate surface. Three replicates were used for each treatment. Petri plates inoculated with either pathogen or antagonistic agent alone were served as control treatment. The radial growth inhibition percentage of the percentage was calculated using Abbott equation (Frolich, 1979) according to the following formula:

I.P= C - T ÷ C× 100……...…… (2)

C= Radial growth of control, T= Radial growth of treatment and I.P= Inhibition%

2.6.2. Using, some resistance inducers and fungicides

Three organic acids, *i.e.* Salicylic acid, Ascorbic acid and Boric acid, were used in this experiment to test their effect on growth of the virulent isolate of *F. solani* f. sp. *phaseoli*. PDA medium amended

with different organic acids, it was added to PDA medium. Each organic acid was added at concentration, 5.0mM based on the active ingredient just before pouring the medium in Petri dishes. Fungicides *i.e.* Topsin M70 (Thiophanate-methyl) and Rizolex-T 50%WP (Tolchlophos-methyl) mixture with PDA medium was poured in sterilized petri plates. After medium solidification, dishes were inoculated with inoculum discs cut from the periphery of 5 day's old *F. solani* f. sp. *phaseoli*, and then incubated at 25±2°C. Three petri plates were devoted for each treatment. Three petri plates containing sterilized non-inoculated soil served as a control treatment. The diameter of developed colonies was measured when fungal mycelium covered the medium surface in Petri dishes according to estimate as mentioned before.

2.7. Effect of some bioagents isolates and chemical stimulants against root rot disease of beans (cv. Nebraska), under greenhouse conditions

In this experiment, the effect of, *T. harzianum*, *T. hamatum*, *B. subtilis, P. fluorescence* and microbial vaccines have been used for *R. leguminosarum* bv. *phaseoli* or Salicylic acid, Ascorbic acid and Boric acid without mixing it comparison with fungicides Topsin M70 and Rizolex-T50%WP and control under greenhouse during season 2019. On the other hand, and studied effect rhizobium inoculation in common beans (cv. Nebraska) plants infected with virulent isolate of *F. solani* f. sp. *phaseoli*, who cause of Fusarium root rot. Soil and infection were prepared by virulent isolate as mentioned before, in addition to treatments in soil infective. Pots (25cm in diameter) were sterilized and preparation of bioagents, organic acids and fungicides as mentioned before. Pots were filled with disinfested soil at the rate of 2.5 kg clay: sand (2:1, v/v). Five healthy common bean, seeds were sown in each pot. Two controls were used; the first control was performed using artificially infested soil with *F. solani* f. sp. *phaseoli* disease (control-1). The second control using sterile soil without inocula control-2 (uninoculated). Three replicates were devoted for each treatment and control and all pots were kept in a greenhouse under natural conditions (day temperature 35°C, night temperature 30°C, 16h photo period) and were irrigated directly after transplanting and subsequently as when necessary. Percentage Incidence (PI) and Disease Severity (DS) of Fusarium root rot disease were assessed 21 days after inoculation for each treatment as mentioned before.

2.8. Effect of combined treatments between different biocontrol agents, rhizobia and chemical stimulants against root rot disease of beans (cv. Nebraska), under greenhouse conditions

Combined treatments between the *R. leguminosarum* bv. *phaseoli* and either *T.harzianum*, *T.hamatum*, *B. subtilis* and *P. florescence* or Salicylic acid, Ascorbic acid and Boric acid comparison mix it up with fungicides Topsin M70 and Rizolex-T50%WP and control under greenhouse during season 2019. On the other hand, studied effect rhizobium inoculation in common beans (cv.Nebraska) plants infected with virulent isolate of *F. solani* f. sp. *phaseoli*, which caused Fusarium root rot. Soil and infection were prepared by virulent isolate as mentioned before and addition of treatments in soil infective. Pots (25cm in diameter) were sterilized and preparation of bioagents, organic acids and fungicides as mentioned before. Pots were filled with disinfested soil at the rate of 2.5 kg clay: sand (2:1, v/v). Five healthy common beans seeds were sown in each pot. Two controls were used; the first control was performed using artificially infested soil with *F. solani* f. sp. *phaseoli* disease control-1 (Rhizobium and artificially infection). The second control (control-2) using sterile soil without inocula (without rhizobium and artificially infection). Three replicates were devoted for each treatment and control and all pots were kept in a greenhouse under natural conditions (day temperature 35°C, night temperature30°C, 16h photo period) and were irrigated directly after transplanting and subsequently as when necessary. Percentage Incidence (PI) and Disease Severity (DS) of Fusarium root rot disease were assessed 21 days after inoculation for each treatment as mentioned before.

2.9. Effect of individual and combined treatments between different biocontrol agents, rhizobia and chemical stimulants on growth characters of common beans (cv. Nebraska), plants under greenhouse conditions

In this trial, experiment was carried out in summer season 2020, under greenhouse conditions to efficient treatments either singly or mixed with rhizobium *R. leguminosarum* bv. *phaseoli* for both *T.harzianum*, *T.hamatum*, *B. subtilis* and *P. florescence* or Salicylic acid, Ascorbic acid and Boric acid comparison with fungicides Topsin M70 and Rizolex-T50%WP and control under greenhouse during

season 2020. On the other hand, studied effect of rhizobium inoculation in common beans (cv. Nebraska) plants infected with virulent isolate of *F. solani* f. sp. *phaseoli*, which caused Fusarium root rot on growth characters. Soil and infection were prepared by virulent isolate as mentioned before and addition of treatments in soil infective. Pots (25cm in diameter) were sterilized and preparation of bioagents, organic acids and fungicides as mentioned before. Pots were filled with disinfested soil at the rate of 2.5 kg clay: sand (2:1, v/v). Five healthy common bean, seeds were sown in each pot. Two controls were used; the first control was performed using artificially infested soil with *F. solani* f. sp. *phaseoli* disease control-1 (Rhizobium and artificially infection). The second control using sterile soil without inocula control-2 (without Rhizobium and artificially infection). Three replicates were devoted for each treatment and control and all pots were kept in a greenhouse under natural conditions (day temperature 35°C, night temperature30°C, 16h photo period) and were irrigated directly after transplanting and subsequently as when necessary. Percentage Incidence (PI) and Disease Severity (DS) of Fusarium root rot disease were assessed 21 days after inoculation for each treatment as mentioned before. Representative sample of five plants was taken by random 45 days after sowing (flowering stage), from each experimental plot for measuring the plant growth characters, as follows: Plant height (cm), Number, dry weight of nodules and dry weight of root (determined at 65ºC for 72 hours using the standard methods as illustrated by A.O.A.C, (1990) at flowering stage for natural infection of common beans (cv. Nebraska) under greenhouse conditions.

2.10. Statistical analysis

Data were subjected to statistical analysis of variance. The experimental design (S) of all studies were a completely randomized with three replications, analysis of variance (ANOVA) of the data was performed with the MSTAT-C statistical package (A) micro-computer program for the design, management, and analysis of agronomic research experiments.

3. Results

3.1. Isolation, identification and preparation of the pathogen and bioagents associated with rhizosphere common beans (*Phaseolus vulgaris* L.)

3.1.1. Source of fungal isolate

The data presented in Table (1) indicate that the field observations for the randomly selected common bean fields are located in five fields *i.e.* Salehia (Sharkia Gov.), Wadi-El Mollak (Ismailia Gov.), Seds (Beni suief Gov.), Derwa (Minia Gov.) and Minfaloat (Assiut Gov.). The fungus that causes common beans root rot it has been identified as *F. solani* f. sp. *phaseoli* based on the characteristics described by Booth (1977).

Table 1: Source of fungal isolates

No. isolate	Geographic origin
1	Sharkia (Salehia)
2	Ismailia (Wadi-El Mollak)
3	Beni-suief (Seds)
4	Minia (Derwa)
5	Assiut (Minfaloat)

3.1.2. Pathogenicity test of the causal pathogen *F. solani* f. sp. *phaseoli* in common beans (cv. Nebraska), at the different surveyed governorates during season 2018

Data in Table (2) indicated that all tested isolates proved to be pathogenic for the common beans (cv. Nebraska) and varying degrees of Fusarium root rot symptoms. As well as, results indicate that the highest percentage of disease incidence and severity in common beans root rot occurred in Sharkia Gov. (Salehia) by about (100.00 and 86.67%, respectively), while recorded Assiut Gov. (Minfaloat) by about (26.67and 11.11%, respectively), and the lowest infection rate occurred during the 2018growing season.

Table 2: Pathogenicity test of the causal pathogen *F. solani* f. sp. *phaseoli* in common beans (cv.Nebraska), at the different surveyed governorates during season 2018

No. of fungal isolates	Disease incidence%	Disease severity%
1	100.00	86.67
2	80.00	75.56
3	60.00	51.11
4	46.67	26.67
5	26.67	11.11
Control	0.00	0.00
General mean	52.22	41.85
L.S.D at 0.05%	21.69	14.63

3.1.3. Antagonistic effect of some microorganisms and chemical stimulants on virulent isolates of *F. solani* f. sp. *phaseoli in* (Calculated as percentage of inhibition in mycelial growth) compared with fungicides

Results in Table (3) reveal that effect of application of *T.harzianum*, *T.hamatum*, *B. subtilis*, *P.fluorescence* and *R. leguminosarum* bv. *phaseoli* or Salicylic acid, Ascorbic acid and Boric acid, on inhibition the mycelial growth of the virulent isolate of *F. solani* f. sp. *phaseoli in vitro*. Data show that treatments mentioned appeared to be antagonistic by different degrees compared with control. *T.harzianum* showed strong inhibited the mycelial growth of the pathogenic fungus by about (63.62 %) compared with other treatments and control.

Table 3: Antagonistic effect of some microorganisms and chemical stimulants on virulent isolates of *F. solani* f. sp. *phaseoli in vitro* (Calculated as percentage of inhibition in mycelial growth) compared with fungicides

Treatments	Inhibition zone (cm.)
T. harzianum	63.62
T.hamatum	59.92
P.fluorescence	56.22
B. subtilis	46.96
R. leguminosarum bv. phaseoli	38.33
Salicylic acid	37.11
Ascorbic acid	29.03
Boric acid	25.96
Topsin M 70	18.55
Rizolex 50% WP	11.18
F. *solani f. sp. Phaseoli* (control)	0.00
General mean	35.17
L.S.D at 0.05%	6.91

3.1.4. Effect of some bioagents isolates and chemical stimulants against root rot disease of beans (cv. Nebraska), under greenhouse conditions

Results in Table (4) reveal that application of *T. harzianum*, *T. hamatum*, *B. subtilis* and *P. fluorescence* or *R. leguminosarum* bv. *phaseoli*, Salicylic acid, Ascorbic acid and Boric acid, on controlling common beans (cv. Nebraska), root rot disease, on infection with Fusarium root rot disease comparison fungicides Topsin M70 and Rizolex 50%WP. Previous treatments gave individually showed remarkable different degrees of infection to beans root rot disease, while *T. harazianum* proved more effective in decreasing percentage of disease incidence and severity by about (13.33 and 11.11%, respectively) surpassed the effect of the fungicides compared with the other treatments and control.

Table 4: Effect of some bioagents isolates and chemical stimulants against root rot disease of beans (cv. Nebraska), under greenhouse conditions

Treatments	Disease incidence%	Disease severity%
T. harzianum	13.33	11.11
T. hamatum	26.67	15.55
P. fluorescence	33.33	20.00
B. subtilis	40.00	22.22
R. leguminosarum bv. phaseoli	46.67	26.67
Salicylic acid	53.33	31.11
Ascorbic acid	60.00	33.33
Boric acid	60.00	57.78
Topsin M 70	73.33	66.67
Rizolex 50% WP	80.00	68.89
F. solani f. sp. Phaseoli **(control)**	93.33	86.67
General mean	52.73	40.00
L.S.D at 0.05%	17.75	11.24

3.1.5. Effect of combined treatments between different biocontrol agents, rhizobia and chemical stimulants against root rot disease of beans (cv. Nebraska), under greenhouse conditions

Data presented in Table (5) indicated that application of combined treatments between *R. leguminosarum* bv. *phaseoli* and either *T. harzianum*, *T.hamatum*, *B. subtilis*, *P. fluorescence*, Salicylic acid, Ascorbic acid and Boric acid comparison mixed with fungicides Topsin M70 and Rizolex 50%WP which gave different degrees of infection to bean root rot disease compared with other treatments and control. On the other hand, was proved that, *T. harazianum* + *R. leguminosarum* bv. *phaseoli* gave more effective in decreasing percentage of disease incidence and severity by about (8.89 and 6.67%, respectively) which was significantly higher than any tested treatments.

Table 5: Effect of combined treatments between different biocontrol agents, rhizobia and chemical stimulants against root rot disease of beans (cv. Nebraska), under greenhouse conditions

Treatments	Disease incidence %	Disease severity %
T. harzianum**+ *R. leguminosarum* bv. phaseoli**	8.89	6.67
***T.hamatum* + *R. leguminosarum* bv. phaseoli**	13.33	13.33
P.fluorescence+ R. leguminosarum bv. phaseoli	26.67	15.55
B. subtilis+ R. leguminosarum bv. phaseoli	33.33	20.00
Salicylic acid+ *R. leguminosarum* bv. *phaseoli*	40.00	24.45
Ascorbic acid+ *R. leguminosarum* bv. *phaseoli*	46.67	28.89
Boric acid+ *R. leguminosarum* bv. *phaseoli*	53.33	35.55
Control-1 (Rhizobium and artificially infection	60.00	53.33
Topsin M 70+ *R. leguminosarum* bv. *phaseoli*	66.67	62.22
Rizolex 50% WP+ R. leguminosarum bv. phaseoli	86.67	68.89
Control-2 (without Rhizobium and artificially infection)	00.00	00.00
General mean	39.60	29.90
L.S.D at 0.05%	22.22	8.01

3.6. Effect of individual and combined treatments between different biocontrol agents, rhizobia and chemical stimulants on growth characters of common beans (cv. Nebraska), plants under greenhouse conditions

Data presented in Table (6 and 7) indicated that application of previous treatments, whether individually or mixed led to improvement and increase growth parameters of common beans (cv. Nebraska) plants, remarkable different degrees, while *T. harazianum* mixed with rhizobia proved more effective in increasing growth parameters of common beans (cv. Nebraska) plants, was better than other treatments and control.

Table 6: Effect of biological control, rhizobia and chemical stimulants on growth characters of common beans (cv. Nebraska), plants under greenhouse conditions

Treatments	Plant height (cm)	Number of nodules /plant	Dry weight of nodules /plant (g)	Dry weight of root (g)	Dry weight of shoot (g)	Nitrogen (N) content
T. harzianum	25	15.33	0.015	1.68	2.48	2.14
T. hamatum	23.67	13.67	0.014	1.64	2.53	1.84
P. fluorescence	18.67	11	0.014	1.42	2.40	1.85
B. subtilis	19.00	11.33	0.015	1.66	2.12	1.78
Control-1 (Rhizobium and artificially infection)	19.67	18.33	0.016	1.94	2.82	2.30
Salicylic acid	15.67	10	0.010	1.27	1.69	1.47
Ascorbic acid	14.67	9.67	0.010	1.18	1.65	1.45
Boric acid	16.33	11	0.012	1.26	1.66	1.47
Topsin M 70	18.67	11.33	0.015	1.73	2.08	1.88
Rizolex 50% WP	13.33	9.67	0.007	1.18	1.88	1.50
Control-2 (without Rhizobium and artificially infection)	9.00	5.33	0.004	0.35	1.33	1.13
General mean	17.61	11.51	0.012	1.39	2.06	1.71
L.S.D at 0.05%	4.62	3.67	0.005	0.42	0.21	0.39

Table 7: Effect of combined treatments between different biocontrol agents, rhizobia and chemical stimulants on growth characters of common beans (cv. Nebraska), plants under greenhouse conditions

Treatments	Plant height (cm)	Number of nodules /plant	Dry weight of nodules/plant (g)	Dry weight of root (g)	Dry weight of shoot (g)	Nitrogen (N) content
T. harzianum* + *R. leguminosarum* bv. *phaseoli	34	22.33	0.028	2.473	4.20	3.18
T. hamatum* + *R. leguminosarum* bv. *phaseoli	33.67	20.33	0.024	2.31	4.16	2.90
P. fluorescence* + *R. leguminosarum* bv. *phaseoli	31.33	17	0.021	2.097	3.73	2.56
B. subtilis*+ *R. leguminosarum* bv. *phaseoli	26	15.33	0.019	1.887	3.42	2.43
Control-1 (Rhizobium and artificially infection)	25	14	0.023	1.897	3.59	2.91
Salicylic acid + *R. leguminosarum* bv. *phaseoli*	20.33	12.33	0.016	1.567	2.74	1.99
Ascorbic acid + *R. leguminosarum* bv. *phaseoli*	19.33	12	0.014	1.207	2.56	1.77
Boric acid + *R. leguminosarum* bv. *phaseoli*	22	12.33	0.014	1.233	2.30	1.83
Topsin M 70+ *R. leguminosarum* bv. *phaseoli*	23	11	0.015	1.643	2.78	2.20
Rizolex 50% WP + *R. leguminosarum* bv. *phaseoli*	16.33	11	0.009	1.21	2.28	1.90
Control-2 (without Rhizobium and artificially infection)	12.33	7.67	0.006	0.733	1.45	1.47
General mean	23.94	14.12	0.017	3.68	3.019	2.29
L.S.D at 0.05%	5.11	4.45	0.004	0.34	0.69	0.43

On the other hand, treatment with *R. leguminosarum* bv. *phaseoli* + *T. harzianum*, gave higher records *i.e.* Plant height (25 and 34 cm), Number of nodules/plant (15.33 and 22.33), Dry weight of nodules/plant (0.015 and 0.028 g), Dry weight of root (1.68 and 2.47 g), Dry weight of shoot 2.48 and 4.20 g) and nitrogen content (N) 2.14 and 3.18, respectively.

4. Discussion

F. solani was isolated originally from naturally diseased beans plant exhibiting topical symptoms of root rot disease according to Booth (1977). Root rots disease in common beans plants is caused by (*Fusarium solani* {Mart.} Sacc. f. sp. *phaseoli* {Burkholder} W. C. Snyder & N.H. Hans (Fsp) (Filion, *et al*., 2003). Root rot of bean and Pre or Post emergence damping-off diseases are caused by single or combination of soil borne fungi of which, *Fusarium solani* Mart. Sacc, *F. oxysporium*, *Rhizoctonia solani* Kuhn, *Sclerotium rolfsii* Sacc as mentioned by both Lewis and Lumsden 2001; Nayaka, *et al*., 2008 and Begum, 2010. *Fusarium solani* is another phytopathogen that causes yield loss in important crops, including olive trees, pen, sweet potato, cucurbits, soybean and common beans (Filion, *et al*., 2003; Gao, *et al*., 2004; Zhang, *et al*., 2006 and Amira, *et al*., 2017).

In present studies, pure cultures of isolated fungi, which belong to the genus *Trichoderma*, were obtained by using hyphal tip isolation technique described by Brown (1924). Coating seeds of many crops with biocontrol agents such as *Trichoderma* spp., *Bacillus subtilus* and *Pseudomonas fluorescence* was the most effective treatments for controlling of seed and root rot pathogens this is consistent with (El-Mohamedy and Abdel-Baky, 2008 and Begum *et al*., 2010).While, others reported that rhizobia able to inhibit significantly growth of pathogenic fungi (Bardin *et al*., 2004; El-Batanony *et al.,* 2007 and Mazen *et al.,* 2008). The effect inhibitory of biocontrol agents against soil-borne fungal pathogens could be explained through direct effect caused by mycoparasitism, production of antibiotics, hydrogen cyanide and siderophores, or indirect effect caused by the induction of plant defense mechanisms, which reduces their susceptibility (Das *et al.,* 2017).

Application of *T.harzianum*, *T.hamatum*, *B. subtilis* and *P. fluorescence* or *R. leguminosarum* bv. *phaseoli*, Salicylic acid, Ascorbic acid and Boric acid, whether individually or mixed led to improvement and increase growth parameters of beans which was significantly higher than any tested treatments are in agreement with the findings of Raschel and Reuszer (1973) who found that addition of nitrogen (Rico23 nutrient solution) at beans increased plant dry weight, percentage and total nitrogen content in plant tops but decreased nodule weight. As well as, Nambiar and Dart (1982) who mentioned that there is an increase in yield at range of 2.8 to 40 percent (60 to 1000 kg pods/ha) on rhizobial inoculation over the control. Furthermore, Poi and Kabi (1983) who found that inoculation significantly increased fresh weight and N2 content of pot grown plants. *R. leguminosarum* bv. *phaseoli* the effectiveness of this strain in improving growth parameters plants such as dry shoot, root weights and the number of pods per plant has recently been reported (Osdaghi *et al*., 2009) and according to (Hungria *et al*., 2015 and Kumara *et al*., 2016) who found that the inoculation of green bean seeds with effective rhizobial strains significantly stimulated. There are several reports on the application of effective rhizobia on beans improved growth and caused yield enhancement in different climatic conditions and soil.

In other studies, the nodules produced by one strain of *Rhizobium* demonstrated high nitrogenous activity even under N treatment condition according to Alagawadi *et al.,* (1993) who found that inoculation of groundnut seeds with *Rhizobium* significantly increased yield over that of the uninoculated control. Inoculation increased nodule number, dry weight of nodules/plant and N content of plants at 120 days after sowing, while an application on a plot with uninoculated plants reduced nodule number and dry weight compared with the uninoculated control these findings are in agreement with the findings of de Jensen *et al.,* (2004) showed that co-inoculation of *Rhizobium tropici* and *Bacillus subtilis* inhibits *Fusarium* sp development, as well as improving shoot dry weight and yield of common bean (CB) plants. Furthermore, N% was higher in plants inoculated with the tested rhizobia. Plants dry matter affected positively with the increasing N levels. Many researchers reported an increase in shoot N of legume plants inoculated with rhizobial strains (Fengxian *et al.,* 2009; Yadav and Verma, 2014; El-lithy *et al*., 2014).

Also, Shahda (2000) mentioned that using benzoic acid, salicylic acid and ascorbic acid significantly reduced disease incidence ranged from 36-74% and exhibited growth promoting effect. Soaking sesame seeds in salicylic acid at rate of 5 mM for 24 h before sowing followed by foliar application after 15 days from sowing gave the best control compared to Benlate against *F. oxysporum* according by (Abdou *et al*., 2001). On the other hand, application of inducer resistance chemicals under field conditions have been proved to increase vegetative growth, yield and quality of many vegetable crops these findings are in agreement with the findings of (Abdel-Mawgoud *et al.*, 2010; Shehata *et al*.,2012; Bakeer, 2014; Abd El-Gawad and Bondok, 2015 and El-Mohamedy *et al*., 2015). Additions

of Salicylic acid, at 200 ppm reduce liner growth of *F. oxysorum*, *F. solani* and *R. solani* from 90 mm in check treatment to 54.93, 48.60 and 65.45 mm, respectively. Results are in agreement with finding of many authors, they mentioned that many chemical plant resistance inducers recorded the highest inhibition of growth of many pathogenic fungi (Abdel-Monaim, 2013 and Bakeer, 2014).

References

Abd El-Gawad, H.G. and A.M. Bondok, 2015. Response of tomato plants to salicylic acid and chitosan under infection with tomato mosaic virus. Am-Euras J. Agric. Environ., Sci., 15(8): 1520-1529.

Abdel-Mawgoud, A.M.R., A.S. Tantawy, M.A. El-Nemr, and Y.N. Sassine, 2010. Growth and yield responses of strawberry plants to chitosan application. Euro. J. Sci. Res., 39(1): 170 177.

Abdel-Monaim, M.F., 2013. Improvement of bio control of damping-off and root rot/wilt of faba bean by salicylic acid and hydrogen peroxide. Plant Path Res Inst. Agric Res Center. Giza Egypt, 41(1): 47-55.

Abdou, El. S., H.M. Abd-Alla, and A.A. Galal, 2001. Survey of sesame root rot/wilt disease in Minia and their possible control by ascorbic and salicylic acids. Assuit J. Agric. Sci., 32(3): 135-152.

Alagawadi, A.R., T.K. Siddaramegowda, and A.F. Habib, 1993. Groundnut crop response to *Rhizobium* inoculation. Madras Agril. J., 70(10): 635-637.

Amira, M.B., D. Lopez, A.T. Mohamed, and *et al.,* 2017. Beneficial effect of *Trichoderma harzianum* strain Ths97 in biocontrolling *Fusarium solani* causal agent of root rot disease in olive trees. Biological Control, 110:70-78.

Aneja, K.R., 1993. Experiments in Microbiology, Plant Pathology and Tissue Culture. Wishwa Prakashan (Wiley Eastern Ltd.), New Delhi, 1-141.

A.O.A.C., 1990. Official Methods of Analysis of Association of Official Agricultural Chemists, 15th: 1045-1106.

Atlas, R.M., 1995. Handbook of Media for Environment Microbiology .CRC. Press, Boca Raton, F1.

Bakeer, A.T., 2014. Effect of combination between inducer resistance chemical and systemic fungicides on management of sweet melon downy mildew. Arch Phytopathology Plant Prot., 47(14): 1716- 1725.

Bardin, S.D., H.C. Huang, J. Pinto, E.J. Amundsen, and R.S. Erickson, 2004. Biological control of *Pythium* damping-off of pea and sugar beet by *Rhizobium leguminous crumb. vice*. Can. J. Bot., 82:291-296.

Baudoin, J.P., F. Camarena, M. Lobo, and G. Mergeai, 2001. Breeding *Phaseolus* for intercrop combinations in Andean highlands. *In:* Cooper H.D., Spillane C. & Hodgkin T., eds Broadening the genetic bases of crop. Oxford, UK: CABI Publishing, 373-384.

Begum, M.M., M. Sariah, A.B. Puteh, and M. Siddiquiy, 2010. Field performance of bio primed seeds to suppress *Colletotrichum truncotum* causing damping off and seedling stand of soybean. Biological Control, 53(1): 18-23.

Booth, C., 1977. Fusarium: Laboratory Guide to the Identification of the Major Species. Kew, UK: Commonwealth Mycological Institute.

Brown, W., 1924. Two mycological methods: II- A method of isolating single strains of fungi bu cutting a hyphal tip. Ann. Bot., 38:404.

Broughton, W.J., G. Hernandez, S. Blair Beebe, P. Gepts, and J. Vander Leyden, 2003. Beans (*Phaseolus* spp.) - model food legumes. Plant and Soil, 252: 55-128.

Burbage, D.A., M. Sasser, and R.D. Lumsden, 1982.A medium selective for *Pseudomonas cepacia* (Abstrac.). Phytopathology, 72:706.

Burke, D.W. and R. Hall, 1991. Compendium of bean diseases. St. Paul, Minnesota, USA, APS Press, 9-10pp.

Change, I.P. and T. Kommedahl, 1968. Biological control of seedling blight of corn by coating kernels with antagonistic microorganisms. Journal of Phytopathology, 58:1395-1401.

Das, K., R. Prasanna, and A.K. Saxena, 2017. Rhizobia: Apotential biocontrol agent for soil-borne fungal pathogens. Folia Microbiologic, 62: 425-435.

de Jensen, C.E., J.E. Kurle, and J.A. Percich, 2004. Integrated management of edaphicand abiotic factors limiting yield of irrigation soubean and dry bean in Minnsota. Field Crops Research, 86:211-224.

Difco Laboratories, 1984. "DIFCO Manual: Dehydrated Culture Media and Reagents for Microbiology," 10th Edition, DIFCO Laboratories Inc., Detroit, Michigan, USA.

Domsch, K.H., W. Games, and A. Traute-eidi, 1980. Compendium of soil fungi. Academic Press A subsidiary of Harcout Brace Jovanovich, Publisher, London, 1:859

El-Batanony, N.H., O.N. Massoud, M.M. Mazen, and M.M. Abd El-monium, 2007.The inhibitory effects of cultural filtrates of some wild *Rhizobium spp*. on some *faba bean* root rot pathogens and their antimicrobial synergetic effect when combined with *Arbuscular mycorrhiza* (Am). World J. of Agricultural Sciences, 3: 721-730.

El-Batanony, N.H., A.M. Bdwy, H.E. Hassan and M.E. El-Lithy, 2015. Biodiversity of Rhizobia that nodulate *Melilotus indicus* (L.)ALL in Egyptian Soils. Egypt. J. Microbiol., 50:101-117.

El-Lithy, M.E., N.H. El-Batanony, S. Moreno and E.J. Bedmar, 2014. A selected rhizobial strain isolated from wild-grown *Medicago mono speliaca* improves the productivity of non- specific host *Trifolium alexandrinum.* Applied Soil Ecology, 73: 134-139.

El-Mohamedy, R.S.R. and M.M.H. Abd-El-Baky, 2008. Effect of seed treatment on control of root rots disease and improvement growth and yield of pea plants. Middle Eastern and Russian Journal of Plant Science and Biotechnology, 2(1-2): 84-90.

El-Mohamedy, R.S.R. and M.A. Ahmed, 2009. Effect of biofertilizers and humic acid on control of dry root rots disease and improvement yield quality of mandarin (*Citrus reticulate* Blanco). Res J Agric Biol Sci., 5(2): 127-137.

El-Mohamedy, R.S.R., M.R. Shafeek, A.M. Shaheen, E.H. Abd El-Samad, Fatma A. Rizk and A.A. Morsey, 2015. Integration between seed bio priming and potassium salts treatments to control of root rot disease and improvement growth and yield of green bean plants. Curr. Sci. Int., 4(2): 155-163.

El-Mougy, Nehal S., 2001. Field application of certain biological and chemical approaches on controlling bean wilt disease. Egypt. J. Phytopathology, 29 (1): 69-78.

El-Mougy, N.S., Nadia, G. and M.M. Abdel-Kader, 2007. Control of wilt and root rot incidence in *Phaseolus vulgaris* L. by some plant volatile compounds. J. Plant Prot. Res., 47(3):255-265.

Fengxian, C., C. GuiQin, W. Xiong, Z. Jing, Y. Xiao Long and L. Hong, 2009. Isolation and application of effective nitrogen fixation rhizobial strains on low-phosphorus acid soils in South China. Chin. Sci. Bull., 54:412-420.

Filion, M., M. St-Arnaud, and S.H. Jabaji-Hare, 2003. Quantification of *Fusarium solani* f. sp. *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using real-time polymerase chain reaction and direct isolations on selective media. Phytopathology, 93:229-235.

Frolich, G., 1979. Phytopathology und pflanzenschutz. VEB. Gustav Fisher Veriag. Jena, 295pp.

Gao, X., T.A. Jackson, K.N. Lambert, S. Li, G.L. Hartman, and T.L. Niblack, 2004. Detection and quantification of *Fusarium solani* f. sp. *glycines* in soybean roots with real-time quantitative polymerase chain reaction. Plant Disease, 88, 2.

Hilal, A.A., Nada, MGA and M.H. Zaky, 2006. Induced resistance against *Sclerotinia sclertiorum* disease in some Umbelliferous medicinal plants as a possible and effective control mean. Egypt J Phytopathology, 34:85-101.

Hungria, M., M. Antonio, and R.S. Nogueira Araujo, 2015. Soybean seed co-inoculation with Brady *rhizobium japonicum*. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. Amer. J. Plant Sci., 6:811-817.

Jackson, M.L., 1958. Soil Chemical Analysis. Prentice-Hall, Inc., USA.

Kumara, P., P.R.C. Pandey, and D.K. Maheshwari, 2016. Bacteria consortium optimization improves nutrient uptake, nodulation, disease suppression and growth of the common bean (*Phaseolus vulgaris*) in both pot and field studies. Rhizosphere, 2: 13-23.

Lewis, J.A. and R.D. Lumsden, 2001. Biocontrol of damping-off of greenhouse grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp. Crop Prot., 20: 49-56.

Long, S.R., 2001. Genes and signals in the *Rhizobium*-legume symbiosis. Plant Physiology*,* 125, 69-72.

Mazen, M.M., N.H. EL-Batanony, M.M. Abd El-Monium, and O.N. Massoud, 2008. The cultural filtrate of *Rhizobium* spp. and *Arbuscular mycorrhiza* is potential biological control agents against root rot fungal diseases of faba bean. Global J. of Biotechnology and Biochemistry, 3:32-41.

Mishra, R.R., 1996. Microorganisms associated with plant roots. In: Soil Microbiology. CBS Publishers and Distributors, New Delhi, 52-82.

Mortier, V., M. Holsters, and S. Goormachtig, 2012. Never too many? How legumes control nodule numbers. Plant, Cell &Environment, 35: 245-258.

Nambiar, P.T.C. and P.J. Dart, 1982. Response of groundnut (*Arachis hypogea* L.) to Rhizobium inoculation. Groundnut Microbiology Technical Report 1, ICRISAT, Patancheru, Hyderabad.

Nayaka, S.C., S.R. Nironjana, A.C. Shankar, and C.N. Mortensen, 2008. Seed bio-priming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisin in maize. Archives of phytopathology and plant protection, 1-19.

Osdaghi, E., A. Alizadeh, M. Shams-bakhsh, and M.R. Lak, 2009. Effect of various fertilizer treatments on growth and yield parameters of common bean. In: Abstract Book of 6th International Congress of Horticulture, 14 July, 2009, Rasht, Iran, 22.

Paul, E.A. and F.E. Clark, 1989. Soil Microbiology and Biochemistry. Academic Press, San Diego, CA, USA, 340 pp.

Poi, S.C. and M.C. Kabi, 1983. Tropical grain legume bulletin No. 28:20-22.

Rao, M.S.L., Y.H. Kulkarmi, S. Lingaraju, and H.L. Nadaf, 2009. Bio priming of seeds: Potential tool in the integrated management of *Alternaria* blight of sunflower .HELTA 32(50): 107-114.

Raschel, A.P. and H.W. Reuszer, 1973. Factor affecting *Rhizobium phaseoli-Phaseolus vulgaris* symbiosis. Pesquisa Agrpecuaria Brasileira Agronomia, 8(8): 287-292.

Saleh, O.I., 1997. Wilt, root rot and seed diseases of groundnut in El-Minia Governorate, Egypt. Egypt Journal of Phytopathology, 25:1-18.

Shahda, W.T., 2000. The use of antioxidants for control of tomato damping-off. Alexandria J Agric Res., 45(1): 307-316.

Shehata, S.A., Z.F. Fawzy, and H.R. El-Ramady, 2012. Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. Aust J. Basic Appl Sci., 6(4): 63-71.

Schmidt, E.L. and A.C. Caldwell, 1967. A practical manual of Soil Microbiology Laboratory Methods. Food and Agric. Organization of the United Nations Soils Bull., 72-75.

Somasegaran, P. and H.J. Hoben, 1994. Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Springer, New York.

Vincent, J.M., 1970. A Manual for the Practical Study of the Root Nodule Bacteria. IBP Handb 15. Blackwell Scientific Publications. Oxford and Edinburgh. U. K.

Vital, D.A.L., E.G. Mejía, V.P. Dia, and G. Loarca-Piña, 2014. Peptides in common bean fractions inhibit human colorectal cancer cells. Food Chemistry, 157: 347-355.

Yadav, A.J. and J.P. Verma, 2014. Effect of seed inoculation with indigenous Rhizobium and plant growth promoting rhizobacteria on nutrients uptake and yields of chickpea (*Cicer rietinum* L.).European Journal of Soil Biology, 63: 70-77.

Zhang, N., K.O. Donnell, D.A. Sutton, F.A. Nalim, R.C. Summerbell, A.A. Padhye, and D.M. Geiser, 2006. Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. Journal of Clinical Microbiology, 44: 2186-2190.