Int. J. Phytopathol. 03 (01) 2014. 49-54

Available Online at ESci Journals
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
http://www.escijournals.net/phytopath

ANTAGONISTIC ACTIVITY OF SOME PLANT GROWTH RHIZOBACTERIA TO FUSARIUM GRAMINEARUM

[a]Mohammed Abdulkareem*, [b]Hadi M. Aboud, [a]Hutham M. Saood, [a]Mohammed K. Shibly
[a] *Pest Plant Control Center, Directorate of Agriculture Research, Ministry of Science and Technology, Iraq.*
[b] *Directorate of Agriculture Research, Ministry of Science and Technology, Iraq.*

A B S T R A C T

Biological control is an efficient and environmentally friendly way to prevent plant disease. *In vitro* technique was used to investigate the ability of *Azospirillum* sp., *Azotobacter chroococcum, Pseudomonas flourscnes, Bacillus subtilis* and *Bacillus pumilus* in order to control *Fusarium graminearum*. Results of dual culture interaction test shows that the colony diameter of *Fusarium graminearum* was significantly reduced (P <0.05) when combined individually with *Azotobacter chroococcum*, *Bacillus pumilus* and *Bacillus subtilis*. However, *Azospirillum* sp. and *Pseudomonas flourscnes* comparing to the control did not affect the growth of the pathogen. Volatile metabolites produced by *Azospirillum* sp. affects *F. graminearum* negatively (P < 0.05) at 3 and 6 days. The culture filtrate of *Azospirillum* sp. and *Bacillus subtilis* at three days measurement reduces the pathogen growth significantly. However, the effect of the former disappeared at 7 day while the latter continued affecting the growth of the pathogen significantly. Present study indicates acceptable levels of antagonistic activity of *Azospirillum* sp., *Azotobacter chroococcum, Bacillus subtilis* and *Bacillus pumilus* against *Fusarium graminearum in vitro* condition.

Keywords: Crown rot, antagonism, *Azospirillum* sp., *Azotobacter chroococcum, Pseudomonas flourscnes, Bacillus subtilis* and *Bacillus pumilus*.

INTRODUCTION

Wheat *(Triticum aestivum* L.) is one of the most important cereal crops in Iraq and worldwide. The one of the main causative fungal diseases of wheat is *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) (Murray and Brennan, 2009). The fungus, in addition to seedling blight and fusarium head blight, causes different seed and soil-borne diseases of seedlings. Moreover, the pathogen also produces mycotoxins including trichothecenes such as deoxynivalenol (DON) which is potent inhibitor of eukaryotic protein biosynthesis and has involved in a number of human and animal mycotoxicoses (Obanora *et al.*, 2013). Therefore, even low levels of these toxins in raw grain can make them unsuitable for human or animal consumption.

Resistant cultivars, chemical pesticides, and crop rotation are the main strategies for controlling the disease. Chemical fungicides are used extensively, but their effectiveness is variable(Ivana *et al.*, 2012). Furthermore, public concern with fungicide residues, as well as pathogen resistance to some pesticides, have increased the concerns of pathologist, ecologist and consumers to find alternative methods for disease management and plant protection(Zamanizadeh *et al.*, 2010). Thus, biological control using antagonistic microbes has become more important in recent years as it is sustainable, safe and environmentally friendly(Dwi *et al.*, 2010). Plant growth promoting rhizobacteria (PGPR) promote plant growth via secreting auxin, gibberellin and cytokinine (Yasar *et al.*, 2010; Yuan *et al.*, 2013). Rhizobacterial organisms also show abilities in reduction soil borne diseases. Several biocontrol bacteria such as *Bacillus subtilis* and *pseudomonas flourscens* have shown to have antagonistic activities against *F. oxysporum* (Sundaramoorthy and Balabaska, 2013). Therefore, the purpose of this study was to evaluate the efficacy of bacteria and their antagonistic ability against F. *graminearum*.

* Corresponding Author:
Email: benmkassy@gmail.com

The objectives were to assess the antifungal activity of the antagonists against pathogenic fungi *in vitro* by means of dual culture, volatiles and non-volatile metabolites. This is the initial step toward the selection of most efficient antagonistic combination *in vivo* under greenhouse and field conditions.

MATERIALS AND METHODS

Fungal and bacterial material: All bacteria *Asospirlium sp, Azotobacter chroococcum, Pseudomonas flourscnes, Bacillus subtilis* and *Bacillus pumilus* were previously maintained on Nutrient Agar (NA) at 4ºC (Huang *et al.*, 2012). The isolates were subcultured on nutrient agar (NA) dishes at 25ºC for 48 h.

Fusarium graminearum was isolated from a field soil cultivated with wheat in the north of Iraq. The culture was grown on potato dextrose agar (PDA) at 25ºC. Preparation of *F. graminearum* inoculum was made as follows: wheat grains were autoclaved (150 g/500 ml Erlenmeyer flask plus 100 ml of distilled water) at 121 ^{0}C for 20 min, and then were inoculated with three 5-mm-diameter PDA plugs excised from an actively growing *F. graminearum* culture. Flasks were then placed in a growth chamber at 25 ºC with 12h photoperiod under fluorescent light to induce sporulation. The cultures were shaken every 2 days for 3 weeks to promote uniform colonization. Inoculum was then air-dried for 7 days at room temperature, milled in a blender. The inoculum contained 3 x10^6 with aid of haemicytometer (Dal Bello *et al.*, 2002).

Dual Culture: The antagonistic activity of all bacteria *Azospirillum* sp.*, Azotobacter chroococcum, Pseudomonas flourscnes, Bacillus subtilis* and *Bacillus pumilus* was studied on *Fusarium graminearum* by dual culture technique (Francisco *et al.*, 2011). On Petri dishes with PDA placing equidistantly a 5 mm-disk of the bacteria and on the other side of the Petri dish, a disk of mycelium of the same diameter of *Fusarium graminearum*. The plates inoculated were incubated at 25 ± 2°C until the growth of control treatment (with only plant pathogen disk), covered the Petri dish. The effect of *bacteria* on plant pathogens was determined by mycelia growth inhibition (Saeideh *et al.*, 2008).

Volatile Metabolites Activity: To determine the effects of volatile metabolites, 5mm-plug of each bacterial isolate was placed on the centre of a Petri dish containing PDA. After incubation for 48h, the lid was replaced by the bottom of a plate containing PDA inoculated with a fresh mycelial plug of the opposed pathogen (Ting *et al.*, 2010). The two plates were sealed together with sticky tape to minimize gas exchange. Controls were prepared in a similar manner but the bottom plate contained PDA without a bacterial plug. All sealed sets of Petri dishes were incubated at 25 ^{o}C colonies was measured at 3, 6, 9 days.

Culture Filtrate Examination: The procedure was as in Perveen K and Bokhari (2012) with modifications. A five mm plug of Bacteria species were inoculated into 100 ml sterilized nutrient ager and incubated at room temperature with 48h shaking at 150 rpm. The culture broth was filtered through filter paper (Whatman no.1) then centrifuged at 6000 rpm for 10 min, and re-filtered through Millipore membrane filter (0.2 µ) to obtain cell-free culture filtrates. Fifteen ml of culture filtrates of each species were mixed with 60 ml of molded PDA, to make the final concentration of culture filtrates 25%. After the agar solidified, mycelial discs of the *F. oxysporum* (5 mm in diameter) obtained from actively growing colonies were placed in the centre of the agar plates. The Petri dishes were incubated at 25°C after 4 days and 7 days that the radial colony growth was measured (Davidson *et al.*, 2012).

Statistical Analysis: The means and standard errors of the data were calculated, and statistically analyzed using the analysis of variance (ANOVA), and Duncan's multiple range tests (P≤0.05) 4 th Genstat. Discovery. The assays of dual culture interaction, volatile metabolites and inhibitory effect of culture filtrate were conducted in 6 replications in complete randomized design.

RESULTS

Dual Culture Examination: The data as in (fig 1) shows that the colony diameter of the pathogen was significantly reduced (P <0.05) when combined with *Azotobacter chroococcum*, *Bacillus pumilus* and *Bacillus subtilis*. However, *Azospirillum* sp. and *Pseudomonas flourscnes* comparing to the control did not affect the growth of *Fusarium*. The antagonism produced by *Azotobacter chroococcum, Bacillus subtilis* and *Bacillus pumilus* was classified as follows: antagonists overgrow two-thirds of the surface of the medium, antagonists and pathogen colonized each half of the surface and nobody seems to dominate the other, and the pathogen colonizes the 2/3 parts of the media surface and resists invasion by antagonists. While the antagonism produced by *Azospirillum* sp. and *Pseudomonas flourscnes* was the plant pathogen overgrows completely to antagonists cover an area total culture media (fig 2).

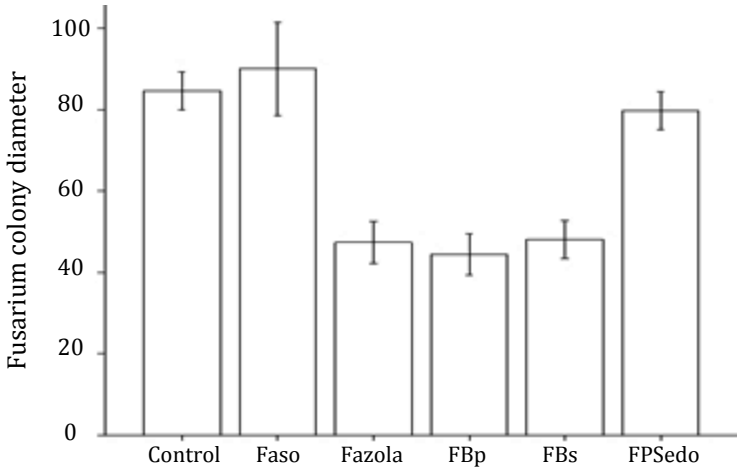

Figure 1. Growth of *Fusarium graminerueam* using dual culture technique. The control was pathogen alone and the treatments were pathogen combined with antagonistic bacteria *Asospirlium sp, Azotobacter chroococcum, Bacillus subtilis, Bacillus pumilus*. and *Pseudomonas flourscnes.* The bars indicates the stander error of means.

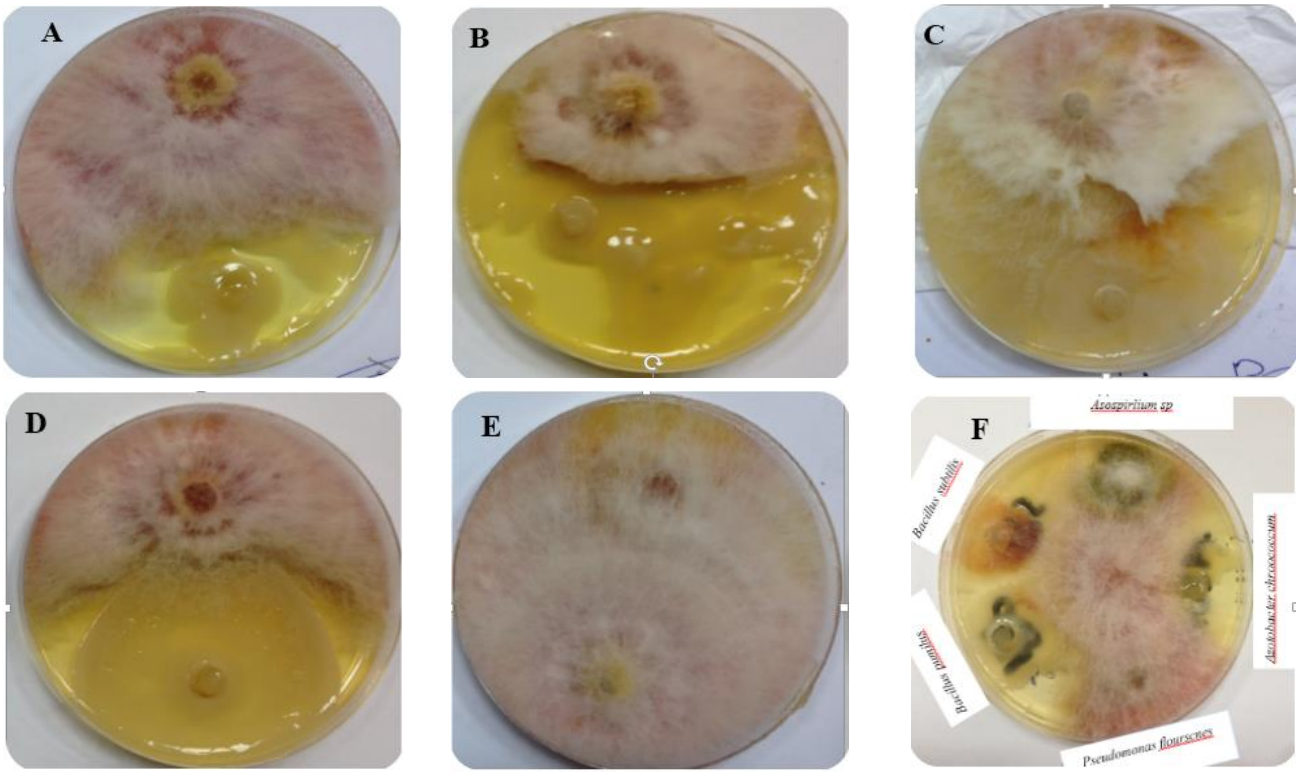

Figure 2. morphology of antagonism produced by, *Azotobacter chroococcum, Bacillus subtilis, Bacillus pumilus* and *Pseudomonas flourscnes* due to dual culture technique with *Fusarium garminearum*. **A**, *Azotobacter chroococcum* occupied 2/3 and **B** 1/3 of the Petri dishes. **C** the occupation 2/3 of the culture by *Bacillus subtilis* and **D** 2/3 of Petri dish by *Bacillus pumilus, **E** Pseudomonas flourscnes overgrown by the pathogen Fusarium garminearum. **F** combination of all bacterial antagonists against Fusarium garminearum. Clockwise from above Asospirlium sp, Azotobacter chroococcum, Pseudomonas flourscnes, Bacillus pumilus* and *Bacillus subtilis.*

Volatile Metabolites Activity: *Azospirillum* sp. affects *Fusarium* negatively ($P < 0.05$) at 3 and 6 days (fig 3). In contrast *Pseudomonas flourscnes* and *basillus subtilis* show no effects on the growth of *fusarium* at either pointed time. *Azotobacter chroococcum* and *Bacillus pumilis* were eliminated due to contamination.

Culture Filtrate Examination: At three days measurement *Azospirillum* sp. and *Bacillus subtilis* affect the pathogen growth significantly. The effect of the former disappeared at 7 day while the latter continued affecting the growth of the pathogen significantly (fig 4 A & B).

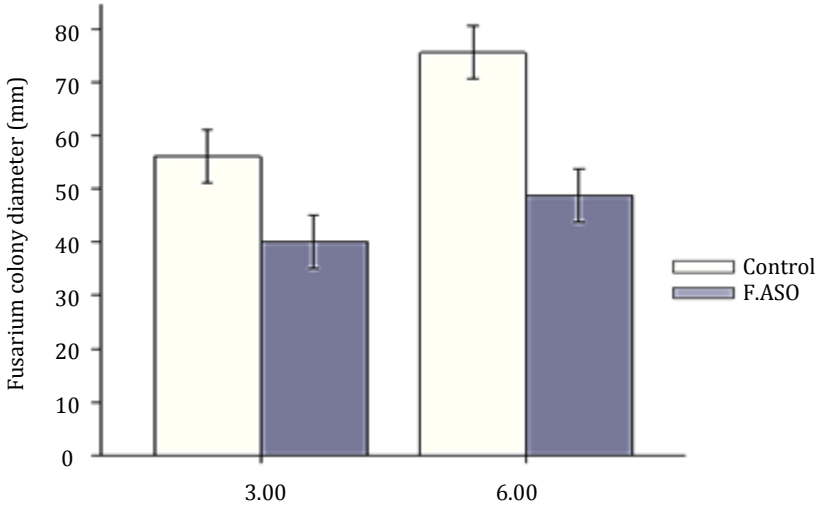

Figure 3. Growth of *Fusarium graminearum* under effects of volatile metabolites of the *Asospirlium sp* (F.ASO). The measurements were taken after 3 and 6 day of inoculation. The bars indicates the stander error of means.

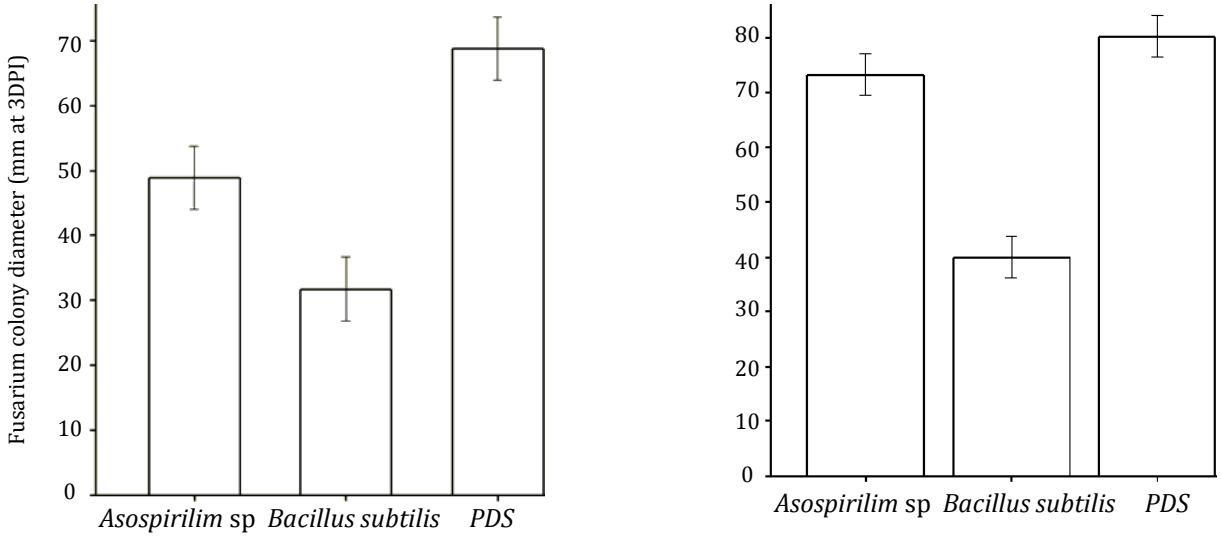

Figure 4. Colony growth of *Fusarium graminearum* on PDA treated by culture filtrate of *Asospirilim* sp. and *Bacillus subtilis,* the control was PDA alone. The bars illustrate stander error of means. The measurement was taken at (A) 3 days after and (B) 7 days after inoculations

DISCUSSION

An alternative promising strategy of chemical pesticides to control plant pests has been the implementation of biological control. Research has repeatedly point out that rhizosphere microorganisms can act as natural antagonists to the wide range of plant pathogens (Al-Mughrabi, 2010; Elshafie *et al.*, 2013). Plant Growth Promoting Rhizobacteria PGPR can act as bioagnets indirectly or directly. Indirectly by promoting growth of infected plants so that the deleterious effects of pathogens maybe prevented or lessen. Directly many researchers have reported that effective colonization by plant growth promoting rhizobacteria (PGPR) contributed to the successful suppression of plant pathogens (Huang *et al.*, 2012).

The use of rapid prescreen techniques allowing testing large number of microorganisms. This has resulted in the widespread use of *in vitro* techniques as the initial stage for potential biocontrol agents. Several reviews indicate the method proved useful for preliminary

selection of biocontol agents (Dal Bello *et al.*, 2002).

The present *in vitro* study shows that *Azotobacter chroococcum* and *Bacillus subtilis* and *Bacillus pumilis* have antagonistic activities against *F. graminearum* by means of dual culture. Furthermore, the culture filtrate of *Bacillus subtilis* and *Azospirillum* sp reduce growth of the pathogen fundamentally that indicating that the secretion of diffusible non-volatile inhibitory substance may involve. Different members of the genus *Azospirillum* secrete siderophores, which shows *in vitro* antifungal activity against *Colletotrichum acutatum* and *in planta* where a reduction of anthracnose symptoms on strawberry plants previously inoculated with *A. brasilense* was evident (María L. Tortora *et al.*, 2011). Evaluation of produced volatile components also shows an appreciable performance on inhibiting mycelia growth of pathogens. The results reported here suggest that *Azospirillum* was more capable of influencing the growth of pathogen in culture filtrate and through production of volatile inhibitors under controlled condition; therefore, it can be used as a biological control agent under field condition.

Bacteria including bacilli has been used for controlling plant pathogens and considered to be PGPR. *Bacillus* sp. produces wide range of metabolites with biological activities, such as antimicrobial, and antiviral activities, that allow the bacterium to stay alive in its natural environment (Sansinenea and Ortiz, 2011). *Bacillus subtilis* is inhibitory to several fungal pathogens and causes extensive lysis of mycelium of *Aspergillus niger*, *Sclerotium rolfsii* and *Bacillus pumilus* is a potent antagonist agains *Rhizoctonia solani* (Harish *et al.*, 1998; Huang *et al.*, 2012). These findings may help explain the role of *Bacillus sp* in the soil ecosystem as biopesticides.

Sundaramoorthy and Balabaskar (2013) showed that *Pseudomonas fluorescens* has an acceptable performance against *Fusarium oxysporum*. However, in the present study *P. fluorescens* shows no effects as on *F. graminearum*. The degree of effectiveness of metabolites may vary according to the nature, quality, quantity of antibiotics secreted by the antagonists(Fekadu and Tesfaye, 2013). In addition, it can be assumed that *F. graminearum* has an acceptable level of tolerance to the substance secreted by *P. fluorescens,* or ability to degrade these substances.

CONCLUSION

Antagonistic interactions show acceptable activity of *Azospirillum* sp*., Azotobacter chroococcum, Bacillus subtilis* and *Bacillus pumilus* against *Fusarium graminearum in vitro* condition. However, antagonist bacteria with the highest level of biocontrol *in vitro* may not act as well as *in vivo* since environmental conditions and competition with other microorganisms. Therefore, it is important that biocontrol potential under field conditions should be further evaluated.

REFERENCES

Al-Mughrabi, K.I. 2010. Biological control of Fusarium dry rot and other potato tuber diseases using *Pseudomonas fluorescens* and *Enterobacter cloacae*. Biological Control. 53. 280-284.

Dal Bello, G.M., C.I. Mónaco, M.R. Simón. 2002. Biological control of seedling blight of wheat caused by *Fusarium graminearum* with beneficial rhizosphere microorganisms. World Journal of Microbiology and Biotechnology. 18. 627-636.

Davidson, J., M.H. Krysinska-Kaczmarek, Mckay A, E.S. Scott. 2012. Comparison of cultural growth and in planta quantification of *Didymella pinodes, Phoma koolunga,* and *Phoma medicaginis var. pinodella,* causal agents of ascochyta blight on field pea (*Pisum sativum*). . Mycologia. 104. 93-101.

Dwi, S., P. Siti, M. Erman. 2010. Control of Fusarium Wilt of Chili With Chitinolytic Bacteria. Hayati Journal of Biosciences. 17. 5-8.

Elshafie, H.S., I. Camele, E. Ventrella, L. Scrano, S. Lovelli, S.A. Bufo, M. Amato. 2013. Use of plant growth promoting bacteria (pgdb) for promoting tomato growth and its evaluation as biological control agent. International Journal of Microbiology Research. 5. 452-457.

Fekadu, A., A. Tesfaye. 2013. Antifungal activity of secondary metabolites of *Pseudomonas fluorescens* isolates as a biocontrol agent of chocolate spot disease (*Botrytis fabae*) of faba bean in Ethiopia. African Journal of Microbiology Research. 7. 5364-5373.

Francisco, D.H.C., M.B.P. Angelica, G.M. Gabriel, C.S. Melchor, R.H. Raul, N.A.G. Cristobal, C.R. Francisco. 2011. *In Vitro* antagonist action of T*richoderma* Strains against *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*. American Journal of Agricultural and Biological Science. 6. 410-417.

Harish, S., K. Manjula, A.R. Podile. 1998. *Fusarium udum* is resistant to the mycolytic activity of a biocontrol strain of *Bacillus subtilis* AF 1. FEMS Microbiology Ecology. 25. 385-390.

Huang, X., N. Zhang, X. Yong, X. Yang, Q. Shen. 2012. Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. Microbiological Research. 167. 135-143.

Ivana, P., V. Jelena, S. Mirjana, K. Dejana, R. Emil, S. Miloš, M.-M. Svetlana. 2012. Impact of Fungicides Used for Wheat Treatment on Button Mushroom Cultivation. Pesticidi i Fitomedicina. 27. 9-14.

María L. Tortora, M.L., V. Díaz-Ricci, R.O. Pedraza. 2011. *Azospirillum brasilense* siderophores with antifungal activity against *Colletotrichum acutatum*. Archives of Microbiology. 193. 275-286.

Murray, G., J. Brennan. 2009. Estimating disease losses to the Australian wheat industry. Australasian Plant Pathology. 38. 558–570

Obanora, F., S. Neateb, S. Simpfendorferc, R. Sabburga, P. Wilsond, S. Chakrabortya. 2013. *Fusarium graminearum* and *Fusarium pseudograminearum* caused the 2010 head blight epidemics in Australia. Plant Pathology. 62. 79–91.

Perveen K, N.A. Bokhari. 2012. Antagonistic activity of Trichoderma harzianum and Trichoderma viride isolated from soil of date palm field against Fusarium oxysporum. African Journal of Microbiology Research. 6. 3348-3353.

Saeideh, J.-P., H. Behzad, S. Akram. 2008. An *in vitro* study on the fungicidal effects of percidin 535® (per acetic acid 15%) against phytopathogenic fungi. Biotechnology. 7. 830-832.

Sansinenea, E., A. Ortiz. 2011. Secondary metabolites of soil *Bacillus* spp. Biotechnology Letters. 33. 1523-1538.

Sundaramoorthy, S., P. Balabaska. 2013. Evaluation of Combined Efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* in Managing Tomato Wilt Caused by *Fusarium oxysporum* f. sp. *lycopersici* (Fol). Plant Pathology Journal,. 12. 154-161.

Ting, A.S.Y., S.W. Mah, C.S. Tee. 2010. Identification of Volatile Metabolites from Fungal Endophytes with Biocontrol Potential towards Fusarium oxysporum F. sp. cubense Race 4. American Journal of Agricultural and Biological Science. 5. 177-182.

Yasar, E., E. Sezai, H. Ayhan, C. Ramazan. 2010. Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. Biological Research. 43. 91-98.

Yuan, J., Y. Ruan, B. Wang, J. Zhang, R. Waseem, Q. Huang, Q. Shen. 2013. Plant Growth-Promoting Rhizobacteria Strain *Bacillus amyloliquefacien*s NJN-6-Enriched Bio-organic Fertilizer Suppressed Fusarium Wilt and Promoted the Growth of Banana Plants. Journal of Agricultural and Food Chemistry. 61. 3774-3780.

Zamanizadeh, H.R., N. Hatami, M.M. Aminaee, F. Rakhshandehroo. 2010. Application of biofungicides in control of damping disease off in greenhouse crops as a possible substitute to synthetic fungicides. International Journal of Environmental Science and Technology. 8. 129-136.