Pak. J. Bot., 57(4): DOI: http://dx.doi.org/10.30848/PJB2025-4(25)

COMPARATIVE EFFICACY OF COMMERCIAL FUNGICIDES AND *TRICHODERMA HARZIANUM* AGAINST *FUSARIUM* WILT IN STRAWBERRY

ABDUL HANNAN[1], SAGHEER ATTA[1], MUHAMMAD AMJID BASHIR [1], SAJJAD HYDER[2], NASIR ALI TAUQIR[3], MUHAMMAD ALI[4] AND IRUM MUKHTAR[5]

[1]*Department of Plant Protection, Ghazi University, Dera Ghazi Khan, Pakistan*
[2]*Department of Botany, G.C.W.U. Sialkot, Pakistan;* [3]*The Islamia University Bahawalpur, Pakistan*
[4]*Sustainable Development Study Centre, G.C.U. Lahore, Pakistan*
[5]*Institute of Oceanography, Minjiang University, Fuzhou, China*

Abstract

Strawberry (*Fragaria ananassa*) cultivation is increasing among Pakistani farmers because of extra profit and demand in local market. This crop is attacked by multiple pathogens responsible for low yield. The current study's objective was to identify a sustainable and a better approach to managing Fusarium wilt caused by *Fusarium oxysporum* f.sp. *fragariae* (Schlecht) in the field. Firstly effectiveness of eight commercial fungicides was evaluated by the poisoned agar technique against colony growth of *F. oxysporum* f.sp *fragariae In vitro*. Among all tested chemicals, four (Score, Avito, Carbendazim and Topsin-M) were found to suppress and inhibit pathogen growth significantly with reference to control. Later, the efficacy of four strains/isolates of *Trichoderma harzianum* was also evaluated through dual culture technique against *F. oxysporum* f.sp *fragaria*e. However, only one strain of *T. harzianum* was found to suppress colony diameter of pathogen. Afterwards, four fungicides and one isolate of *T. harzianum* were assessed in the greenhouse. Finally, three chemicals (Score, Carbendazim and Topsin-M) and a biocontrol isolate were applied in the field after artificial inoculation of pathogen in a randomized complete block design (RCBD). All three chemicals reduced disease severity of Fusarium wilt with varying degrees of success. The mean results of field trials indicated that Carbendazim and Score gave 72.0%, *T. harzianum* 80.0%, while Topsin-M showed 88.0% protection value against Fusarium wilt disease in the filed after 42 days. Topsin-M was most effective, followed by *T. harzianum* and can be applied to manage Fusarium wilt of strawberry confidently.

Key words: Biological management, Chemicals, Dual culture, Inoculation, Poisoned agar.

Introduction

Cultivation of strawberry (*Fragaria ananassa* Duch.) has entered a critical phase of increase in Pakistan, but multiple factors like climate of the region, size of the fruit, and diseases restrict its quantity and quality. Among diseases, soil-borne fungal pathogens are the most common in a strawberry crop, *viz*. *Phytophthora nicotinae* (Mingzhu *et al*., 2013), *P. fragariae* (Wilcox *et al.*, 1993), *Verticillium dahliae* (Thomas, 1932), *Fusarium oxysporum* (Koike *et al.*, 2009), *Rhizoctonia fragariae* (Hussain & Mckeen, 1963), *R. solani* (Fang *et al*., 2013) and *Pythium* spp (Martin & Loper, 1999). Although many diseases have been reported but Fusarium wilt caused by *Fusarium oxysporum* f. sp. *fragariae* is one of the emerging diseases in strawberry growing areas of Pakistan (Veesar *et al.*, 2015). The introduction of better cultural practices as acclimatized varieties and disease-free suckers can reduce disease problems in the long run, while appropriate pesticide uses are quick and effective ways to overcome disease before crop damage occurs. Several researchers have used pre-planting chemicals like methyl bromide (Koike *et al.*, 2009) and managed disease through fumigation. On the other hand, biological control organisms also showed a promising effectiveness against *F. oxysporum* f. sp. *fragariae* under controlled experimental conditions and including *Bacillus Velezensis*, *B. subtilis* and *Trichoderma harzianum* (Moon *et al*., 1995; Okayama *et al*., 1991; Tezuka & Makino, 1991; Nam *et al*., 2009; Zhang *et al*., 2012). The main symptoms of strawberry wilt are drying and withering of older leaves, stunting of plants, and reduction in fruit setting, wilting of foliar part and eventually plants collapsed and perished (Koike *et al.*, 2009). Therefore, keeping in view, the frequent reports by framers of strawberry wilting in local areas, the current research was designed to manage this disease with all possible practical options presently available. The disease can be managed by applying commonly available fungicides and biological control, i.e., *Trichoderma harzianum*. The differences in cultivar susceptibility have not been recorded in Pakistan. A significant strawberry variety (Chandler) was selected and subjected to investigate. The study was initiated with the principal objective to develop a long-lasting and economical method to manage fusarium wilt in the strawberry crop. By overcoming these disease-related problems and improving the crop's quality, Pakistan can export strawberries to foreign countries, primarily Middle Eastern Countries.

Methodology

Collection of diseased specimen: The symptomatic samples were gathered and examined from different locations of Lahore (Longitude :74.34 and Latitude :31.55) and Okara (Longitude: 73.459396 and Latitude: 30.808500) after complaints of strawberry growers. All samples were transported in sterile polythene bags in an icebox, and an accession number was assigned before storing at 4ºC.

Isolation, identification and preservation of pathogen: The fungus was isolated by following the method of Joshi, 2013. Firstly, all infected leaves and lateral roots were trimmed off, leaving only the main stem and primary root. The stem was surface sterilized by dipping in 10% bleach (NaOCl) solution for 4 to 5 minutes and was dried out on paper towels. Thin wedges (2 to 4 mm) were cut from one side of the stem near the root/stem junction, including

xylem tissue with each wedge using sterile technique. Four wedges were placed on each potato dextrose agar (PDA) plates. The plates were incubated under fluorescent lights until sufficient growth from the plant samples was observed. Later fungal mycelium was transferred onto sterilized PDA plates and incubated at 25ºC for 14 days for purification. The fungus was identified based on the colony and morphological characteristics of conidia following Barnett and Hunter (1998) and Watanabe (2010).

Pathogenicity test: Four-week-old seedlings of strawberry suckers (ver. Chandler) were transplanted to earthen pots containing sterilized soil. Plants were irrigated with sterile distilled water and covered with polythene bags for one day before inoculation. The conidial suspension (1×10^8 spores/mL) prepared from 7-day old culture was atomized, 10 mL per plant and plants were again covered with polythene bags for two days, ensuring enough humidity for the establishment of the pathogen. Inoculated plants were then shifted to the greenhouse and periodically observed for symptom development. In case of disease, a re-isolation of the fungus from infected plants was done after two weeks, and the new isolate was compared with the original one, thus fulfilling Koch's postulates if identical.

Collection, identification and preservation of biocontrol agent: Four isolates/strains of *Trichoderma harzianum* were collected from different sources and vegetable growing fields in Faisalabad and Okara. Cultural characteristics including growth rate, colony colour and appearance were recorded. These characteristics were taxonomically suitable features for Trichoderma spp. (Samuels *et al.*, 2002). The isolates were identified on PDA following Rifai (1969) and preserved on a 2% PDA medium at 25ºC. These isolates were evaluated for antagonistic potential against *F. oxysporum* f. sp. *fragariae* using a dual culture technique (Fig. 2). Mycelial growth of the fungal pathogen and *Trichoderma harzianum* isolates was recorded after 8, 12 and 16 days.

***In vitro* evaluation of chemicals:** Commonly available commercial fungicides were evaluated by the poison agar method at 100, 200, 300 and 400 ppm for all formulations (Borum & Sinclair, 1968) in Department of Plant Pathology, University of Agriculture Faisalabad Pakistan. The description of fungicides, including their chemical name, active ingredient, trade name, and mode of action, is described in Table 1. The calibrated concentration of respective fungicides was mixed after autoclaving and before pouring the medium at 55ºC. PDA plates without any fungicide served as control. After solidification of medium, a one-centimeter discs cut from a pure culture of the pathogen were placed in the centre of the poisoned agar plates and were incubated at 25ºC for three weeks with four replications for each treatment. The mycelial growth of the pathogen was recorded in millimeters until the fungus's growth on control plates completely filled. Each fungicide's protective value was assessed by determining the percentage reduction in colony diameter over control by the following formula:

$$\text{Percentage inhibition of mycelial growth} = \frac{\text{Colony diameter in control} - \text{Colony diameter in treatment}}{\text{Colony diameter in control}} \times 100$$

Table 1. List of fungicides applied against Fusarium wilt in strawberry.

S. No.	Trade name	Active ingredient	Formulation	Distributor	Dose per acre
1.	Topsin-M	Thiophanate Methyl	70% WP	Arysta LifeScience	200 g
2.	Aliette	Fosetyl Aluminium	80% WP	Bayer Crop Science	800 g
3.	Sulfex Gold	Sulfur	80% WG	Jaffer Agro Services	600 g
4.	Score	Difenoconazol	250 SC	Syngenta Pakistan	200 mL
5.	Hexaconazole	Hexaconazole	5% EC	Arysta LifeScience	250 mL
6.	Evito	Fluoxastrobin	480 SC	Arysta LifeScience	120 mL
7.	Nativo	Tebuconazole + Trifloxystroben	75 WP	Bayer Crop Sciences	160 g
8.	Carbendazim	Carbendazim	50% WP	Sungrow Agro	250 g

Pot experiments: After the *In vitro* evaluation, fungicides and *T. harzianum* isolates with the best fungal growth inhibiting potentials were selected for greenhouse experiments. Artificial inoculation of pots was carried out with 10 mL of a freshly prepared conidial suspension (1.0×10^6 spores/mL) of *F. oxysporum* isolated from strawberry plants. The conidial suspension was counted and adjusted using a haemocytometer, whereas controls were kept un-inoculated. Moreover, 25 g of organic matter (semi-decomposed dhaincha leaves) was added to each pot. The experiments were conducted with a completely randomized design with 4 replications to evaluate the efficacy of selected fungicides and biocontrol isolate under controlled conditions.

Management of disease under sick field: Comprehensive field experiments were conducted in Okara during winter 2019, following randomized block design with four treatments (Three chemicals and one bio-agent) and replicated thrice. Fresh suckers of the commonly grown variety Chandler were transplanted to 30x10 cm in an 8.0 m^2 size plot for each treatment/replication and NP fertilizer applied at 160:80 (kg/ha). Inoculum of *F. oxysporum* f. sp. *fragariae* was prepared on boiled chickpea grains in polythene bags (20x30 cm, autoclavable) and was applied at 500g/plot. The initial application of selected chemicals and biocontrol agents was applied together with first irrigation during transplanting, whereas further two applications were made two weeks apart before recording disease incidence.

The data were analyzed statistically. A lower than 5% level of significance for the null hypothesis of no correlation was used to interpret treatment differences using Duncan's Multiple Range (DMR) test.

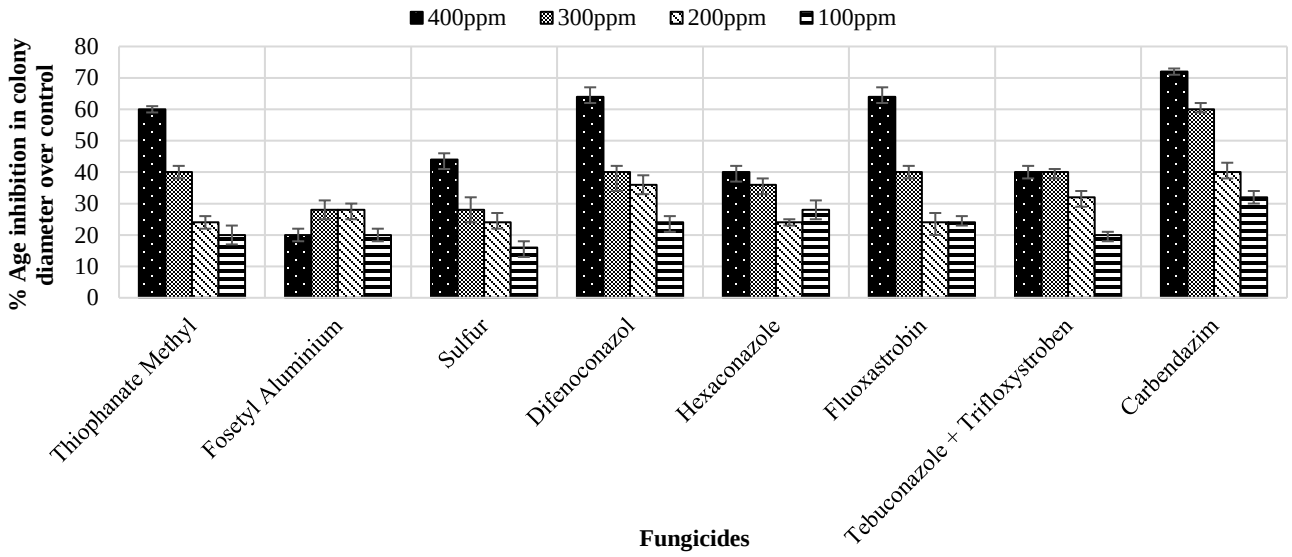

Fig. 1a. *In vitro* evaluation of chemicals using the poison agar technique after 8 days of incubation.

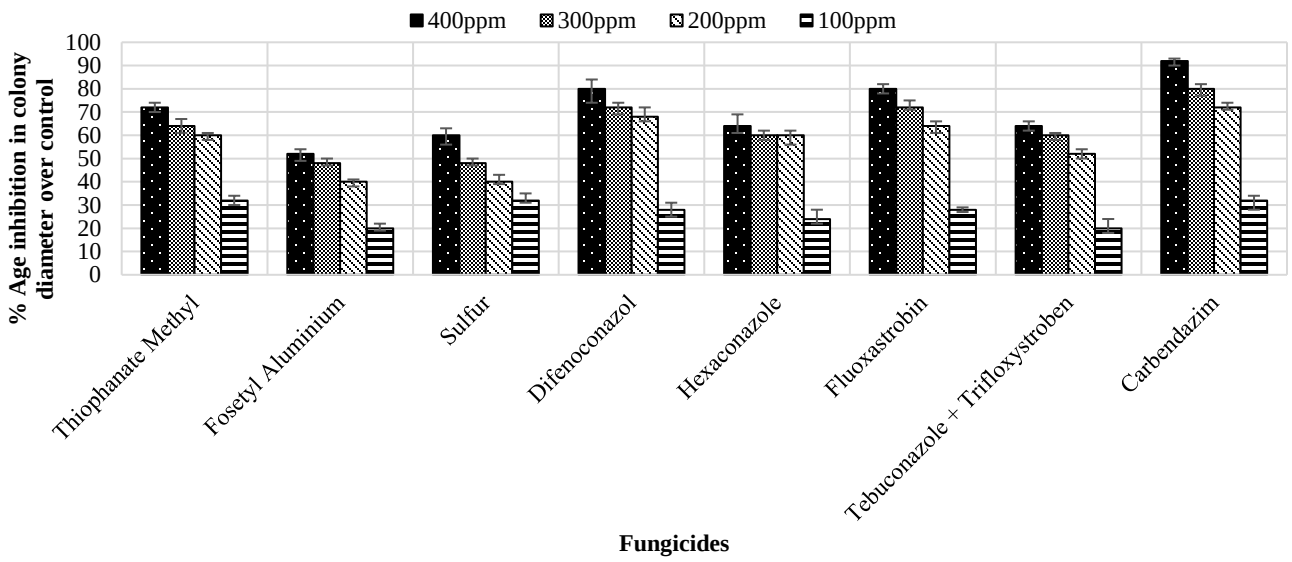

Fig.1b. *In vitro* evaluation of chemicals using the poison agar technique after 12 days of incubation.

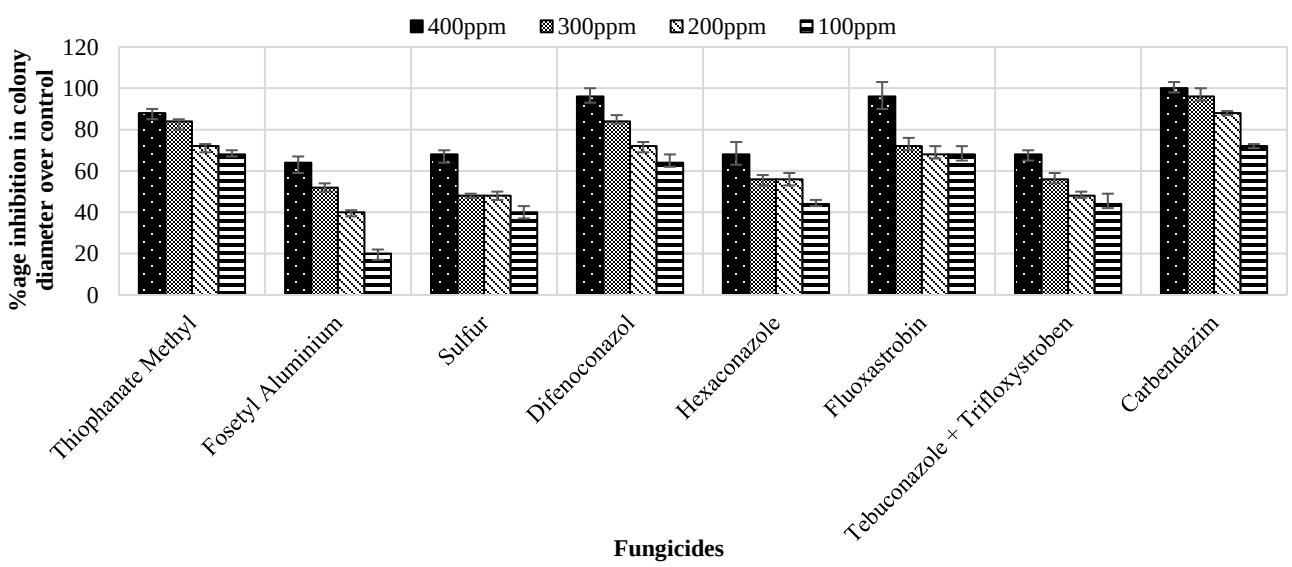

Fig. 1c. *In vitro* evaluation of chemicals using the poison agar technique after 16 days of incubation.

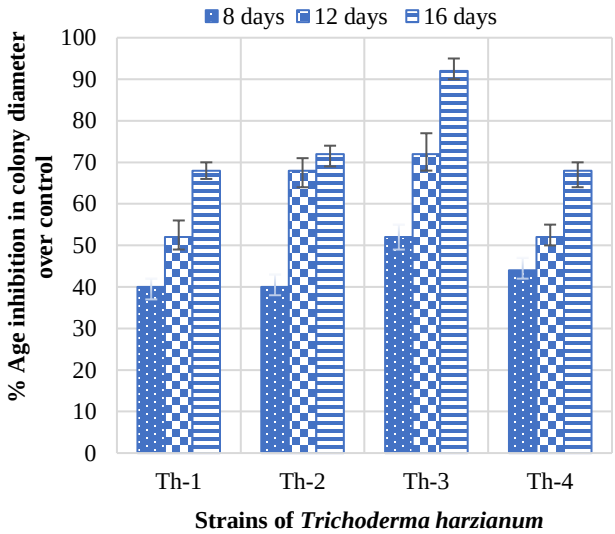

Fig. 2. *In vitro* evaluation of biological agent through dual culture.

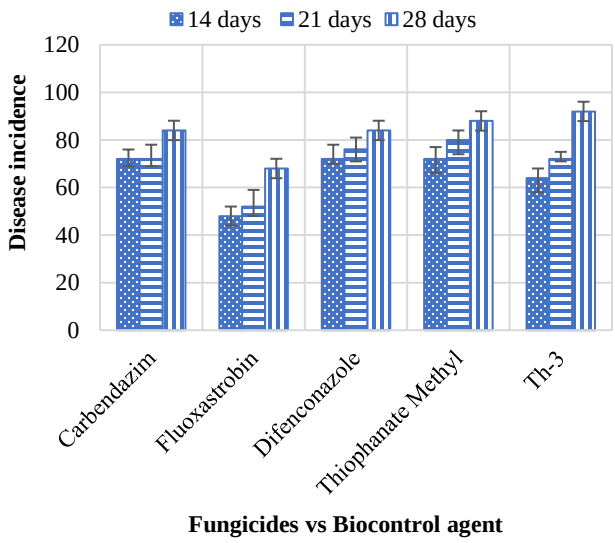

Fig. 3. Evaluation of fungicides and biocontrol agent under greenhouse conditions.

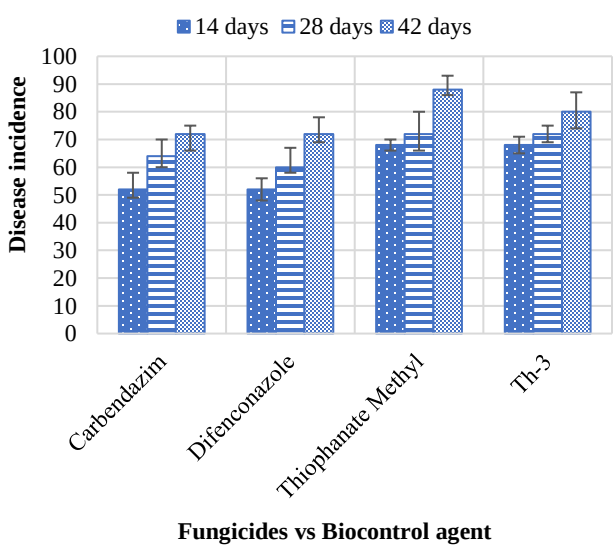

Fig. 4. Evaluation of chemicals and biocontrol agent under field conditions.

Results and Discussion

Isolation, identification and preservation of pathogen and biocontrol agent: The isolated colonies of *F. oxysporum* f. sp. *fragariae* were pigmented with a reddish-purple colour and surrounded by a pinkish to white mycelium (Fig. 5A). The mycelia of *F. oxysporum* f. sp. *fragariae* were delicate and white with a pinkish or purple tinge (Fig. 5A). This pathogen produced three types of spores i.e., macroconidia (Fig. 5C), microconidia (Fig. 5D) and chlamydospores. The pathogen was identified by examining colony characters and shape of micro and macro-conidia, according to Barnet and Hunter (1998) and Watanabe (2010). The microconidia were plentiful, oval to ellipsoid, straight and slightly curved, 5 to12 x 2.2 to 3.5 µm, and non-septate, whereas macroconidia were sparse to abundant with 3 to 5 septations and pointed at both ends. The three-septate macroconidia measure 27 to 46 x 3 to 5 µm while five-septate macroconidia measure 35 to 60 x 3 to 5 µm. The three-septate macroconidia were most abundant.

All isolates of *T. harzianum* were identified following Rafai (1969), where 1 to 2 concentric rings were formed with production of green conidia on PDA (Fig. 5B). The conidial production was denser in the plate's centre than at the margins. Micromorphological characteristics of two-week-old cultures of *T. harzianum* grown on PDA were examined. Conidia were globose to sub-globose, and size measured by micrometry (2.8x2.6 µm) (Fig. 5E).

***In vitro* Evaluation of chemicals and *Trichoderma harzianum*:** A total of eight fungicides (Table 1) were tested with four doses for all fungicides, their effective concentration in reducing pathogen growth varied (Fig. 1a and 1b). Based on *In vitro* efficacy, test fungicides were divided into 2 groups. The promising group consists of Difenoconazole, Carbendazim, Fluoxastrobin and Thiophanate Methyl. These appeared more effective than the others, even at low concentrations. In the carbendazim treatments, the fungal pathogen completely failed to grow at 400 ppm, whereas 96 and 88% reduction in colony diameter of *F. oxysporum* was recorded at 300 and 200 respectively after 16 days of incubation (Fig. 1c). Similarly, Fluoxastrobin and Difenoconazole also suppressed the pathogen to 96% at 400 ppm, while Thiophanate Methyl inhibited the growth up to 88% at 400 ppm after 16 days of incubation. The fungicides Fosetyl Aluminium, Sulfur, Hexaconazole and Tebuconazole + Trifloxystroben, comprised the 2nd group, found less effective against *F. oxysporum*. Aliette was the least effective and showed 20, 40, 52 and 64% reduction at 400 ppm after 16 days, whereas its efficacy was even lower than the other at low doses. Sulfur, Hexaconazole and Tebuconazole + Trifloxystroben also showed little effect on the pathogen's growth. Despite the health hazards of chemical control, it is a fast and standard method to manage crop diseases, and eight chemicals were tested *In vitro* using the poison agar technique. A positive correlation was observed between concentration and inhibition of the pathogen's colony growth. Generally, high concentration of fungicides was more effective than low doses. There have been several

reports regarding the *In vitro* evaluation of fungicides against *F. oxysporum* f.sp *fragariae*. Our results confirm existing published results by Maitlo *et al*., (2014) where carbendazim and thiophanate-methyl were most promising among 12 test chemicals against *F. oxysporum.* Moreover, similar results have also been reported by Akhtar *et al*., (2017), Poddar *et al*., (2004), Khan *et al*., (2012) and Sultana & Ghafar (2010). Our investigations indicate that four fungicides efficiently inhibit the pathogen's growth (*In vitro*) and are probably effective against the same pathogen under agricultural field conditions; therefore, the four most effective fungicides (*In vitro*) were preceded further to greenhouse studies.

The dual-culture test of the biocontrol agent (Fig. 5F) *T. harzianum* (Th_3) isolated from vegetable fields resulted in 92.0% inhibition over control after 16 days of incubation. Th_3's inhibition increased after each incubation interval, 52 and 72% after 8 and 12 days, respectively. In comparison, the isolates purified from the dung and rice field exhibited 68% of growth inhibition after 16 days. Fusarium wilt is a newly reported disease in Pakistan and can become a future threat for strawberry growers as it is a well-known soil and nursery soil-borne pathogen. The current experiments were conducted with the idea that bio-control agents may effectively be used against the Fusarium wilt and considered an excellent alternative to hazardous fungicides (Maltby *et al.*, 2009). Although fungicides are widely used, most of these are considered hazardous to the farmer, the environment and might change soil microbial populations to the negative and should be replaced by more eco-friendly biological management (Frampton *et al*., 2006). Our findings were entirely in agreement with number of scientists who found that the isolates *T. harzianum* isolated from different rhizospheres were found to manage the plant pathogens as well (Burr *et al*., 1998, Postma *et al*., 2003 and Saikia *et al*., 2003). Merkuz & Getachew (2012) found that Trichoderma isolates were very effective against *F. oxysporum*. Trichoderma's species were found superior to *Aspergillus niger* and *Bacillus subtilis* as biocontrol agents (Dubey *et al.,* 2007).

Four fungicides and one isolate of *T. harzianum* (Fig. 3) showing better inhibition were selected to be evaluated in greenhouse experiments where suckers were transplanted in artificially inoculated earthen pots, while controls were kept without biocontrol agents added.

The results indicated that all fungicidal treatments appeared to be effective compared to controls and minimum disease incidence was recorded. Finally, three chemicals and one isolate of *T. harzianum* were kept for field trials since Fluoxastrobin exhibited the least protection value (68%). The results revealed that Thiophanate Methyl showed 88%, *T. harzianum* 80%, while Carbendazim and Difenoconazole gave 72% protection value after 42 days in the field (Fig. 4).

Although there are many chemical control reports of Fusarium wilt in strawberry but pathogen management by *T. harzianum* has not been reported yet. The fungicides which efficiently reduced the pathogen growth *In vitro* are supposed to be effective against the same pathogen under field trials with varying degrees of success. Although carbendazim was leading to inhibit the colony growth *In vitro* but performance of Thiophanate Methyl was better in the field and greenhouse. Some researchers have reported that seed treatment with carbendazim reduce disease incidence significantly and increased yield (Kamdi *et al.*, 2012). The current study concludes that fungicide applications (Thiophanate Methyl, Difenoconazole and Carbendazim) can be useful tools for Fusarium wilt control. The application of *T. harzianum* was also a worthy alternative and can be a more environmentally friendly tool for managing the soil borne plant pathogen in future.

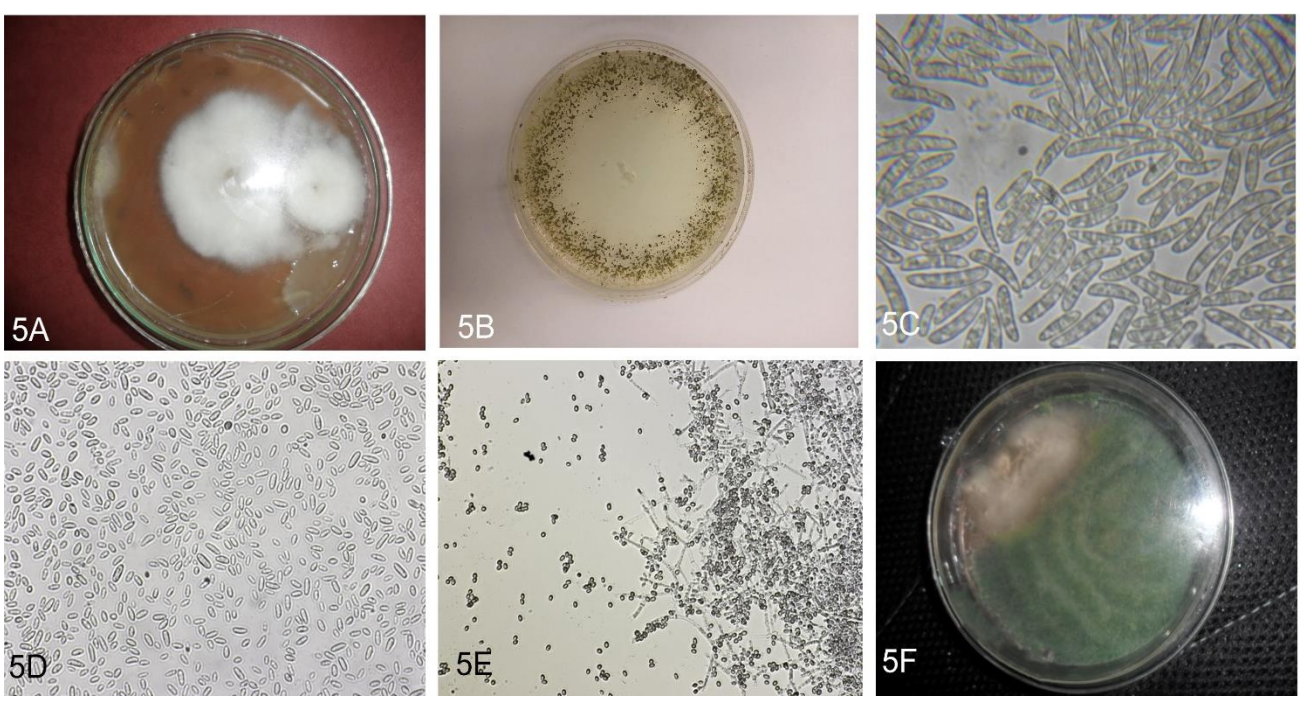

Fig. 5. (A-F) Colony of *Fusarium oxysporum* (5A) Colony of *Trichoderma. harzianum*; (5B) Macroconida of *Fusarium oxysporum* f. sp. *fragariae*; (5C) Microconidia of *Fusarium oxysporum* f. sp. *fragariae*; (5D) Conidia and mycelia of *T. harzianum* (5E) Dual Culture (5F).

Acknowledgement

This work was done with a start-up grant from the Higher education Commission (HEC) of Pakistan. We also thank the First Fungal Culture Bank of Pakistan University, Punjab Lahore, for providing biocontrol culture and pathogen identification.

References

Akhtar, T., Q. Shakeel, G. Sarwar, S. Muhammad, Y. Iftikhar, M.I. Ullah, M. Mubeen and A. Hannan. 2017. Evaluation of fungicides and biopesticides for control of fusarium wilt of tomato. *Pak. J. Bot*., 49(2): 769-774.

Barnett, H.L. and B.B. Hunter. 1998. *Illustrated genera of imperfect fungi*, 4th Edition. The American Phytopathological Society, St. Paul, MN, USA, p. 130.

Borum, D.F. and J.B. Sinclair. 1968. Evidence for systemic fungicides protection against *Rhizoctonia solani* with Vitavax in cotton seedlings. *Phytopathol.*, 58: 976-980.

Burr, A., A. Ortuno and T. Armero. 1998. Phosphate solubilizing effect of *Aspergillus niger* and Pseudomonas. *Microb. Espan.*, 30: 113.

Dubey, S.C., M. Suresh and B. Singh. 2007. Evaluation of Trichoderma species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. *Biol. Cont*., 40: 118-127.

Fang, X., P.M. Finnegan and M.J. Barbetti. 2013. Wide variation in virulence and genetic diversity of binucleate Rhizoctonia isolates associated with root rot of strawberry in Western Australia. *PloS One.*, 8(2): 558-77.

Frampton, G.K., S. Jänsch, J.J. Scott-Fordsmand, J. Römbke and P.J.V. Brink. 2006. Effects of pesticides on soil invertebrates in laboratory studies: A review and analysis using species sensitivity distributions. *Environ. Toxic. Chem*., 25: 2480-2489.

Hussain S.S. and W.E. Mackeen.1963. *Rhizoctonia fragaria* spp. nov. in relation to strawberry degeneration in southwestern Ontario. *Phytopathol.* 53: 532-540.

Joshi, M., R. Srivastava, A.K. Sharma and A. Prakash. 2013. Isolation and characterization of *Fusarium oxysporum*, a wilt causing fungus, for its pathogenic and non-pathogenic nature in tomato (*Solanum lycopersicum*). *J. Appl. Nat. Sci.*, 5(1): 108-117.

Kamdi, D.R., M.K. Mondhe, G. Jadesha, D.N. Kshirsagar and K.D. Thakur. 2012. Efficacy of botanicals, bio-agents and fungicides against *Fusarium oxysporum* f. sp. *ciceri*, in chickpea wilt sick plot. *Ann. Biol. Res*., 3(11): 5390-5392.

Khan, H.S.I., M. Saifulla, A.S.N. Nawaz, P.R. Somashekharappa and R. Razvi. 2012. Efficacy of fungicides and biocontrol agents against *Fusarium oxysporum* f. sp. *ciceris* causing wilt of chickpea. *J. Environ. Ecol.*, 3: 570-572.

Koike, S.T., S.C. Kirkpatrick and T.R. Gordon. 2009. Fusarium wilt of strawberry caused by *Fusarium oxysporum* in California. *Plant Dis.*, 93(10): 1077-1077.

Maitlo, S.A., R.N. Syed, M.A. Rustamani, R.D. Khuhro and A.M. Lodhi. 2014. Comparative efficacy of different fungicides against Fusarium Wilt of Chickpea (*Cicer ariatinum* L.). *Pak. J. Bot.*, 46(6): 2305-2312.

Maltby, L., T.C.M. Brock and P.J. Brink. 2009. Fungicide risk assessment for aquatic ecosystems: importance of interspecific variation, toxic mode of action, and exposure regime. *Environ. Sci. Tech*., 43: 7556-7563.

Martin, F.N. and J.E. Loper. 1999. Soil-borne plant diseases caused by *Pythium* spp.: Ecology, epidemiology, and prospects for biological control. *Crit. Rev. Plant Sci.*, 18(2): 111-181.

Merkuz, A. and A. Getachew. 2012. Epidemic of Fusarium wilt (*Fusarium oxysporum*f. sp. *ciceri*) of chickpea at wilt sick plot in adetethiopia. *Int. J. Curr. Res*., 4(05): 135-141.

Mingzhu, L., M. Inada, H. Watanabe, H. Suga and K. Kageyama. 2013. Simultaneous detection and quantification of *Phytophthora nicotianae* and *P. cactorum*, and distribution analyses in strawberry greenhouses by duplex real-time PCR. *Microb. Environ*., 28(2): 195-203.

Moon, B.J., H.S. Chung and H.C. Park. 1995. Studies on antagonism of *Trichoderma* species to *Fusarium oxysporum* f. sp. *fragariae:* V. Biological control of *Fusarium* wilt of strawberry by a mycoparasite, *Trichoderma harzianum*. *Kor. J. Plant Pathol.*, 11: 298-303.

Nam, M.H., M.S. Park, H.G. Kim and S.J. Yoo. 2009. Biological control of strawberry *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *fragariae* using *Bacillus velezensis* BS87 and RK1 formulation. *J. Microbiol. Biotechnol.*, 19: 520-524.

Okayama, K., H. Kobatake and T. Kodama. 1991. Selection and effect of antagonists on *Fusarium* wilt of strawberries. *Bull. Nara Agric. Exp. Stn*., 22: 17-22.

Poddar, R.K., D.V. Singh and S.C. Dubey. 2004. Management of chickpea wilts through combination of fungicides and bioagents. *Indian Phytopathol*., 57: 39-43.

Postma, J., M. Montanari and J.F. Van den Boogert. 2003. Microbial enrichment to enhance disease suppressive activity of compost. *Eur. J. Soil Biol.*, 39: 157-163.

Rifai, M.A. 1969. A revision of genus Trichoderma. *Mycol. Papers*., 116: 1-5.

Saikia R., T. Singh, R. Kumar, J. Srivastava, A.K. Srivastava, K. Singh and D.K. Arora. 2003. Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* in chickpea. *Microbiol. Res*., 158: 203-213.

Samuels, G.J., S.L. Dodd, W. Gams, L.A. Castleburry and O. Petrini. 2002. Trichoderma species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycol.*, 94: 146-170.

Sultana, N. and A. Ghaffar. 2010. Effect of fungicides, microbial antagonists and oilcakes in the control of *Fusarium solani*, the cause of seed root, seedling and root infection of bottle gourd, bitter gourd and cucumber. *Pak. J. Bot.*, 42(4): 2921-2934.

Tezuka, N. and T. Makino. 1991. Biological control of *Fusarium* wilt of strawberry by nonpathogenic *Fusarium oxysporum* isolated from strawberry. *Ann. Phytopath. Soc. Jpn*., 57: 506-511.

Thomas, H.E. 1932. Verticillium wilt of strawberries. *Univ. Calif. Agr. Expt. Sta. Bul.*, 530.

Veesar, M.A., A.A. Ujjan, Y.F. Kazi, J.A. Ujan and S. Shahzad. 2015. New host record of *Fusarium oxysporum* Schlecht., in Pakistan causing wilt of strawberry (*Fragaria ananssa* Duch) and *In vitro* control. *Int. J. Biol. Biotech.*, 12: 423-429.

Watanabe, T. 2010. Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species. CRC Press. USA.

Wilcox, W.F., P.H. Scott, P.B. Hamm, D.M. Kennedy, J.M. Duncan, C.M. Brasier and E.M. Hansen. 1993. Identity of a Phytophthora species attacking raspberry in Europe and North America. *Mycol. Res.*, 97(7): 817-831.

Zhang, Y., T. Fan, W. Jia, W. Zhang, Q. Liu, B. Li and L. Zhang. 2012. Identification and characterization of a *Bacillus subtilis* strain TS06 as bio-control agent of strawberry replant disease (*Fusarium* and *Verticillium* Wilts). *Afr. J. Biotechnol*., 11: 570-580.

(Received for publication 12 May 2024)