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BIOLOGICAL CONTROL OF *FUSARIUM SOLANI*, THE CAUSAL AGENT OF GRASS ROOT AND CROWN ROT USING GRASS RHIZOBACTERIA

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Abstract

Grass growing areas are spread all over of the world. *Fusarium* species are considered as main pathogenic fungi in the grasslands. The purpose of the present study was to evaluate the antagonistic activity of different bacterial isolates obtained from grasses rhizosphere against *Fusarium solani*, the causal agent of grass root and crown rot disease. *Fusarium solani* was isolated from infected grass plants in Qom province, central Iran. Pathogenicity of the fungal isolates was confirmed on the grass seedlings in greenhouse conditions. Thirty-seven rhizobacterial strains were isolated from healthy grasses in the same areas where fungal pathogen was isolated. Dual culture test was conducted between all the rizobacteria and *Fusarium solani* strains *in vitro*. Among the 37 bacterial strains, two strains including EMJ2 and EMJ7 showed the best antagonistic activity of 98.2% and 46.4% inhibitor, respectively, against fungal strains *in vitro*. Based on the biochemical and phylogenetic analyses, EMJ2 and EMJ7 strains were identified as *Pseudomonas chlororaphis* and *P. putida*, respectively. The later can be considered as biocontrol agents against the grass root and crown rot disease in upcoming studies.

Key words: *Fusarium solani*, Rhizosphere, Grass, *Pseudomonas* spp.

Introduction

Grasslands have extensively developed during the recent decades in Iran. (Kafi and Kaviani, 2001). The narrow-leaf plants that are planted as grass are of millet or grains species and are divided into two groups of cold and warm season grasses. In Iran, Baren Burg class grasses are mainly grown in urban areas and sport complexes (Anonymous, 2004). Due to the high cost of establishment and preservation of grasses, knowledge from the grass diseases and managing them is of high importance. Pathogenic fungi including *Fusarium* sp., *Pythium* sp., *Rhizoctonia* sp. and *Bipolaris* sp. are common constraints on grass plants (Smiley *et al.*, 1992). However, among them, *Fusarium* species are considered the most destructive pathogens as the causal agents leaf spot and blight at the aerial parts and root rot in grass plants (Smiley and Thompson, 1985). *Fusarium* species have also been frequently involved in seedling diseases (Smiley *et al.*, 1992).

Regarding the principles of sustainable agriculture in urban areas, biological control of plant diseases in grassland are of high interest in the recent years. Bacteria in the rhizosphere region have reported to control fungal diseases such as Phytophthora root rot (Muthuraju *et al.*, 2006). Furthermore, *Fusarium solani*, *F. oxysporum* and F. *moniliforme* have successfully controlled using the rhizobacterial strains (Gond *et al.*, 2015 and Kim *et al.*, 2015).

Fusarium species are among soil-borne fungi that can cause diseases in various products. Several attempts have been made to develop sustainable methods based on the biological control of these fungi (Papavizas, 1985). On the other hand, the concerns associated with increased use of pesticides are growing (Curtis, 1995). The use of biological control methods can be an appropriate alternative for the traditional methods of plant disease control (Mercado-Blanco and Lgtenberg, 2014; Hardoim *et al.*, 2008). Bacteria such as *Pseudomonas* spp. and *Bacillus* spp. are among the best soil microorganisms that are considered in the biological control on a wide range of plant diseases (Umashankar *et al.*, 2010). For instance, *F. oxysporum f.* sp. *cubense* and *Fusarium graminearum* were successfully subsided by antagonistic bacteria (Aparna and pious, 2015; Jocham *et al.*, 2006).

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This purpose of the present study was to evaluate rhizobacterial strains isolated from grass rhizosphere to biologically control the grass root and crown rot disease caused by *F. solani*.

Materials and Methods

Sampling, isolation and purification of pathogenic fungi

Surveys were conducted in various grassland areas where suspected to be infected by the crown and root rot pathogen in Qom province, central Iran. Grass roots, crowns and leaves were surface sterilized by dipping into 0.5% sodium hypochlorite for 20 seconds followed by two to three rinses in sterile distilled water (SDW). Small pieces of tissues were cut with a sterile scalpel and spotted on PDA medium Nash-snyder (Burgress *et al.*, 1994). The plates were incubated at 25-27°C for 3-7 days and examined for the growth of fungal mycelia. Pure cultures of fungal mycelia were obtained by serial dilution method. A piece of culture medium along with the fungal mycelia were suspended in 20 ml SDW. The resulted suspension was serially diluted until 10^{-4} and 100 µl of the last dilution was spread on a water agar medium. The grown tips from a single spore were transferred onto a PDA medium and maintained to be used. In order to produce Sprodoxume, CLA medium and agar clove leaves (Fisher *et al.*, 1982) were used.

Identification of fungal pathogen

In order to identify the fungal species, a single-spore pure culture of all isolates was sub-cultured on PDA medium to determine the morphological characteristics, as well as the features of macro conidia and micro conidia. Further morphological characteristics such as the conidiophore length, presence of false heads or chains of micro conidia on monophialides or polyphialides were determined as described in the literature (Leslie, 2006). Eventually, to identify the *Fusarium* species illustrated keys (Summerell Burgress *et al.*, 1994; Nelson *et al.*, 1983) were used.

Pathogenicity test

Pathogenicity test of fungal isolates were conducted on commercial grass seed lot BARENBRUG® (the Netherlands). For this purpose, the seeds were surface sterilized with sodium Hypocrite and washed with sterile distilled water. Seeds were grown in 20cm diameter pots and they were maintained in the greenhouse at ambient conditions (25-28°C, 14 h., natural light). The spore suspension of the representative isolates were prepared from 5 days old culture on PDA medium. Totally ten plants were inoculated with each fungal isolate. The same numbers were considered as control plants and treated in the same manner, except DSW was used instead of the spore suspension. Koch's postulates were accomplished by re-isolating the inoculated fungal isolates on PDA, from the inoculated plants showing symptoms. Confirmation of the re-isolated fungi was made using the morphological method as described above.

Isolation of antagonistic bacteria from the rhizosphere of grass

To isolate appropriate rhizobacteria from grasslands, surveys were conducted on apparently healthy grass plants in Qom province. Samples were collected from rhizosphere, containing the roots and a thin layer of soil. Small pieces of root tissues were cut with a sterile scalpel and macerated in a few drops of SDW using a sterile mortar and pestle. A loopful of the resulting suspension was streaked onto nutrient agar (NA) media as described by Schaad *et al*. (2001). The plates were incubated at 25-27°C for 48-72 h., and examined for the growth of bacterial colonies. Furthermore, for each sample, one gram of rhizosphere soil was rinsed in 10 ml of distilled water and shook for 30 minutes. Different concentrations were made by serial dilution until the dilution of 10^{-5}. Then, 50 µl of dilutions of 10^{-5} and 10^{-6} were streaked on NA medium and incubate as described above. Pure cultures of bacterial strains were obtained by colony sub-culturing. The obtained bacterial strains were re-suspended in SDW and stored at 4°C for further use. For the long-term storage, the strains were maintained in 15% glycerol at -70°C.

Dual culture and antagonistic evaluation of the rhizobacteria

A 1×10^8 CFU/ml bacterial suspension was prepared for all strains to be tested for antagonistic activity. For each of them, a 25 µl of suspension was spotted onto PDA plates in three corners of an imaginary triangle and the plates were incubated for 48 h., at 27°C. After 48 hours, a 5 mm disk of seven days old culture of *F. solani* strains were placed in the center of the Petri dishes. The same procedure was performed in control treatments, but SDW was used instead of rhizobacterial strains.

A clear zone of inhibition around tested fungal mycelia after five days was considered indicative of antagonism. The width of inhibition zones was measured as the distance between the edge of the bacterial colonies and the outer edge of the fungal mycelium. Each treatment was replicated three times.

The percentage of the inhibition of the bacteria from the growth of pathogenic fungi was calculated using the following formula:

Table 1: Physiological and biochemical characteristics of rhizobacterial strains with highest antagonistic activity.

Characteristics	EMJ7	EMJ2
Gram reaction	Negative	Negative
Pigment	-	Orang
Nitrate reduction	-	+
Levan production	+	+
Oxidase	+	+
Arginine dihydrolase	+	+
Pectolytic activity	-	-
HR on Tobacco	-	-
Growth at 41°C	-	-
Growth at 4°C	+	+
Gelatin liquefaction	-	+
Growth at: L-arabinose	+	-
D-galactose	+	+
Trehalose	-	+
Saccharate	+	+
Sorbitol	-	-
Butylamine	+	-
Meso- inositol	-	+
*(+) Reaction Positive, (-) Reaction negative		

$$\text{Percentage of inhibition} = \frac{C - P}{C} \times 100$$

Where,

c = fungal growth in the control treatment (mm)

p = fungal growth in the treatment group (mm)

Phenotypic characterization of the selected rhizobacterial strains

Two representative bacterial strains which showed highest antagonistic activity (Table 1), were subjected to standard biochemical and physiological tests (Schaad *et al*., 2001). Gram reaction, oxidase and catalase activity, aerobic/anaerobic growth (O/F), levan production, growth in NaCl 0.5%, 3% and 7%, growth at pH 4 and 8.5 and colony characteristics on King *et al*., B medium were determined.

Phylogenetic analysis

The 16S rRNA gene was sequenced and phylogenetically compared with those of other rhizobacteria in two representative strains (EMJ2 and EMJ7). For PCR reactions, Universal PCR Kit, Ampliqon® Taq DNA Polymerase Master Mix Red (Ampliqon A/S, Odense, Denmark) was applied according to the manufacturer's recommendations. For each strain, a 20 µl PCR including 50 ng total DNA and 1 µl of each primer (10 pmol×µl^{-1}) (fD1: AGAGTTTGATCCTGGCT CAG, rP2: ACGGCTACCTTGTTACGACTT; Weisburg *et al*., 1991) were used. The certificated PCR products were sent to Bioneer Corporation (http//:www.Bioneer.com) to be sequenced and the resulted sequences were analyzed with the BLAST program (http://blast.ncbi.nlm.nih.gov/). Sequences were aligned with the Clustal W program (Larkin *et al*., 2007) using the MEGA 6.06 software (Tamura *et al*., 2013) and partial sequences were deposited in the NCBI GenBank® and assigned accession numbers. Phylogenetic analysis was performed using the neighbor-joining method using Jukes-Cantor model (Hall, 2011) with MEGA 6.06 software and phylogenetic trees were constructed with bootstrapping (2000 replications) (Tamura *et al*., 2013).

Results

Identification of the fungal isolates

According to the cultural and morphological characteristics observed and compared to the identification keys, three pathogenic isolates were identified as *F. solani*. The most distinct characteristics applied in the identification of species were white sparse mycelia and no pigment production in the agar. Macro conidia were relatively wide, stout, slightly curved with rounded ends and 3-4 transverse walls. Micro conidia were often single cell or two cells (roughly stretched) which are formed on a single false heads on long monophialides recognizable as round transparent circles under microscope. Chlamydospores were visible at short chains.

Pathogenicity test

Grass plants inoculated with fungal isolates obtained from Ghom province initially exhibited chlorosis on the leaves. When the roots and crown of the plants were examined, the roots showed discoloration at the root tips with the reduction in size of the roots when compared to controls plants four weeks post inoculation.

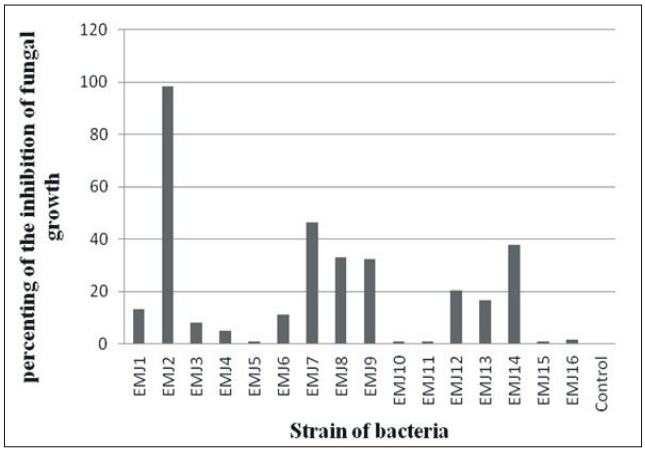

Fig. 1: Antagonistic activity of rhizobacterial strains tested for the inhibition of the growth of *Fusarium solani in vitro*.

Table 2: Duncan grouping of rhizobacterial strains tested for their antagonistic activity against *Fusarium solani*.

Strains of bacteria	Percentage of the inhibition of fungal growth within ten days	Classif-ication
EMJ2	98.2	A
EMJ7	46.4	B
EMJ15	45.3	B
EMJ14	37.8	C
EMJ8	33.2	D
EMJ9	32.3	D
EMJ12	20.3	E
EMJ13	16.5	F
EMJ1	13.2	F-G
EMJ6	11.3	G-H
EMJ3	8.10	H-I
EMJ2	6.4	I
EMJ4	5	I
EMJ16	1.5	J
EMJ11	1	J
EMJ5	1	J
EMJ10	1	J
Control	0	K

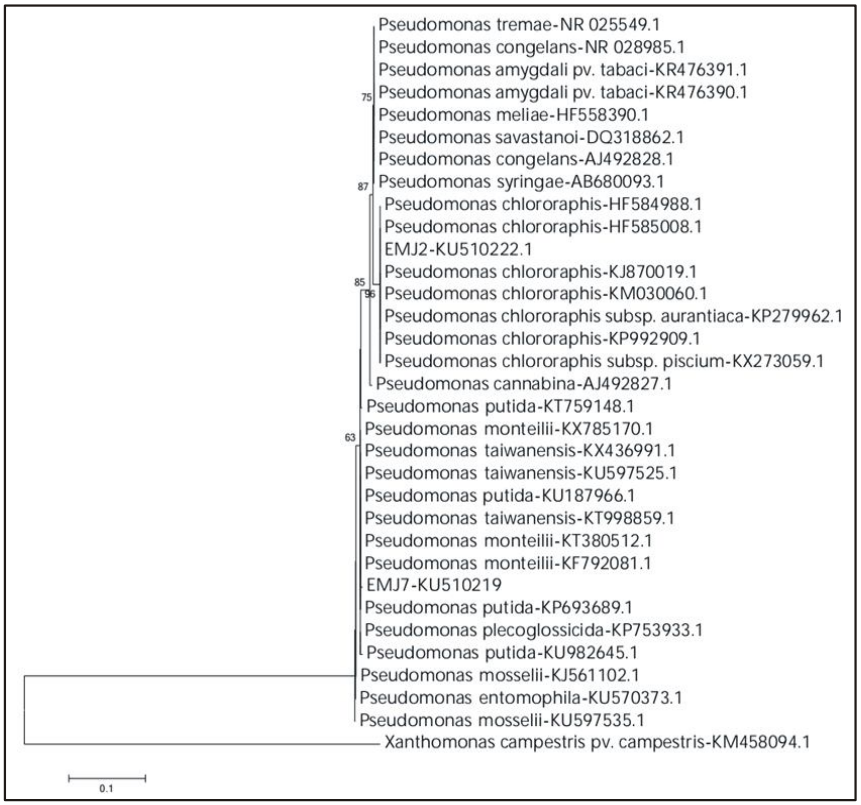

Fig. 2: Phylogeny of 16S rRNA gene of EMJ2 and EMJ7 strains obtained in this study with other species of *Pseudomonas* spp. Neighbor joining method using Jukes-Cantor model was used. Percentage bootstrap values >50% from 2000 samplings were indicated. *Xanthomonas campestris* pv. *campestris* was used as an out-group cluster. Phylogeny of the 16S rRNA gene sequences indicated that the EMJ2 and EMJ7 strains belong to *P. chlororaphis* and *P. putida* species, respectively. Bar 0.1 substitutions per site.

Antagonistic activity

EMJ2 and EMJ7 strains had the best antagonistic activity against *F. solani* on PDA medium with the inhibition of 98.2% and 46.4% respectively. In addition, EMJ5 and EMJ10 strains have showed the lowest antagonistic activity with the percentage of 1%. Table 2 shows the Analysis of Variance (ANOVA) for the antagonistic activity of the tested rhizobacteria. Fig. 1 shows the percentage of the inhibition of the fungi growth by the rhizobacterial strains isolated from the rhizosphere of the grass.

Phenotypic and phylogenetic characterization of antagonistic rhizobacteria

The results of phenotypic tests were summarized in table 1. The results showed that two selected strains including EMJ2 and EMJ7 belong to *Pseudomonas* spp. The 16S rRNA gene was amplified and sequenced in two strains with the most antagonistic activity as described above. Construction of phylogenetic tree with the partial sequences of 16S rRNA gene revealed that EMJ2 and EMJ7 strains belong to *Pseudomonas chlororaphis* and *P. putida*, respectively (Fig. 3), with the homology of 98% with those of standard strains (Fig. 2). Obtained sequences were deposited in the GenBank and assigned accession numbers. 16S rRNA gene for strains EMJ2 and EMJ7 are assigned accession numbers KU510222.1 and KU510219, respectively.

Discussion

In this study, we report high antagonistic activity in two bacterial strains against *F. solani*, the causal agent of grass root and crown rot disease. Among the main constraints of grasslands in Iran, *Fusarium* root and crown rot considered as a restriction for grass cultivation in urban areas in Iran. The results of this study can be considered as a useful biocontrol potential in the upcoming studies.

Plant growth-promoting rhizobacteria (PGPR) live in the rhizosphere, where they occupy approximately 5 to 17% of the total root surface. The most widely studied genera include *Bacillus*, *Pseudomonas*, *Azospirillum* and *Rhizobium*. These microorganisms have beneficial effects on seed

germination, seedling emergence and plant growth (Giassi*et al.,* 2016). A primary mode of suppression of phytopathogens and indirect promotion of plant growth by *Pseudomonas* is production of secondary metabolites such as phenazines, pyrrolnitrin, pyoluteorin, 2,4-diacetylphloroglucinols (DAPG), etc. in the rhizosphere of plants (Gross and Loper, 2009). Phenazines are nitrogen containing heterocyclic antibiotics secreted by *Pseudomonas* spp., possessing broad spectrum activity. Role of phenazines, in biocontrol of a number of phytopathogens, secreted by various species of *Pseudomonas* has been demonstrated in several studies (Jain and Pandey, 2016a).

Pseudomonads are important due to their applications in plant growth improvement by showing antagonism against phytopathogens, induction of systemic resistance, production of various growth promoting compounds and solubilization of inorganic phosphorus (Jain and Pandey, 2016b). The genus *Pseudomonas* sp. is known to comprise a huge diversity of species with the ability to thrive in different habitats, including those considered as agricultural areas and grasslands. Several studies have been conducted to investigate the antagonistic activity of *Pseudomonas* strains against particular plant diseases and several species have been introduced with high biocontrol capability. Among them, *P. chlororaphis* and *P. putida* are the commonly accepted and mostly used species. Recently a psychrotolerant, wide pH tolerant and halotolerant strain of *P. chlororaphis*, isolated from wheat rhizosphere growing in a mountain location in Indian Himalayan Region, has been investigated for its antimicrobial potential with particular reference to phenazine production and plant growth promoting traits. The inhibition of phytopathogens in diffusible biocontrol assays was recorded in an order: *Alternaria alternata* > *Phytophthora* sp. > *Fusarium solani*> *F. oxysporum* (Jain and Pandey, 2016c).

P. chlororaphis produced phenazine, which was active against Gram-positive bacteria and actinomycetes with varying antimicrobial activity with respect to the solvents used. Furthermore, extracts of *P. chlororaphis* sub sp. *aureofaciens* and *P. chlororaphis*, have been reported for antimicrobial activity against Gram-positive bacteria namely *Bacillus subtilis*, *Paracoccus pratrophus* and *Staphylococcus aureus*, respectively (Mezaache-Aichour *et al.,* 2012).

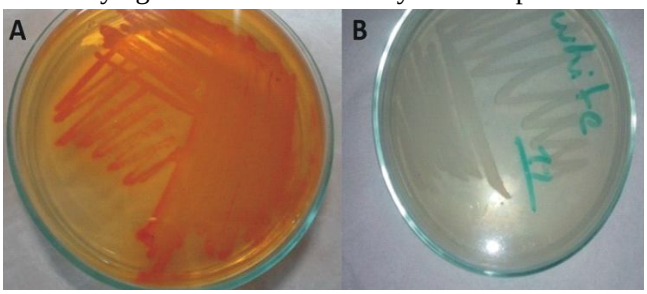

Fig. 3: Colonies of *Pseudomonaschlororaphis* (A) and *P. putida* (B) strains on NA medium identified in this study.

The antagonistic activity of bacterial strains were shown to be more applicable when they used in the rhizosphere of the host plants. In this study, 37 isolates were obtained recovered from rhizosphere of healthy grass plants. Among them, 15 isolates were selected according to their phenotypic characteristics. With increasing demand for biofertilizers and biopesticides, bacterial isolates with wide high antagonistic activity can be referred as potential strains for the management of plant diseases. The *P. chlororaphis* EMJ2 in the present study, possessed beneficial traits related to biocontrol of a destructive plant pathogen *F. solani,* which makes the bacterium a potential agent for field applications especially in urban ecosystem for sustainable and eco-friendly crop production.

References

Amargar, N.,V. Macheret and G. Lagurre (1997). *Rhizobium gallicum* sp. *Nov.* and *Rhizobium giardinii* sp. *Nov* from *Phaseolus vulgaris nodules*. *International Journal of Bacteriology*., **470:** 996-1066.

Anonymous (2004). Principals of Planting Lawn, The Tehran Parks and Green Spaces Organization Publication, 209.

Aparna Ch. S. and Th. Pious (2015). Isolation and Identification of Shoot-Tip Associated Endophytic Bacteria from Banana cv. Grand Naine and Testing for Antagonistic Activity against *Fusarium oxysporum* f. sp. *Cubense*. *American Journal of Plant Sciences*., **6:** 943-954.

Ayers, S.H., P. Rupp and W.T. Johnson (1919). A study of the alkali-forming bacteria in milk. University Seoul Department Agricultural Bulletin. 782.

Barzegar, F. and Z. Banihashemi (2011). diagnosis and pathogenesis of pathogenic fungi of grass in Shiraz green space. *Plant Disease.*, **47:** 361-377

Burgess, L.W., B.A. Summerell, S. Bullock, K.P. Gott and D. Bakhous (1994). Laboratory manual for *Fusarium* research. Fusarium Research Laboratory. Department of Crop Science, University of Sydney and Royal Botanic Gardens, 133.

Curtis, C.R. (1995). The public & pesticides, exploring the interface. NAPIAP, USDA. 96.

Fisher, N.L., L.W. Burgess and P.E. Nelson (1982). Carnation leaves as asubstrate and preserving cultures of *Fusarium* species. *Phytopathology.*, **72:**151-153.

Giassi, V., C. Kiritani and K.C. Kupper (2016). Bacteria as growth-promoting agents for citrus rootstocks. *Microbiological Research.*, **190:** 46-54.

Gond, S.K., M.S. Bergen, M.S. Torres, J.F. White and R.N. Kharwar (2015). Effect of bacterial endophyte on expression of defense genes in Indian popcorn against *Fusarium moniliforme*. *Symbiosis*., **66:** 133-140.

Gross, H and J.E. Loper (2009). Genomics of secondary metabolite production by *Pseudomonas* spp. *Natural Product Reports*., **26:** 1408-1446.

Hall, B.G. (2011). Phylogenetic trees made easy, a how to manual. Fourth edition, Sinaure Associate Inc. publishers. Sunderland, Massachusetts, USA. 282.

Hallmann, J., A. Quadt-Hallmann., W.F. Mahaffee and J.W. Kloepper (1997). Bacterial endophytes in agricultural crops. *Canadian Jornal of Microbiology.*, **43:** 895-914.

Hardoim, P.R., L.S. van Overbeek and J.D.V. Elsas (2008). Properties of Bacterial Endophytes and Their Proposed Role in Plant Growth. *Trends in Microbiology.*, **16:** 463-471.

Jain, R. and A. Pandey (2016). A phenazine-1-carboxylic acid producing polyextremophilic *Pseudomonas chlororaphis* (MCC2693) strain, isolated from mountain ecosystem, possesses biocontrol and plant growth promotion abilities. *Microbiological Research.*, **190:** 63-71.

Jochum, C.C., L.E. Osborne and G.Y. Yuen (2006). Fusarium head blight biological control with *Lysobacter enzymogenes* strain C3. *Journal of Biological Control.*, **39:** 36-344.

Kafi, M and S.H. Kaviani (2001). Turf constraction and maintenance management, Shaghaigh Rousta publication, 230.

Khorshidi Kashani, K., M. Abolfathi and Y. Rezaei Danesh (2009). *Fusarium* root rot and crown of the grass. *Scientific-Research Journal of Protection and Conservation of Forests and Rangelands.*, **11:** 45-31.

Kim, Y.G., H.K. Kang, C.H. Seo, H.B. Lee and Y. Park (2015). Antagonistic activities of novel peptides from Bacillus amylo liquefaciens PT14 against *Fusarium solani* and *F. oxysporum*. *Journal of Agriculture Food Chem.*, **63:** 10380-10387.

Klement, Z., C.L. Farkas and L. Lovrekorich (1964). Hypersensivity reaction induced by phytopathogenic bacteria in the tobaco leaf. *Journal of Phytopathology.*, **54:** 474-47.

Larkin M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wil, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins (2007). Clustal W and Clustal X version 2. *Bioinformatics.*, **23:** 2947-2948.

Leslie, J.F. and B.A.Summerell (2006). The *Fusarium* Laboratory Manual. Black Well, USA. 388.

Lim, H.S., Y.S. Kim and S.D. Kim (1991). *Pseudomonas stutzeri* YPL-1 Genetic Transformation and Antifungal Mechanism against *Fusarium solani*, an Agent of Plant Root Rot. *Appl. Environ. Microbiol.*, **57:** 510-6.

Mercado-Blanco, J. and B.J.J. Lugtenberg (2014). Biotechnological Applications of Bacterial Endophytes. *Current Biotechnology*., **3:** 60-75.

Mezaache-Aichour, S., A. Guechi, J. Nicklin, D. Drider, H. Prevost and R.N. Strange (2012). Isolation, identification and antimicrobial activity of *Pseudomonas* isolated from the rhizosphere of potatoes growing in Algeria. *Journal of Plant Pathology*., **94:** 89-98.

Muthuraju, R., N. Umashankar and K. Nagaraju (2006). Biological control of Rot (*Phytophthora infestans*) on Tomato by Selective Antagonists. *Journal of Soil Biology and Ecology*., **26:** 85-93.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas (1983). *Fusarium* species, An illustrated manual for identification. Pennsylvania State University Press, University Park, 193.

Papavizas, G.C. (1985). *Trichoderma* and *Gloicladium* : Biology, ecology and potential for biocontrol. *Ann. Rev. Phytopathol.*, **23:** 23-54.

Schaad, N.W., J.B. Joens and W. Chun (2001). Laboratory Guide for Identification of Plant Pathogenic Bacteria. A.P.S. USA 373.

Smiley, R.W. and D.C. Thompson (1985). Soil and atomospheric moistures associated with *Fusarium* crown rot and foliar blight of *Poa pratensis*. *Plant Disease*., **69:** 294-297.

Smiley, R.W., P.H. Dernoeden and B.B. Claeke (1992). Compendium of Turf grass Diseases. 2nd ed., APS Press. St. Paul. USA.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution.*, **30:** 2725-2729.

Umashankar, N., A.S. Devakumar, H.R. Reveendra and R. Krishnamurthy (2010). *Environment and Ecology.*, **28:** 1111-1115.

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane (1991). 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*., **173:** 697-703.

Yu., X., C. Ai., L. Xin and G. Zhou (2011). The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. *European Journal of Soil Biology.*, **47:** 138-145