International Journal of
Molecular Microbiology

A Peer-reviewed Journal

Research Article 2018 | Volume 1 | Issue 1 | 29-37

Article Info

Open Access
Citation: Hashmi, I.H., Aslam, A., Farooq, T.H., Zaynab, M., Munir, N., Tayyab, M., Abbasi, K.Y., 2018. Antifungal Activity of Biocontrol Agents against Corm Rot of *Gladiolus grandiflorus* L. Caused by *Fusarium oxysporum*. Int. J. Mol. Microbiol., 1(1): 29-37.

Received: February 24, 2018

Accepted: June 12, 2018

Online first: August 6, 2018

Published: August 15, 2018

***Corresponding author:**
Ali Aslam;
Email:
mianaliaslam538@gmail.com

Scan QR code to see this publication on your mobile device.

Antifungal Activity of Biocontrol Agents against Corm Rot of *Gladiolus grandiflorus* L. Caused by *Fusarium oxysporum*

Irfan Haider Hashmi[1], Ali Aslam[1,2*], Taimoor Hassan Farooq[3], Madiha Zaynab[4], Nigarish Munir[5], Muhammad Tayyab[6], Karim Yar Abbasi[1]

[1]Institute of Horticultural Sciences, University of Agriculture, 38000, Faisalabad, Pakistan.
[2]Graduate School of Chinese Academy of Agricultural Sciences, 100081, Beijing, China.
[3]College of Forestry,[4]College of Life Science, [5]Institute of Horticultural Biotechnology, [6]College of Crop Science, Fujian Agriculture and Forestry University, 350002, Fuzhou, China

Abstract
Field experiment was conducted to analyze the prospects of two isolated bacteria *Bacillus subtilis*, *Pseudomonas florescence*, and one fungal isolate *Trichoderma harzianum* to evaluate corm rot of *Gladiolus grandiflorus* L. caused by pathogenic fungus *Fusarium oxysporum*. Experiment was arranged in RCBD with 5 replicates. Suspensions of these agents individually and in the combination of two and three were applied to the plants at the seedling stage. All treatments provide significant results against disease incidence severity in the field. These bio control agents restrain the disease in stem and rots. *B. subtilis* showed more significant result against the disease severity % and number of lesion corms^{-1}. *B. subtilis* treatment significantly enhanced the root and shoot length of Gladiolus plant. The present experiment revealed that corm rot of *Gladiolus grandiflorus* L. can be efficiently managed by using bio controls agents.
Keywords: *Gladiolus grandiflorus* L., Biocontrol agents, *Fusarium oxysporum*, Corm rot.

INTRODUCTION

Gladiolus *(Gladiolus grandiflorus L.)* superlative ornamental cut flower commercially cultivated all around the world (Pragya *et al.,* 2010). *Fusarium* corm rot being a tremendously serious disease of gladiolus cause foliage distressing in the field along with corm destruction in store houses. This disease also appeared in storage house and as well as after the planting of corms (Riaz *et al.,* 2010; Sharma and Tripathi, 2008). Soil-borne fungus affects the gladiolus cultivation causing above ground plant parts to wilt, with yellowing symptoms, pre mature dying of leaves and producing rotted corms (Dallavalle *et al.,* 2002), faded flowers, stained leaves, wrecked corms and undersized plant growth resulting in poor crop production and yield outcome (Ram *et al.,* 2004). Fungus infestation in corms and soil as macro-conidia, micro-conidia, mycelia, and chlamydospores cause necrosis and death of the plant. In the field, underdeveloped appearance occurs due to severe infection along with complete breakdown due to soft rot. The productivity of gladiolus is greatly affected by *Fusarium* corm rot, being the leading ornamental crop of the country. For sustainable, eco-friendly integrated disease management strategies, the trend is shifting from chemical peptides to naturally occurring compounds. One such alternative for protecting crops from disease is biocontrol. The level and consistency of control are therefore greatly enhanced due to multiple modes of action, a more stable rhizosphere community and effectiveness over a wider range of environmental conditions (De Boer *et al.,* 1999; Iqbal and Ashraf, 2017; Larkin and Fravel, 1999). Varying combinations of multiple and compatibility of biocontrol agents have been reported against the disease control. This strategy is eco-friendly and it's leading toward the search of alternatives against the chemical pesticides. These include mixtures of fungi (Paulitz and diseases. Proceedings of a UCLA colloquim held at Frisco, 1990), bacteria (Shanmugam *et al.,* 2002), yeasts (Janisiewicz, 1996), bacteria and fungi (Janisiewicz, 1988) and bacteria and yeast (Janisiewicz *et al.,* 1995). These practices influence nutrient availability, disease response, pathogen viability and distribution and release of biologically active substances from soil microorganisms and crop residues. Application of binary and multiple mixtures mimic the natural situation more closely and broaden the spectrum of biocontrol activity. The past decade has seen the implementation of these strategies, now the interest is focused on new microbial combinations capable of enhanced performance on plant health with respect to the application of single species (Souza Júnior *et al.,* 2017). The productivity of gladiolus is greatly affected by *Fusarium*corm rot, as it is the leading corm crop of the country. The aim of this study was an evaluation of biocontrol agents *Trichoderma harzianum, Bacillus subtilis and Pseudomonas florescence)* against *F. oxysporum* f.sp. gladioli.

MATERIALS AND METHODS

Samples collection

Both healthy and diseased samples were collected. The numbers of same-sized corms were labeled with the name of the area and these samples were stored at 4°C (Figure 1 a,b).

(a) (b)

Fig. 1. Collection of diseased samples (a) leaves (b) corms.

Isolation, identification, purification, and multiplication

Potato dextrose agar media was prepared and autoclaved at 121°C at 15 psi for 20 minutes and poured into Petri plates in laminar flow. The infected corms were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogen in Nematology Laboratory, University of Agriculture Faisalabad. The infected portions were cut into small pieces of about 1 cm in size. 0.1% of mercuric chloride ($HgCl_2$) solution was used for surface sterilization for 1 min and rinsed thrice and then placed in sterilized petri plates. The petri plates were incubated at 25 + 2°C for 72 h for the growth of fungus. These samples were again purified with the same procedure. Fungal isolates were again purified with PDA slants plugged with cotton balls. These slants were incubated at 25+2°C for 15 days for purifying the fungus culture. Isolated fungus was identified morphologically with explanations described previously (Booth, 1971; Iqbal *et al.,* 2016; Massey, 1926; McCulloch, 1944).

Fusarium oxysporum f.sp. gladioli comprised of white to peach pale salmon or purple mycelium. Microconidia were abundant, hyaline ovoid to ovate. Macroconidia were scarce, 3-septate. Chlamydospores were hyaline usually vacuolated and spherical.

Multiplication of biocontrol agents

These biocontrol agents (*B. subtilis* and *P. fluorescens*) were cultured on Nutrient Broth while *T. harzianum* was cultured on PD Broth and Agar medium.

Extraction of aliquots

15 days old cultures of three biocontrol agents (*B. subtilis, P. fluorescens,* and *T. harzianum*) were used in this experiment. 20 ml of each solution was taken into a 50 ml centrifuge tube and centrifuged at 6000 rpm for 10 minutes. The supernatant was collected for further use.

Preparation of sick plot

A plot of 12 x 12 was roughed. The soil was thoroughly plowed 30-40 cm deep and was exposed to the sun for at least 48 hours. Blocking was done within the plot, ridges and furrows were prepared. After that pathogen culture in the form of spore suspension was added to soil to make it inoculated with Fusarium. Corms were sown 6 inches apart and about 4 inches deep with the pointed end facing up covered with soil and pressed firmly (Figure 2).

Fig. 2. Preparation of sick plot.

Evaluation of biocontrol agents in field conditions

Corms treated with $HgCl_2$ for surface disinfectant were sown on ridges 6 inches apart from each other and about 4 inches deep with the pointed end facing up covered with soil and pressed firmly. After 30 days of sowing inoculum of *Fusarium oxysporum* f.sp. gladioli were applied in the root zone at $4x10^7$ spores/ml. At seedling stage suspension of three biocontrol agents (*B. subtilis, P. fluorescens and T. harzianum*) individually as well as in the combination of two and three was applied to the plants. The experiment was arranged in RCBD with 5 replications. The healthy plants without biocontrol agents were kept under control. Data was recorded on regular basis along with cultural practices during the entire period of study.

Data recording and statistical analysis

Data were recorded on the basis of disease and growth parameters during the experiment and after the harvesting of plants about 110 days after plantation. Disease parameters included disease severity and a number of lesions while the growth parameters were corm diameter (cm) and corm weight (g). Data were subjected to ANOVA and significant differences among the treatments were portioned by Least significant difference test (LSD) at probability levels of $P<0.01$ (Steel et al., 1997).

RESULTS

Disease severity (%)

Disease severity of leaves and spikes is shown in figure (3 a,b). The results regarding effects of different biocontrol agents (*Pseudomonas florescence* + *Bacillus Subtilis* + *Trichoderma harzianum*) and their combinations (Baci+Pf), (Baci+Tr), (Pf+Tr) and (Baci+Pf+Tr), (F+Pf+Tr), (F+Baci+Tr) on disease severity % are illustrated in Figure 4. All observations were processed statistically and the results obtained depict significant results. Biocontrol application in treatment (*P. fluorescens*) suppressed the maximum disease severity to 1.26 % followed by 1.33 % in treatment (*B. Subtilis*) and 1.53 % in treatment (*T. harzianum*). Combination of two biocontrol agents gave disease severity ratings of 1.13 % in treatment (Baci+Pf), 1.06 % in both (Baci+Tr) and (Pf+Tr) while combination of three biocontrol agents gave the maximum suppression in disease severity (0.50 %) in treatment (Baci+Pf+Tr) followed by 0.8 % in treatment (F+Pf+Tr) and 0.60 % in (F+Baci+Tr) as compared to 3.66 % in control treatment.

(a) (b)

Fig. 3. Disease severity on (a) Spikes (b) Leaves

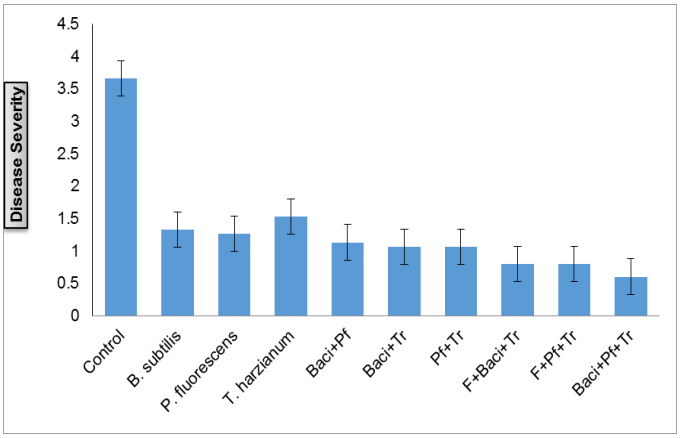

Fig. 4. Effects of biocontrol agents on disease severity.
Baci: *Bacillus Subtilis* ; Pf: *Pseudomonas fluorescens* ; Tr: *Trichoderma harzianum* ; F: Fusarium

Number of lesions corm^{-1}

The results regarding the effect of different biocontrol agents (*Pseudomonas florescence* + *Bacillus Subtilis* + *Trichoderma harzianum*) and their combinations (Baci+Pf), (Baci+Tr), (Pf+Tr) and (Baci+Pf+Tr), (F+Pf+Tr), (F+Baci+Tr) on a number of lesions corm^{-1} are illustrated in Figure 5. The corms treated with treatment (*B. subtilis*) showed the minimum no of infection lesions (10.60) followed by 10.80 in treatment (*P. fluorescens*), 12.33 in treatment (*T. harzianum*). Combination of two biocontrol agents gave minimum infection lesions corm-1 (8.0) in treatment (Baci+Pf) followed by (8.20) and (8.76) in treatments (*Pf+Tr*) and (Baci+Tr) respectively while combination of three biocontrol agents gave the maximum suppression in infection lesions corm^{-1} (6.97) in treatment (*Baci+Pf+Tr*) followed by (7.20) in treatment (*F+Baci+Tr*), 8.6 in treatment (*F+Pf+Tr*) and as compared to 28.6 % in control treatment.

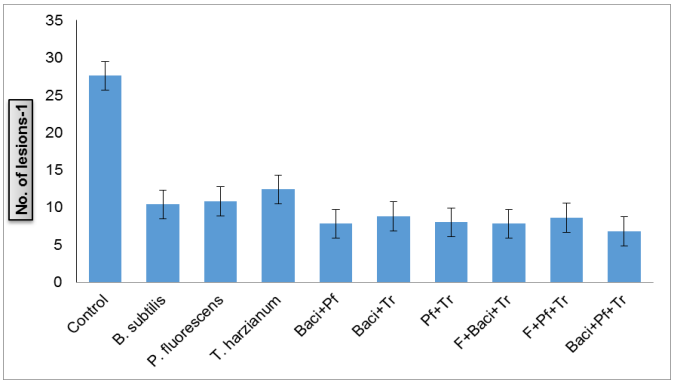

Fig. 5. Effects of biocontrol agents on No. of lesions^{-1}.
Baci: *Bacillus Subtilis* ; Pf: *Pseudomonas fluorescens* ; Tr: *Trichoderma harzianum* ; F: Fusarium

Number of cormel clumps^{-1}

The results regarding the effect of different biocontrol agents (*Pseudomonas florescence* + *Bacillus Subtilis* + *Trichoderma harzianum)* and their combinations *(Baci+Pf), (Baci+Tr), (Pf+Tr)* and *(Baci+Pf+Tr), (F+Pf+Tr), (F+Baci+Tr)* on a number of cormel clumps^{-1} are illustrated in Figure 6. All observations depict significant results. The maximum no. of cormels 16.80 in treatment (*B. subtilis*) followed by 14.20 in treatment (*P. fluorescens*), 14.20 in treatment (*T. harzianum*). Combination of two biocontrol agents had a significant effect on of no. cormel clumps^{-1} and formed (21.6) in treatment *(Baci+Tr)* followed by (19.2) and (18.82) in treatments *(Pf+Tr)* and *(Baci+Pf)* respectively while combination of three biocontrol agents gave the maximum no. of cormel clumps^{-1} (26.2) in treatment *(Baci+Pf+Tr)* and was regarded as best followed by (22.4) in treatment *(F+Baci+Tr)*, 21.8 in treatment *(F+Pf+Tr)* as compared to 10.2 in control treatment.

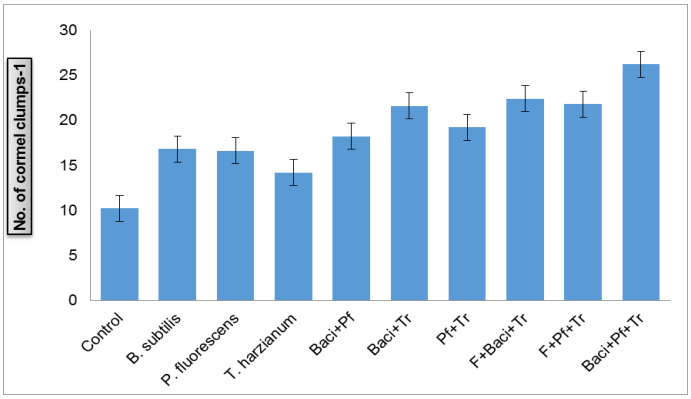

Fig. 6. Effects of biocontrol agents on No. of cormel clumps^{-1}.
Baci: *Bacillus Subtilis* ; Pf: *Pseudomonas fluorescens*; Tr: *Trichoderma harzianum* ; F: Fusarium

Plant height (cm)

The results regarding effects of different biocontrol agents (*Pseudomonas florescence* + *Bacillus Subtilis* + *Trichoderma harzianum)* and their combinations *(Baci+Pf), (Baci+Tr), (Pf+Tr)* and *(Baci+Pf+Tr), (F+Pf+Tr), (F+Baci+Tr)* on Plant Height (cm) are illustrated in Figure 7. All observations showed significant results. Maximum plant height (67.48) was found in treatment (*B. subtilis*) followed by 66.96 in treatment (*P. fluorescens*), 63.50 in treatment (*T. harzianum*), as compared to 50.68 in control treatment. The effect of bio control agents when applied in combination of two biocontrol agents gave maximum plant height (69.10) in treatment *(Baci+Pf)* followed by 68.32 in *(Pf+Tr)*, 67.46 in *(Baci+Tr)* while combination of three biocontrol agents gave the maximum plant height 82.10 in treatment *(Baci+Pf+Tr)* and was the best amongst all biocontrol treatments followed by 79.20 and 73.33 in

treatments *(F+Pf+Tr)* and *(F+Baci+Tr)* respectively as compared to 50.68 in control treatment.

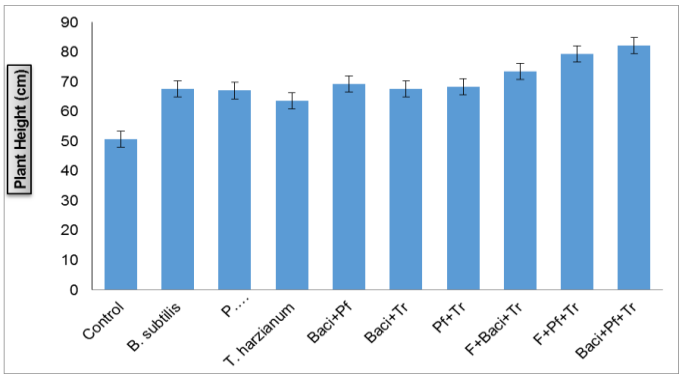

Fig. 7. Effects of biocontrol agents on plant height.
Baci: *Bacillus Subtilis* ; Pf: *Pseudomonas fluorescens* ; Tr: *Trichoderma harzianum* ; F: Fusarium

The weight of corm (g)

The results regarding effects of different biocontrol agents (*P. florescence + Bacillus Subtilis + Trichoderma harzianum)* and their combinations *(Baci+Pf), (Baci+Tr), (Pf+Tr)* and *(Baci+Pf+Tr), (F+Pf+Tr), (F+Baci+Tr)* on the weight of corm (g) are illustrated in Figure 8. Maximum weight of corm (31.73) was found in treatment (*P. fluorescens)* followed by 30.56 in treatment (*B. subtilis)*, 24.02 in treatment (*T. harzianum*). Combination of two biocontrol had a significant effect on weight of corm as maximum weight observed (45.51) in treatment *(Baci+Pf)* followed by (42.46) and (40.71) in treatments *(Baci+Tr)* and *(Pf+Tr)* respectively while combination of three biocontrol agents gave the maximum corm weight (53.21) in treatment *(Baci+Pf+Tr)* and was regarded as best followed by (48.20) in treatment *(F+Baci+Tr)*, 47.83 in treatment *(F+Pf+Tr)* as compared to 18.6 in control treatment.

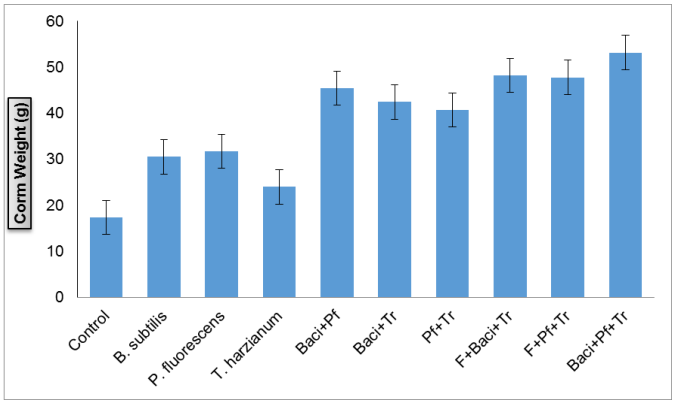

Fig. 8. Effects of biocontrol agents on corm weight.
Baci: *Bacillus Subtilis*; Pf: *Pseudomonas fluorescens*; Tr: *Trichoderma harzianum* ; F: Fusarium

Diameter of corm (cm)

The results regarding effects of different biocontrol agents (*Pseudomonas florescence + Bacillus Subtilis + Trichoderma harzianum*) and their combinations (*Baci+Pf*), (*Baci+Tr*), (*Pf+Tr*) and (*Baci+Pf+Tr*), (*F+Pf+Tr*), (*F+Baci+Tr*) on the weight of corm (g) are illustrated in Figure 9. The maximum diameter of corm (3.88) was recorded in treatment (*B. subtilis*) followed by 3.31 in treatment (*P. fluorescens*), 3.11 in treatment (*T. harzianum*). The effect of biocontrol agents when applied in combination of two biocontrol agents gave maximum diameter of corm (4.93) in treatment (*Pf+Tr*) followed by 4.60 in (*Baci+Pf*), 4.36 in (*Baci+Tr*) while combination of three biocontrol agents gave the maximum corm diameter 5.41 in treatment (*Baci+Pf+Tr*) and was the best amongst all biocontrol treatments followed by 4.88 and 4.72 in treatments (*F+Pf+Tr*) and (*F+Baci+Tr*) respectively as compared to 2.68 in control treatment.

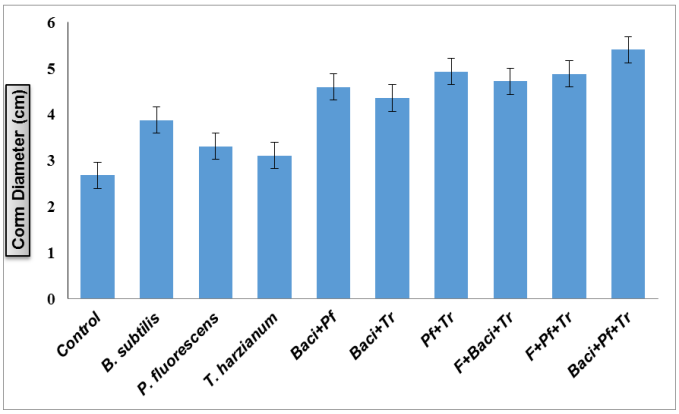

Fig. 9. Effects of biocontrol agents on corm diameter.
Baci: *Bacillus Subtilis* ; Pf: *Pseudomonas fluorescens*; Tr: *Trichoderma harzianum* ; F: Fusarium

DISCUSSION

To minimize the disease effects, the use of beneficial microorganisms as biopesticides is considered as one of the most promising methods in crop management practices and improves product quality (Kanwal *et al.,* 2016). In this experimental study, biocontrol agents along with their combination were used for integrated management of corm rot of gladiolus. Results indicated that *B. subtilis* was the most effective to significantly reduce the disease severity %, a number of lesion corms^{-1} and also enhanced number of cormel clumps^{-1}, size of corm and weight of corm. These results were compared with (Mohamed and Gomaa, 2000), who checked the efficacy of two biocontrol agents i.e. *Bacillus subtilis* and *T. harzianum* as soil or corm soaking treatment in controlling *Fusarium* disease of gladiolus. *B.subtilis* significantly reduced the disease index in wheat and exhibited the best control against the important plant pathogens especially in *Fusarium* (Gao *et*

al., 2014). Another study revealed the bio-efficiency of *B. subtilis* against powdery mildew (*Leviellula taurica*) and early blight (*Alternaria solani)* of tomato and also reported improved plant growth due to abilities to produce phytohormones, solubilizing minerals and vitamins and direct inhibition of pathogen growth (Basamma *et al.,* 2017). *B. subtilis* has a great potential for plant growth promotion and biological control, it induces resistance against Fusarium in pepper, and it also increased the average yield per plant (Yu *et al.,* 2011). Another study reported that *T.harzianum*, *T. viride,* and *T. virens* not only lowered the disease incidence but also improve the plant growth and corm formation (Sharma *et al.,* 2005). *Bacillus subtilis* is a prominent antifungal agent against various plant pathogens as it resides and surrounds the root of plants. The antibiotics production is the principal mechanism for fungicidal action by any biological agent (Alsohiby *et al.,* 2016; Khalid *et al.,* 2016; Loeffler *et al.,* 1986). This bacterium strains not only produce antibiotics and enzymes (Malik *et al.,* 2017) but also known to produce volatile substances which make them to inhibit the growth of a range of organisms. Different seed treatments of *B. subtilis and Trichoderma* spp. with pigeon pea effectively controlled pigeon pea wilt and improved the yield in a considerable manner (Nakkeeran and Renuka Devi, 1997). The production of lytic enzymes (pectinase, glucanase, chitinase), indole 3 acetic acid, salicylic acid, HCN and secondary metabolites including antibiotics is probably one of the most important mechanisms of *Pseudomonas* antifungal properties. Many effective antibiotics such as pyoluteorin, pyrrolnitrin, and phenazine were synthesized by *Pseudomonas* spp. The production of these compounds is directly or indirectly correlated with biocontrol activity. It was also documented that *P. fluorescens* isolates significantly inhibit the growth of *F. oxysporum* in chickpea crop (Kandoliya and Vakharia, 2013). A study reported that *P. fluorescens* was efficient in controlling Fusarium infection in water melon and allowing normal seedling growth of both root and shoot. It also improved the productivity and yield of the crop. And this method is cost-effective, easily applicable and may accumulate beneficiary results on a long turn. Moreover, environmentally it is a better choice than chemical methods (Salman *et al.,* 2017).

Amongst combination of two biocontrol agents treatments *(B. subtilis + P. fluorescens)* controlled the fungal biomass and suppressed the diseased lesions on corms while (*B. subtilis + T. harzianum)* enhanced corm weight, corm diameter and no of cormel clumps^{-1} followed by (*P. fluorescens + T. harzianum).* The results are in agreement with some previous studies of (Nosir et al., 2011) who used *Aneurinobacillus migulanus* in a combination with *T. harzianum* and found that Fusaric acid (FA) secretions were prevented in the infected corm of gladiolus moreover, T22 prevented the frequency of lesion on gladiolus corms and fully concealed the fungal hyphen. It was reported that other strains of *Trichoderma* i.e *T. viride* (61.34%), *T. virions* (66.280%), *onigiri* (72.50%) were less effective in controlling the disease as compared to *T. harzianum* (76.08%) being the most efficient of all (Kulkarni *et al.,* 2007). On the other hand, *Pseudomonas fluorescens and Bacillus subtilis* were least effective. *Trichoderma harzianum* acted as a mycoparasite and suppressed the *F. oxysporum* growth as well as another soil-borne fungus, notably wilt fungus (Khan and Gupta, 1998; Leben *et al.,* 1987; Papavizas, 1985).

CONCLUSION

A systematic and eco-friendly biological control approach was adopted to manage *Fusarium corm* rot in *Gladiolus grandiflorus* with the application of biocontrol agents both individually as well as in combination which expressed significant management/control of disease as compared to control as, commercialization of bio fungicides has marked a considerable boost in recent years. Bio pesticides have gained popularity regarding safety and eco-friendliness, for effectiveness, with the increased demand, particularly over the past decade.

ACKNOWLEDGMENTS

Authors are thankful for technical and research support provided by the University of Agriculture, Faisalabad, Pakistan.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

REFERENCES

Alsohiby, F.A.A., Yahya, S., Humaid, A.A.J.P.M., 2016. Screening of soil isolates of bacteria for antagonistic activity against plant pathogenic fungi. PSM Microbiol., 1(1): 5-9.

Basamma, H., Shripad, K.J.E., Ecology, 2017. Compatibility of *Bacillus subtilis* (Ehrenberg) Cohn with chemical fungicides. Environ. Ecol., 35(3B): 2137-2141.

Booth, C.J.T.g.F., 1971. The genus fusarium. The genus Fusarium.

Dallavalle, E., D'Aulerio, A.Z., Verardi, E., Bertaccini, A., 2002. Detection of RAPD polymorphisms in Gladiolus cultivars with differing sensitivities to *Fusarium oxysporum* f. sp. gladioli. Plant Mol. Biol. Report., 20(3): 305-306.

De Boer, M., van der Sluis, I., van Loon, L.C., Bakker, P.A.J.E.J.o.P.P., 1999. Combining fluorescent Pseudomonas spp. strains to enhance suppression of fusarium wilt of radish. Eur. J. Plant Pathol.,105(2): 201-210.

Gao, X., Han, Q., Chen, Y., Qin, H., Huang, L., Kang, Z.J.B.s., technology, 2014. Biological control of oilseed rape Sclerotinia stem rot by *Bacillus subtilis* strain Em7. Biocont. Sci. Technol., 24(1): 39-52.

Iqbal, M.N., Ali, S., Anjum, A.A., Khushi, M., Ali, M.A., Wang, S., Khan, W.A., Iahtasham, K., Ali, M., Abid, M., Muhammad, I., Aftab, A., Asfa, A., Firasat, H., 2016. Microbiological risk assessment of packed fruit juices and antibacterial activity of preservatives against bacterial isolates. Pak. J. Zool., 48(6): 1695-1703.

Iqbal, M.N., Ashraf, A.J.P.M., 2017. Antagonism in Rhizobacteria: Application for Biocontrol of Soil-borne Plant Pathogens. PSM Microbiol., 2(3): 78-79.

Janisiewicz, W., Bors, B.J.A., Microbiology, E., 1995. Development of a microbial community of bacterial and yeast antagonists to control wound-invading postharvest pathogens of fruits. Appl. Environ. Microbiol., 61(9): 3261-3267.

Janisiewicz, W.J.P., 1988. Biocontrol of postharvest diseases of apples with antagonist mixtures. Phytopathol., 78(2): 194-198.

Janisiewicz, W.J.P., 1996. Ecological diversity, niche overlap, and coexistence of antagonists used in developing mixtures for biocontrol of postharvest diseases of apples. Phytopathol., 86(5): 473-479.

Kandoliya, U., Vakharia, D.J.L.R., 2013. Antagonistic effect of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. ciceri causing wilt in chickpea. Legume Res., 36(6): 569-575.

Kanwal, F., Rashid, F., Ashraf, A., Iqbal, M.N., Xiao, S.J.P.B.R., 2016. A Comparative Study on Isolation and Identification of *Bacillus thuringiensis* from Different Localities of Gujranwala City. PSM Biol. Res., 1(1): 34-38.

Khalid, Z.Z., Rashid, F., Ashraf, A., Iqbal, M.N., Hussain, F.J.P.M., 2016. Isolation and Screening of Antibiotic producing Bacteria from Soil in Lahore City. PSM Microbiol., 1(1): 1-4.

Khan, M., Gupta, J., 1998. Antagonistic efficacy of Trichoderma species against *Macrophomina phaseolina* on eggplant. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 105(4): 387-393.

Kulkarni, S.P., Hegde, Y.R., Meena, B., Kulkarni, S.J.I.J.o.C.S., 2007. Screening of biocontrol agents and varieties against wilt of gladiolus. Indian J. Crop Sci., 2(1): 235-236.

Larkin, R.P., Fravel, D.R.J.P., 1999. Mechanisms of action and dose-response relationships governing biological control of Fusarium wilt of tomato by nonpathogenic Fusarium spp. Phytopathol., 89(12): 1152-1161.

Leben, S., Wadi, J., Easton, G., 1987. Effects of *Pseudomonas fluorescens* on potato plant growth and control of *Verticillium dahliae*. Phytopathol., 77(11): 1592-1395.

Loeffler, W., Tschen, J.S.M., Vanittanakom, N., Kugler, M., Knorpp, E., Hsieh, T.F., Wu, T.G.J.J.o.P., 1986. Antifungal Effects of Bacilysin and Fengymycin from Bacillus subtilis F‐29‐3 A Comparison with Activities of Other Bacillus Antibiotics. J. Phytopathol., 115(3): 204-213.

Malik, H., Hadi, S., Shahzad, M.I., Irfan, M., Ali, S., Muhammad, A.J.P.M., 2017. Isolation and Identification of Protease Enzyme Producing *Bacillus subtilis* from Soil. PSM Microbiol., 2(1): 15-19.

Massey, L.J.P., 1926. Fusarium rot of gladiolus corms. Phytopathol., 16: 509-523.

McCulloch, L.J.P., 1944. A vascular disease of gladiolus caused by Fusarium. Phytopathol., 34: 263-287.

Mohamed, F., Gomaa, A.J.A.A.S., 2000. Effect of some bio agents and agricultural chemicals on Fusarium wilt incidence and growth characters of gladiolus plants. Annals Agricul. Sci., 38: 883-906.

Nakkeeran, S., Renuka Devi, P.J.P.D.R., 1997. Seed-Borne Microflora of Pigeon Pea and Their Management. Plant Dis. Res., 12: 197-200.

Nosir, W., McDonald, J., Woodward, S., 2011. Impact of biological control agents on fusaric acid secreted from *Fusarium oxysporum* f. sp. gladioli (Massey) Snyder and Hansen in *Gladiolus grandiflorus* corms. J. Indust. Microbiol. Biotechnol., 38(1): 21-27.

Papavizas, G., 1985. Trichoderma and Gliocladium: biology, ecology, and potential for biocontrol. Annual review of Phytopathology, 23(1): 23-54.

Paulitz, T.J.N.d.i.b.c.A.f.s.a.p., diseases. Proceedings of a UCLA colloquim held at Frisco, C., January 20-27, . 1990. Biochemical and ecological aspects of competition in biological control. Proceedings of a UCLA colloquim held at Frisco, Colorado, January 20-27, 713-724.

Pragya, R., Bhat, K.V., Misra, R.L., Singh, S.K., Ranjan, J.K., 2010. Genetic relationships of gladiolus cultivars inferred from fluorescence based AFLP markers. Scientia Horticult., 123(4): 562-567.

Ram, R., Manuja, S., Dhyani, D., Mukherjee, D., 2004. Evaluations of fortified fungicide solutions in managing corm rot disease of gladiolus caused by *Fusarium oxysporum*. Crop Prot., 23(9): 783-788.

Riaz, T., Nawaz Khan, S., Javaid, A., 2010. Management of corm-rot disease of gladiolus by plant extracts. Nat. Prod. Res., 24(12): 1131-1138.

Salman, M., Shahin, N., Abu-Khalaf, N., Jawabrih, M., Rumaileh, B.A., Abuamsha, R., Barghouthi, S.A.J.J.o.P.S., 2017. Antagonistic Activity of

Pseudomonas fluorescens against *Fusarium oxysporum* f. sp. Nievum Isolated from Soil Samples in Palestine. J. Plant Stud.,6(2): 1.

Shanmugam, V., Senthil, N., Raguchander, T., Ramanathan, A., Samiyappan, R.J.P., 2002. Interaction of *Pseudomonas fluorescens* withRhizobium for their effect on the management of peanut root rot. 30(2): Phytopara.,169-176.

Sharma, N., Tripathi, A., 2008. Integrated management of postharvest Fusarium rot of gladiolus corms using hot water, UV-C and *Hyptis suaveolens* (L.) Poit. essential oil. Postharvest Biol. Technol., 47(2): 246-254.

Sharma, S., Chandel, S., Tomar, M.J.I.P.D.M.S.P., Jodhpur, India, 2005. Integrated management of Fusarium yellows of gladioli snyder and Hans under poly house conditions. Integrated Plant Disease Management Scientific Publishers, Jodhpur, India, 221-229.

Souza Júnior, I.T.d., Schafer, J.T., Corrêa, B.O., Funck, G.D., Moura, A.B.J.R.C.A., 2017. Expansion of the biocontrol spectrum of foliar diseases in rice with combinations of rhizobacteria. Revista Ciência Agronômica, 48(3): 513-522.

Steel, R., Torrie, J., Dickey, D., 1997. Principles and procedures of statistics: a biometrical approach., 3rd edn (McGraw-Hill: New York).

Yu, X., Ai, C., Xin, L., Zhou, G.J.E.J.o.S.B., 2011. The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. Eur. J. Soil Biol., 47(2): 138-145.