

Full Length Research Paper

Determination of mycoflora of pea (*Pisum sativum*) seeds and the effects of *Rhizobium leguminosorum* on fungal pathogens of peas

Hulya Ozgonen* and Merve Gulcu

Department of Plant Protection, Agricultural Faculty, Suleyman Demirel University, 32260, Isparta, Turkey.

Accepted 29 April, 2011

Mycoflora of pea seeds and the effectiveness of *Rhizobium leguminosorum* on important seed-borne pathogens were determined. Mycoflora were changed according to seed groups with or without surface sterilization. The most common isolated fungi were *Fusarium* spp., *Alternaria* spp., *Macrophomina phaseolina*, *Phytophthora megasperma*, *Rhizoctonia solani* and *Sclerotium rolfsii*. *R. leguminosorum* showed antagonistic effect on *Fusarium oxysporum* and *P. megasperma* by 66.3 and 62.1%, respectively, in dual culture test *in vitro*. *R. leguminosorum* application reduced disease severity of *P. megasperma*, *R. solani*, *F. oxysporum* and *S. rolfsii* by 52.9, 50, 67.9 and 52%, respectively. Results indicated that *R. leguminosorum* showed effects on plant pathogenic fungi and could be used as biocontrol agent.

Key words: Seed mycoflora, dual culture, *Rhizobium leguminosorum*, biocontrol pathogenic fungi, disease severity.

INTRODUCTION

Pea (*Pisum sativum*) which is the most widely grown legume in Turkey is consumed when dried, fresh or frozen. Production of peas has been restricted due to various reasons. Seed and soil-borne diseases are considered as important factors for yield loss. Nowadays, different non-chemical approaches are adopted in disease management which has benefits to plant growth by using microorganisms (Dar et al., 1997). Particularly, the establishment of beneficial bacteria on root system has long been of major interest in agricultural production. *Rhizobium* spp. are symbiotic microorganisms that form nodules fixing of free nitrogen of air in roots of legumes and improve soil fertility.

The rhizobial bacteria species including *Rhizobium leguminosorum* is able to maintain long-term viability in natural conditions and can be easily established around the plant roots (Jones et al., 2007). It has been reported that symbiotic relationship enhance plant growth as in the case of inoculation of *Rhizobium* species which affected

seed germination and promoted the plants growth under controlled and field conditions (Pena-Cabrales and Alexander, 1983; Önder and Özkaynak, 1994; Chabot et al., 1996; Noel et al., 1996). Antoun et al. (1998) revealed that *Rhizobium* and *Bradyrhizobium* species had a potential of growth promoting rhizobacteria on even non-legumes, especially radishes. *Rhizobium* species are known to affect the plant growth directly by producing phytohormones like indole acetic acid (Wang et al., 1982; Wiehe et al., 1994). It was also reported that *Rhizobium* species increased tolerance to abiotic stress such as drought (Athar, 1998). In addition, different studies revealed that inoculation with *Rhizobium* species increased plant tolerance to some plant pathogens (Rabie, 1998; Sturz et al., 2000; El-Batanony et al., 2007; Huang and Erickson, 2007; Mazen et al., 2008).

In this context, it is important to control these diseases timely, especially seed-borne ones and it is necessary to develop different control strategies such as supportive and traditional methods. The aim of this study was to determine the seed-borne fungal pathogens in peas and to evaluate the effects of *R. leguminosorum* on some of these seed-borne fungal pathogens in controlled

*Corresponding author. E-mail: hozgonen@gmail.com

conditions.

MATERIALS AND METHODS

Mycoflora isolations were performed using four different groups of seeds. Peas (*P. sativum*) cv Araka was used in all experiments. *R. leguminosarum* was isolated from nodules of bean root according to Mostasso et al. (2002) for effective studies.

Mycoflora isolations and identifications in pea seeds

Potato dextrose agar (PDA) was used for fungi isolations. Four different groups of seeds were divided into two parts and thirty seeds were randomly selected in each. One group of seed was cultured on PDA directly without surface sterilization using 5 seed for one Petri dish with 6 replicates and the other groups were surface sterilized with 1% NaOCl for 5 min. Cultures were incubated for one week at 24°C. Number of fungal colonies were determined and rates of isolation were calculated for each species. Fungal colonies were identified using macroscopic and microscopic criteria (Alexopoulos, 1966; Nelson et al., 1983; Barnett and Hunter, 1998; Simmons, 2008).

Pathogenicity tests

Pea seeds were sown in 15 cm diameter pots containing autoclaved growing medium with a mixture of soil, sand and pumice (1/1/1, v/v/v). Inoculation procedure were performed for *Fusarium* spp. using wheat culture of 5 g, for *Phytophthora megasperma*, zoospore suspension of 10 ml in each pot, for *Rhizoctonia solani*, sand-corn meal medium of 5 g, for *Sclerotium rolfsii* and sclerot directly (50 to 55 mg) for each pot. *Macrophomina phaseolina* was maintained on PDA for 10 days, spore suspension was prepared from formed microsclerote with 10^6 microsclerotes ml⁻¹ and 10 ml suspension was added to each pot. After maintaining on PDA, *Alternaria*, *Aspergillus* and *Penicillium* species were inoculated, preparing spore suspensions with 10^6 spores ml⁻¹ and 10 ml in each pot.

In pathogenicity test, 5 pots were used for fungal agents with 3 seeds in each. Control plants with only the seed planted were included in the test and same maintenance procedures were applied. Pots were placed at 24 ± 2°C with 16 h photoperiod in a growth room. Seed germination rate, dead plants in subsequent periods, non-germinated seeds and necrosis on root and crown were recorded.

Dual culture tests

Dual culture tests consisted of two parts which are reciprocal culturing and volatile compound test. Effect of *R. leguminosarum* *in vitro* were performed on PDA in 9-cm Petri plates by applying a dual culture technique (Sadfi et al., 2001; Fakhrunnisa and Ghaffar, 2006). *R. leguminosarum* were streaked across the centre of the plate, with a second streak made at right angles to the first. Four discs, 5 mm in diameter cut from the edge of a 7 day-old culture of *P. megasperma*, *R. solani*, *Fusarium oxysporum* and *S. rolfsii* were placed at each side of the *R. leguminosarum* and 2.5 cm distance was left between the two microorganisms. Plates were incubated at 25°C for one week. Percent growth inhibition of fungal agents after 7 days was calculated by the formula $(R1 - R2) / R1 \times 100$, where, R1 is the fungal radial growth (mm) in the direction opposite to the antagonist and R2 is the radial growth toward the antagonist (Whipps, 1987). Growth inhibition (GI) was measured on a scale of

0 to 3 (Korsten et al., 1995), where 0 = no growth inhibition, 1 = 1 to 25% growth inhibition, 2 = 26 to 50% growth inhibition and 3 = 51 to 75% growth inhibition.

To determine the effect of volatile compounds, sealed plate method was used (Fernando et al., 2005). *Rhizobium* isolate was maintained in yeast mannitol agar and incubated at 25°C for two days. *P. megasperma*, *R. solani*, *F. oxysporum* and *S. rolfsii* were maintained on PDA in the centre of the plate with a 6 mm disc in 9 cm diameter Petri dishes. Petri dish containing microorganisms were inverted and placed over the rhizobial culture. The two plates were sealed together with parafilm and incubated at 25°C. Petri dishes containing yeast mannitol agar without *Rhizobium* was placed over the PDA medium inoculated with the microorganisms as a control. The experiment was conducted with 5 replications. The radial growth was measured after five days.

Pot experiment

Pea seeds were placed on the two layers of blotting paper moistened with sterile distilled water in Petri dishes and it facilitated germination at 24°C. The mixture of soil : sand : pumice (1:1:1, v:v:v) used as growing medium was autoclaved at 121°C and 1 kPa twice for one hour each time. Germinated seeds were sown in 15 cm diameter pots including growing medium. Pots were placed at 24 ± 2°C with 16 h photoperiod in a growth room.

R. leguminosarum was cultured on yeast mannitol agar (YMA) at 24°C for one week (Ames-Gottfred et al., 1989). Bacterial suspension was prepared using *Rhizobium* cultures and the concentration was adjusted to 10^6 cells ml⁻¹ using a spectrophotometer, and 10 ml suspensions were applied to the pots. *R. leguminosarum* was applied to the soil around the roots immediately after 10 days culture of the plant.

Inoculation procedures following fungal agents

P. megasperma was grown on PDA at 28°C for seven days and placed under fluorescent light for sporulation. Culture plates were incubated in sterile distilled water for 40 min at 4°C then for 30 min at room temperature. Zoospores were collected by filtering the suspension and zoospore concentration was adjusted to 10^6 spores ml⁻¹. A 10 ml suspension was applied to the soil around the root (Sunwoo et al., 1996). Symptoms were evaluated 10 days after inoculation, based on a 0 to 5 scale (Andres et al., 2006) where: 0 = no visible disease symptom, 1 = brownish lesion beginning to appear on stems and approximately 30% of the entire plants were diseased, 2 = 31 to 50% of the entire plants were diseased, 3 = 31 to 50% of the entire plants were diseased, 4 = 71 to 90% of the entire plants were diseased, 5 = plant died.

F. oxysporum was grown at 25°C for 7 days on PDA and for inoculation, wheat culture was prepared. Grains were boiled to soften, and then cooled and 200 g amounts were placed in Erlenmayer and sealed with cotton and aluminium foil. Grains were autoclaved for 30 min. After cooling, the 1 cm diameter 5 mycelial discs of *F. oxysporum* were placed on medium and incubated at 25°C for 15 days. Application of *Fusarium* wheat culture provided enough mycelial development using 5 g in each pot. *F. oxysporum* wilt symptoms was evaluated using 0 to 4 scale according to Kraft and Boge (2001), where: 0 = no disease symptoms, 1 = epinasty, shedding of primer leaves, 2 = wilting at second and third leaf stage, 3 = wilting of entire plant, 4 = dead plant.

R. solani was maintained on PDA at 25°C for 7 days. For inoculation, sand-corn meal medium was used (Porter and Merriman, 1983). Sand-corn meal medium included 237 g of sand, 13 g of corn meal and 50 ml of distilled water in 500 ml Erlenmayer flasks and it was autoclaved. After cooling, five mycelial discs of *R. solani* were placed in the medium and incubated for 3 weeks at

Table 1. Fungal flora of pea seeds without surface sterilization and rate of isolations (%).

Isolated fungi	Isolation rate (%)			
	Group 1	Group 2	Group 3	Group 4
<i>Fusarium</i> spp.	45	30	40	20
<i>Penicillium</i> spp.	10	12	9	19
<i>Aspergillus</i> spp.	14	21	16	16
<i>Rhizopus</i> spp.	1	3	1	2
<i>Alternaria</i> spp.	15	20	18	18
Yeasts colonies	7	9	6	11
Bacteria colonies	8	5	10	14

25°C. Five grams of the medium was applied around roots for each pot. For evaluation of disease symptoms, 0 to 5 scale was used (Mc Coy and Kraft, 1984), where: 0 = no visible symptoms, 1 = <25% disease of entire plant, 2 = 25% diseased plant, 3 = 25 to 50% diseased plant, 4 = 50 to 75% diseased plant, 5 = >75% diseased plant.

S. rolfsii was maintained on PDA which facilitated the formation of sclerot. After 3 weeks, selected sclerots were added to soil (25 to 30) (approximately 50 to 55 mg). For evaluation of disease symptoms, a 0 to 5 scale was used (Lotunde-Dada 1993), where: 0 = no visible symptom, 1 = a small number of leaf wilt symptoms in plants, 2 = slight infection, mycelial mass only on the surface of the soil, 3 = moderate infection, wilting and blight, mycelial mass around stem, 4 = severe infection, advanced wilt, sclerot forming around crown, 5 = dead plant.

The following treatments with four replications and five plant in each treatment were used according to completely randomized block design: *R. leguminosarum* (Rl), *F. oxysporum* (Fo), *P. megasperma* (Pm), *R. solani* (Rs), *S. rolfsii* (Sr), *F. oxysporum* + *R. leguminosarum* (Fo + Rl), *P. megasperma* + *R. leguminosarum* (Pm + Rl), *R. solani* + *R. leguminosarum* (Rs + Rl), *S. rolfsii* + *R. leguminosarum* (Sr + Rl), and control (C).

Diseases index was calculated using scale values. The data were subjected to analysis of variance (F-test). Means were compared using Fisher's LSD test at P = 0.05 (Gomez and Gomez, 1983).

RESULTS

Fungal flora of seeds

Isolations were performed to determine the origin of the fungal flora in pea seeds with or without surface sterilization. Fungal profile and ratios were changed in seeds with or without surface sterilization. The most commonly isolated fungi were *Fusarium* species from seeds placed on PDA directly without surface sterilization for all groups with high ratio (Table 1), followed by *Alternaria* species, the other isolated fungal species belonged to the genus *Penicillium*, *Aspergillus* and *Rhizopus*. Apart from these fungal species, yeast and bacteria colonies were counted and determined for these seeds on the surface microflora.

Different fungal species were obtained from seeds with surface sterilization (Table 2), however, genus *Fusarium*

was the most isolated. The other species isolated include *Phytophthora* sp., *S. rolfsii*, *R. Solani* and *Macrophomina* sp.

Fusarium species were identified as *F. Oxysporum* and *F. solani*. Chromist *Phytophthora* sp obtained from two groups of seeds were identified as *P. megasperma*. Three *Aspergillus* spp. were obtained and determined as *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus ochraceus*. All examined *Alternaria* colonies isolated from the all groups of seeds were identified as *Alternaria alternata*.

Pathogenicity tests

Pathogenicity tests were conducted using pea seeds and evaluating seed germination and symptoms in plant after germination, and results are summarized in Table 3. Ratio of germinated seeds and infected plants were recorded. *F. oxysporum* and *F. solani* influenced the germination of seeds by 46.6 and 33.3% in plants and caused infection in plants by 53.3 and 50%, respectively.

R. solani and *S. rolfsii* also reduced the rate of seed germination by 66.6% and germinated seeds also had infection depending on the disease development. *Alternaria* species were isolated from all seed samples and reduced the seed germination, and caused the disease by 26.6 and 20%, respectively.

Aspergillus spp. and *Penicillium* spp showed signs of infection in pea plants, but a negative impact on seed germination was not observed in these plants. Seeds germination rate was only 20% for both fungal species.

In vitro studies

R. leguminosarum tested were the most effective in dual culture against *F. oxysporum* and *P. megasperma* with 66.3 and 62.1% growth inhibition belonging to scale 3, respectively. The effectiveness was found to be lower than *R. solani* and *S. rolfsii* belonging to scale 2 and 1, respectively (Table 4). In volatile compound test, *R.*

Table 2. Fungal flora of pea seeds with surface sterilization and rate of isolations (%).

Isolated fungi	Isolation rate (%)			
	Group 1	Group 2	Group 3	Group 4
<i>Fusarium</i> spp.	65	67	60	45
<i>Phytophthora</i> sp	7	-	5	-
<i>Sclerotium rolfsii</i>	5	2	-	-
<i>Rhizoctonia solani</i>	5	7	10	8
<i>Alternaria</i> spp.	5	5	8	3
<i>Macrophomina phaseolina</i>	-	5	-	7
<i>Aspergillus</i> spp.	3	6	5	10
Others	10	8	12	27

Table 3. Summary of fungal inoculation test.

Inoculated fungi	Seed germination rate (%)*	Infected plant (%)	Symptom
<i>Fusarium oxysporum</i>	46.6	53.3	Dead plants
<i>F. solani</i>	33.3	50	Yellowing and wilting
<i>Phytophthora megasperma</i>	33.3	66.6	Yellowing and wilting
<i>Rhizoctonia solani</i>	66.6	33.3	Dead plants
<i>Sclerotium rolfsii</i>	66.6	33.3	Dead plants
<i>Machrophomina phaseolina</i>	33.3	26.6	Necrosis on root and stem
<i>Alternaria alternata</i>	26.6	20	
<i>Aspergillus</i> spp.	20	-	-
<i>Penicillium</i> spp.	20	-	-
Control (-)	10	-	-

*The seed germination rate were evaluated using 15 seeds and ratio of infected plants were determined after germination of seeds.

Table 4. Antagonistic effect of *R. leguminosarum* on fungal pathogens in dual culture.

Fungi	Growth inhibition (%)	GI value	Effect of volatile compound (%)
Pm	62.1	3	44.4
Rs	57.4	2	40.9
Fo	66.3	3	63.2
Sr	42.5	1	43.75

Pm, *P. Megasperma*; Rs, *R. Solani*; Fo, *F. Oxysporum*; Sr, *S. Rolfsii*.

leguminosarum showed maximum effect on *F. oxysporum* by 63.2%. The effects on the other microorganisms changed between 40.9 and 44.4%.

Determination of the effects of *R. leguminosarum* on fungal pathogen under pot conditions

The effects of *R. leguminosarum* on fungal pathogens are summarized in Table 5. The disease severity of *F. oxysporum* and *P. megasperma* inoculated plants were 70 and 68%, respectively. For Rhizobium treated plants, the disease severity was lower with 32 and 22.5%, respectively. The disease severity of *R. solani* and *S. rolfsii* were 60 and 50%, respectively, while in Rhizobium treated plants, the disease severity decreased to 30 and

24%.

DISCUSSION

Fusarium species were found to be importantly obtained from seeds cultured on PDA directly and with surface sterilisation in isolations. Saprophyte flora were also obtained from the surface of seeds. More pathogenic fungi were isolated from the seeds with surface sterilization and they include, *R. solani*, *S. rolfsii* and a chromist *P. megasperma* in addition to *Fusarium* species. In pathogenicity test, *A. alternata*, *Aspergillus* spp. and *Penicillium* spp. were non-pathogenic, whereas *F. oxysporum* and *M. phaseolina* were pathogenic. *R. solani*, *P. megasperma* and *S. rolfsii* showed typical

Table 5. The effects of *R. leguminosarum* on diseases in peas.

Treatment	Disease index	Disease severity (%)	Effect (%)*
Pm	3.4	68	-
Pm + Rl	1.6	32	52.9b
Rs	3.0	60	-
Rs + Rl	1.5	30	50b
Fo	2.8	70	-
Fo + Rl	0.9	22.5	67.9a
Sr	2.5	50	-
Sr + Rl	1.2	24	52b

Pm, *P. Megasperma*; Rs, *R. Solani*; Fo, *F. Oxysporum*; Sr, *S. Rolfsii*.

*Means within column followed by different letters are significantly different (P = 0.05) according to Fisher's LSD test.

symptoms on plants. Esen and Erkilic (2004) determined the fungal flora of first and second crops of soybean, showing that *Fusarium verticilloides* (*Fusarium moniliforme*), *Fusarium avenaceum*, *A. alternata*, *A. niger*, *A. flavus*, *A. ochraceus* and *Penicillium* spp. were obtained by isolation from harvested seeds. According to pathogenicity tests, *Aspergillus* spp. and *Penicillium* spp were not pathogenic, however, seed germinated and plant growth was adversely effected by fungus. Results indicated that *Fusarium* spp. and *M. phaseolina* were the potential pathogens in Çukurova soybean growing areas (Esen and Erkilic, 2004).

In this study, *R. leguminosarum* was the most effective on *F. oxysporum* and *P. Megasperma*, reducing the mycelial growth *in vitro*. Similarly, volatile compounds of *R. leguminosarum* had the same effect on both pathogens. Volatile compound had certain effect against *S. rolfsii* in contrast to lower effect of dual culture. Arfaoui et al. (2006) revealed that some isolates of *Rhizobium* were more effective against *F. oxysporum* f.sp *ciceris* of chickpea with 60% growth inhibition *in vitro*. In another study, *Rhizobium* spp. reduced the mycelial growth of *F. oxysporum* f.sp *phaseoli* *in vitro* (Buonassisi et al., 1986). *Rhizobium meliloti* inhibited growth of *M. phaseolina*, *R. solani* and *F. Solani*, while *Bradyrhizobium japonicum* inhibited *M. phaseolina* and *R. Solani*, producing zones of inhibition *in vitro* (Ehteshamul-Haque and Ghaffar, 2008). *Rhizobium* isolates were effective not only for soil borne pathogen but also, the leaf disease as indicated *in vitro* antibiosis assays that showed that *Rhizobium* strains from chickpea nodules are effective via antagonism against blight disease of chickpea caused by *Ascochyta rabiei* (Küçük and Kivanç, 2008).

In this study, *Rhizobium* application reduced the disease severity of all tested fungi under pot conditions. These results are similar to those obtained by Buonassisi et al. (1986) which indicated that *Rhizobium* spp. reduced the disease severity of *F. oxysporum* f.sp *phaseoli* in bean. *Rhizobium* can be effective when it is not alone and also the increasing effect was obtained when dual inoculation was done with mycorrhizal fungi and other

beneficial microorganisms (Rabie, 1998). Another study was conducted under field conditions, and *R. meliloti*, *R. leguminosarum* and *B. japonicum* were used either as seed dressing or as soil drench to reduce infection of *M. phaseolina*, *R. solani* and *Fusarium* spp., in legumes such as soybean, mungbean and non-legumes such as sunflower and okra (Ehteshamul-Haque and Ghaffar, 2008). In addition, Sheikh et al. (2006) reported that *Bacillus thuringiensis* and *R. meliloti* with and without available nursery fertilizers were used to study their effect on the growth and suppression of soil borne root infecting fungi which include *M. phaseolina*, *R. solani* and *Fusarium* spp., on mungbean and okra plants, and applications gave a significant decrease in infection by root infecting fungi by providing better plant growth. In another study, *R. meliloti* and *R. leguminosarum* had complete control against *Fusarium* wilt in bushbean (Khalequzzaman and Hossain, 2007).

Consequently, *Fusarium* species were isolated predominantly and may also be important in field conditions with *P. megasperma*, *R. solani* and *S. rolfsii* from pea seeds. Existing *R. leguminosarum* isolate was significantly effective against pathogens *in vitro* and in pot conditions.

REFERENCES

- Alexopoulos CJ (1966). Einführung in die Mykologie. Gustav Fischer Verlag. Stuttgart.
- Ames-Gottfred NP, Christie BR, Jordan DC (1989). Use of the Chrome Azurol S Agar Plate Technique To Differentiate Strains and Field Isolates of *Rhizobium leguminosarum* biovar *trifolii*. Appl. Environ. Microbiol., 55(3): 707-710.
- Andres JL, Rivera A, Fernandez J (2006). Virulence of Spanish *Phytophthora nicotiana* isolates towards *Capsicum annuum* germplasm and pathogenicity towards *Lycopersicon esculentum*. Spanish J. Agric. Res., 4(3): 248-254.
- Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalende R (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). Plant Soil., 204: 57-67.
- Arfaoui A, Sifi B, Boudabous A, El Hadrami I, Cherif M (2006). Identification of *Rhizobium* isolates possessing antagonistic activity against *Fusarium oxysporum* f.sp *ciceris*, the causal agent of

- Fusarium* wilt of chickpea. J. Plant Pathol., 88(1): 67-75.
- Athar M (1998). Drought tolerance by lentil rhizobia (*Rhizobium leguminosarum*) arid and semiarid areas of Pakistan. Lett. Appl. Microb., 26: 38-42.
- Barnett HL, Hunter BB (1998). Illustrated Genera of Imperfect Fungi. 4th edition. APS Press.
- Bounnasi AJ, Copeman RJ, Pepin HS, Eaton GW (1986). Effect of *Rhizobium* spp. on *Fusarium oxysporum* f.sp. *phaseoli*. Can. J. Plant Pathol., 8: 140-146.
- Chabot R, Antoun H, Cescas MP (1996). Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. Plant Soil., 184: 311-321.
- Dar GH, Zargar MY, Beigh GM (1997). Biocontrol of rot rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. Microb. Ecol., 34: 74-80.
- Ehteshamul-Haque S, Ghaffar A (2008). Use of Rhizobia in the Control of Root Rot Diseases of Sunflower, Okra, Soybean and Mungbean. J. Phytopathol., 138(2): 157-163
- El-Batanony NH, Massoud ON, Mazen MM, Abd El-Monium MM (2007). The inhibitory effects of culture filtrates of some wild *Rhizobium* spp. on some faba bean root rot pathogens and their antimicrobial synergetic effect when combined with arbuscular mycorrhiza. World J. Agric. Sci., 3(6): 721-730.
- Esen H, Erkilic A (2004). Çukurova'da Soya Ekim Alanlarında ve Hasat Sonrası Tohumlarda Görülen Fungal Etmenlerin Belirlenmesi. Türkiye I. Bitki Koruma Kongresi Bildirileri (194). 8-10 Eylül, Samsun.
- Fakhrunnisa MHH, Ghaffar A (2006). *In vitro* interaction of *Fusarium* spp. with other fungi. Pak. J. Bot., 38(4): 1317-1322.
- Fernando WGD, Ramarathnam R, Krishnamoorthy AS, Savchuk S (2005). Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol. Biochem., 37: 955-964.
- Gomez AK, Gomez AA (1983). Statistical Procedures for Agricultural Research. 2nd Ed. Wiley, New York.
- Huang HC, Erickson RS (2007). Effect of seed treatment with *Rhizobium leguminosarum* on *Pythium* damping-off, seedling, height, root nodulation, root biomass, shoot biomass and seed yield of pea and lentil. J. Phytopathol., 155: 31-37.
- Jones KM, Konayashi H, Davies BW, Taga ME, Walker GC (2007). How rhizobial symbionts invade plants: the *Sinorhizobium* (Indash) Medicago model. Nature Rev. Microbiol., 5: 619-633.
- Khalequzzaman KM, Hossain I (2007). Effect of seed treatment with *Rhizobium* strains and biofertilizers on foot/root rot and yield of bushbean in *Fusarium solani* infested soil. J. Agric. Res., 45(2): 151-160.
- Korsten L, De Jager ES, De Villiers EE, Lourens A, Wehner FC (1995). Evaluation of bacterial epiphytes isolated from avocado leaf and fruit surfaces for biocontrol of avocado postharvest diseases. Plant Dis., 79: 1149-1156.
- Kraft JM, Boge V (2001). Root characteristics in pea relation to compaction and *Fusarium* root rot. Plant. Dis., 85: 936-940.
- Kucuk C, Kivanc M (2008). Preliminary characterization of *Rhizobium* strains isolated from chickpea nodules. Afr. J. Biotechnol., 7(6): 772-775.
- Lotunde-Dada AO (1993). Biological control of southern blight disease of tomato caused by *Sclerotium rolfsii* with simplified mycelial formulations of *Trichoderma koningii*. Plant Pathol., 42: 522-529.
- Mazen MM, El-Batanony, NH, Abd El-Monium MM, Massoud ON (2008). Cultural Filtrate of *Rhizobium* spp. and Arbuscular Mycorrhiza are Potential Biological Control Agents Against Root Rot Fungal Diseases of Faba Bean. Global J Biotech. Biochem., 3 (1): 32-41
- Mc Coy RJ, Kraft JM (1984). Resistance in *Pisum sativum* to epicotyl rot caused by *Rhizoctonia solani*. Plant Dis., 68: 491-493.
- Mostasso L, Mostasso FL, Diasa BG, Vargas MAT, Hungria M (2002). Selection of bean (*Phaseolus vulgaris* L.) rhizobial strains for the Brazilian Cerrados. Field Crops Res., 73(2-3): 121-132.
- Nelson PE, Toussoun TA, Marassa WFO (1983). *Fusarium* species, an illustrated manual for identification. Pennsylvania State University. University Park and London.
- Noel TC, Sheng C, Yost CK, Pharis RP, Hynes MF (1996). *Rhizobium leguminosarum* as a plant growth promoting rhizobacterium: direct growth promoting of canola and lettuce. Can. J. Microbiol., 42: 279-283.
- Onder M, Ozkaynak I (1994). Bakteri aşılama ve azot uygulanmasının bodur kuru fasulye çeşitlerinin tane verimi ve bazı özellikleri üzerine etkileri. Tr. J. Agric. For., 18: 463-471.
- Pena-Cabriaes JJ, Alexander M (1983). Growth of *Rhizobium* in unamended soil. Soil Sci. Soc. Am. J., 47: 81-84.
- Porter IJ, Merriman PR (1983). Effects of soil solarisation of soil on nematode and fungal pathogen and two sites in Victoria. Soil Biol. Biochem., 15: 34-44.
- Rabie GH (1998). Induction of fungal disease resistance in *Vicia faba* by dual inoculation with *Rhizobium leguminosarum* and vesicular-arbuscular mycorrhizal fungi. Mycopathologia., 141: 159-166.
- Sadfi N, Cherif M, Fliss I, Boudabous A, Antoun H (2001). Evaluation of bacterial isolates from salty soils and *Bacillus thuringiensis* strains for the biocontrol of *Fusarium* dry rot of potato tubers. J. Plant Pathol., 83: 101-118.
- Sheikh LI, Dawar S, Zaki MJ, Ghaffar A (2006). Efficacy of *Bacillus thuringiensis* and *Rhizobium meliloti* with nursery fertilisers in the control of root infecting fungi on mung bean and pka plants. Pak. J. Bot., 38(2): 465-473
- Simmons EG, (2008). *Alternaria: An Identification Manual*. ASM Press.
- Sturz AV, Christie BR, Nowk J (2000). Bacterial endophytes potential role in developing sustainable system crop production. Plant. Sci., 19(1): 1-30.
- Sunwoo JY, Lee YK, Hwang BK (1996). Induced resistance against *Phytophthora capsici* in pepper plants in response to DL-β-amino-n-butyric acid. Eur. J. Plant Pathol., 102: 663-670.
- Wang TL, Wood EA, Brewin NJ (1982). Growth regulators, *Rhizobium* and nodulation in peas. Indole-3-acetic acid from the culture medium of nodulating and non-nodulating strains of *R. leguminosarum*. Plant., 155: 343-349.
- Whipps JM (1987). Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. New Phytol., 107: 127-142.
- Wiehe W, Hecht-Buchholz CH, Hoeflich G (1994). Electron microscopic investigations on root colonization of *Lupinus albus* and *Pisum sativum* with two associative plant growth promoting rhizobacteria, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* bv. *trifolii*. Symbiosis., 17: 15-31.