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THE EFFICIENT USE OF DIFFERENT FUNGAL BIOAGENTS FOR ECO-FRIENDLY MANAGEMENT OF FUSARIUM WILT DISEASE OF TOMATO

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A B S T R A C T

Tomato (*Lycopersicon esculentum* L.) is one of the most important vegetables, used as condiments, salad and cooked with various recipes. It is a good source of vitamin C, A, calcium, iron etc. However, per acre yield in Pakistan is quite lower as compared to other countries of the world due to various factors, including diseases. Tomato crop suffers by approximately 200 diseases worldwide, among them, Fusarium wilt of tomato is highly destructive fungal disease and has caused losses up to 49.5% in Pakistan. Early strategies to manage this devastating disease include the use of cultural, physical, and chemical control are suggested, but none of these provided positive impact except cultural methods. Keeping in view the importance and losses caused by Fusarium wilt of tomato the studies on its management with fungal bioagents was conducted. For this. different bioagents were tested in lab and fields against *Fusarium oxysporum* f.sp. *lycopersici*. The highest inhibition 89.63% was noted by *Trichoderma harzianum* followed by Neurospora sp. 87.40% in the poisoned food method. In pot experiments, the *T. harzianum* was found as highly successful then *Neurospora* sp., *Chaetomium subaffine* and *Arthrinium* sp. providing minimum disease incidence and lowest mortality percent at higher and medium concentrations. The highest plant biomass and lowest root infection percent were noted in *T. harzianum* followed by *Neurospora* sp., *C. subaffine* and *Arthrinium* sp. at medium and lower doses. The lowest response was recorded by *Nigrospora sphaerica* and *Dermateaceae* sp. In in-vivo trials, the highest response was observed in the treatment of *T. harzianum* followed by *Neurospora sp., Arthrinium sp., N. sphaerica*, respectively. The highest fruit yield was recorded by *T. harzianum* 6.66 (kg) and Neurospora sp. 5.66 (kg). Interestingly, *Neurospora sp.*, *Arthrinium* sp., *N. sphaerica*, and *Dermateaceae* sp. have been first time recognized in the current study as potential bioagents against fusarium wilt of tomato. Based on the findings, it is suggested that *T. harzianum* and *Neurospora* sp., may be used as potential bioagents for ecofriendly management of tomato wilt disease.

Keywords: Tomato, wilt, *Fusarium oxysporum* f. sp. *lycopersici,* bioagent, eco-friendly management.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is recognized as an important vegetable food crop produced all over the world. The warm climate regions are more suitable for its cultivation. South America was the initial origin of tomato and is now cultivated in diverse parts of the world (Gerszberg *et al.*, 2015; Saavedra *et al.*, 2017). It is used as fresh fruit and salad whereas many fruit items are also prepared from tomatoes such as tomato juice, tomato ketchup, soup, drinks, and many other dishes (Bjarnadottir, 2015). It contains 95.3% water, niacin and 0.07% calcium, vitamins A, C and E and a rich source of nutrients such as Na, K, Fe, and antioxidants especially lycopene and salicylate. All have great importance in the human metabolic system (Afzal

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et al., 2013; Domínguez *et al*., 2020).

It is grown in 144 countries around the world. Among them, China, the United States, Turkey, India and Egypt are the most important tomato producers in the world. Pakistan has a planting area of 58,400 hectares and a yield of 550,000 tons, with an average yield of 9,400 tons per hectare (FAO, 2019). Sindh province is a major tomato producing region of Pakistan, where it is cultivated on 21 thousand hectares in area with general production of 153.3 thousand tons and 7.3 thousand tons yield per hectare. This common yield is at a low level as compare to different tomato cultivating nations. In terms of tomato yield per hectare, USA is on top producing 96.80 t/ha followed by China 59.25 t/ha, Egypt 40.96 t/ha, India 24.65t/ha and Pakistan 9.44 t/ha respectively (GOP, 2019).

The tomato plant is highly susceptible to various biotic and abiotic diseases at different critical growth stages from seedling stage to maturity (Yasin *et al*., 2017). Tomatoes are very sensitive to about 200 diseases caused by pathogenic fungi, nematodes, bacteria and viruses during cultivation or postharvest storage (Parker *et al*., 1997; Singh *et al*., 2017). Plant diseases are major challenge in crops although our greatest efforts to fight them. In tomato crop early blight, anthracnose, bacterial wilt, bacterial canker, tomato spotted wilt, verticillium wilt and bacterial wilt diseases are considered important threats (Dodson, 2002; Van Esse *et al*., 2020).

Among the fungal wilt diseases, tomato wilt caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans, that almost affect the vascular bundle and causing production losses between 30% to 40% and it may even reach up to 90% (Gupta, 2006; Nirmaladevi *et al*., 2016). The drooping, yellowing, wilting, and dying of the lower leaves, regularly on one part of the plant are the common symptoms of this disease (Maurya *et al*., 2019).

Fusarium wilt is a very challenging disease (Khan *et al.,* 2020). Various control strategies are suggested for this fungus and the disease is mostly controlled by the uses of fungicides (Song *et al*., 2004). Recently Patiyal *et al*. (2020) assessed six different fungicides against fusarium wilt disease of tomato *in-vitro* conditions and recommended Custodia fungicide as a possible control measure. The overuse and misuse of many fungicides harm the environment and enhances the resistance to pathogen populations. The chemicals do not only affect the nutritional substances of tomatoes but also affect the quality and output of soil (Ozgonen, 1999; Singh *et al*., 2017). However, due to the development of new pathogenic races efforts to control the disease have limited success.

The use of cultural, physical and chemical control including early methods to deal with this destructive plant disease. None of these methods were able to generate a clear impact, except for the cultural and resistance varieties approaches, which are the main prevention (Ajilogba and Babalola, 2013). Now the Scholars and scientists are looking for eco-friendly alternative disease management plans (Javaid *et al*., 2015). As the environment requires more rigorous regulations and the use of chemicals to control plant diseases has become an expensive treatment and may also decrease the number of useful microorganisms in the soil, so the biological treatment of plant diseases has become more and more attractive (Cook, 1993). Biological management of tomato fusarium wilt has become another potential disease control strategy (Alabouvette *et al*., 1998). Several studies deal with biological management of fungal diseases by using nonpathogenic fungal bioagents and beneficial bacteria that reached their uses in the field and green house refer to high efficacy (Bubici *et al*., 2019).

The purpose of this study was to explore the alternative and eco-friendly management of fusarium wilt by using efficient fungal bioagents for sustainable tomato production. Based on the above-mentioned facts it was planned to use fifteen promising biocontrol agents for the eco-friendly management of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) to minimize the economic loss in tomato.

MATERIALS AND METHODS

***In-vitro* assay of different biocontrol agents**: The experiments were conducted at Sindh Agriculture University Tando Jam, Sindh Pakistan from June to October 2016 and 2017. The test fungus *Fusarium oxysporum* f.sp. *lycopersici* (FOL) was isolated from roots and branches of the tomato (*Lycopersicon esculentum* L.) plant showing the fusarium wilt typical symptoms. The pure cultures of fifteen different bioagents viz., *Pestalotiopsis humus, Arthrinium* sp., *Dermateaceae* sp., *Neurospora* sp*., Alternaria* sp*., Hypocrea lixii, Fusarium cf. solani, Trichoderma harzianum, Colletotrichum gloeosporioides, Chaetomium subaffine, Corynespora cassiicola, Nigrospora sphaerica, Pestalotiopsis mangiferae, Xylariaceae* sp. and *Botryosphaeria parva* were brought from State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences No. 1, Beijing 100101, China and purified in potato dextrose agar (PDA) medium. The dual culture method was used to screen all bioagents against the test pathogens. For this, the 5 mm disc of the test fungus and biocontrol agents were inoculated at opposite sides of each other into a 90 mm Petri dish containing sterilized PDA. The plate inoculated with the pathogenic fungus was used as control. The inoculated plates were incubated at 25±1°C. Drew a straight line in the center of the two colonies with permanent markers and the data of the colony diameter of the test pathogen and biocontrol fungus was recorded after every 24 hours till one week. . The following formula was used to determine the percent inhibition of radial growth (PIRG) of all tested bio-control agents (Abd-El-Khair *et al*., 2010; Jomduang and Sariah, 1997).

$$\text{PIRG} = \frac{\text{R1 - R2}}{\text{R1}} \times 100,$$

Among them, R1 is the radial growth of the test fungus on the control plate (without any antagonistic fungi), and R2 is the radial growth of the test fungus on the treated plate (toward the antagonistic colony).

Effect of different bioagents on plant growth and disease development: Pot experiment assay: The seeds of locally grown tomato cultivar 'Desi local' were sterilized with 5% sodium hypochlorite for 2 minutes followed by washing with sterile water twice. Ten seeds per pot were grown in each earthen pot measuring 20 cm diameter contained 2-kilogram steam-sterilized soil. The soil was incorporated with the test pathogen suspension of 10^5 conidia ml^{-1}. The culture of selected antagonistic fungi at different concentrations i.e.10^3, 10^4 and 10^5 conidia ml^{-1} with the help of hemocytometer were also amended in these pots containing sterilized soil. The seeds were slightly covered with a tinny layer of soil. The study design was a complete randomized design (CRD) with five replicates. The pots were kept in a greenhouse and irrigated with sterilized water. The percentages of disease incidence (DI%) and mortality of plants were recorded when the initial disease symptoms appeared. The plants were uprooted and cleaned with tap water after 40 days of sowing and the data of the root infection percentage (RI%) and plant biomass (plant height + weight) were noted. The RI% data was recorded by using the following formula:

$$\text{RI\%} = \frac{n}{N} \times 100,$$

where RI% is root infection percentage, n - number of root pieces colonized by the fungus, N – total number of pieces studied.

Field experiment assay: The six-best evaluated bioagents *in-vitro* trials were selected for the field experiment. The trial was laid down as RCBD having five replications. The plot size was maintained as five rows of ten meters each. The distance between row to row and plant to plant was kept 75 and 15cm, respectively. The culture of bioagents was multiplied on sand maize meal water medium (90g sand, 10 g maize meal, 20 ml distilled water). Tomato seeds were treated with bioagents solution of 10^6 conidia ml^{-1} at the rate of 1 ml 10 g^{-1} (Maurya *et al.,* 2019). The field was irrigated up to 50% moisture-holding capacity. The DI%, mortality, RI%, plant biomass (plant height + weight) and fruit yield (g) were recorded.

STATISTICAL ANALYSIS

Statistical analysis of the data was carried out using software *Statistix*, version 8.1. To judge the treatment effect, ANOVA was used. For average separation, the least significant difference (LSD) test was used, and the significant difference between treatments was used $P<0.05$.

RESULTS

***In-vitro* efficacy of different bioagents:** The *in-vitro* dual culture assay of fifteen various bioagents, i.e., *Trichoderma harzianum, Neurospora* sp., *Chaetomium subaffine, Arthrinium* sp, *Nigrospora sphaerica*, *Dermateaceae* sp., *Colletotrichum gloeosporioides, Pestalotiopsis mangiferae, Pestalotiopsis humus*, *Botryosphaeria parva*, *Xylariaceae* sp., *Alternaria* sp., *Corynespora cassiicola, Fusarium* cf. *solani* and *Hypocrea lixii* exhibited significant variation among the treatments. The data of the colony diameter of the test pathogen and biocontrol fungus was recorded after every 24 hrs till one week. All bioagents inhibited the mycelial colony growth of tested fungus ranging from 22.59% to 89.63%. The significantly maximum inhibition 89.63% was observed by *T. harzianum* followed by *Neurospora* sp. 87.40%, whereas, there were no significant differences among *C. subaffine, Arthrinium* sp., *N. sphaerica, Dermateaceae* sp. and *C. gloeosporioides*. The lowest inhibition 22.59% was recorded by *Hypocrea lixii* followed by *F. cf. solani* 23.33%, *C. cassiicola* 23.70% and *Alternaria* sp. 39.25% respectively (Figure 1). The *Xylariaceae* sp., *B. parva*, *P. humus* and *P. mangiferae* performed moderate efficacy against the test fungus.

Effect of different bioagents on plant growth and disease development: Pot experiment effect: The disease incidence of tomato plants was significantly decreased with the application of bio-control agents as compared to the control. DI% was recorded higher in all untreated plants. However, it was decreased with the increase in concentrations of antagonistic fungi. The *T. harzianum* was found as highly effective which provided the lowest 18.66 and 24.33 DI% at their higher and medium (10^5 and 10^4 conidia g^{-1} of soil) concentrations. The *Neurospora sp.* was recorded as the second most effective antagonistic fungus followed by *C. subaffine* and Arthrinium sp., while the

Dermateaceae sp. was recorded as less effective as compared to others, whereas *N. sphaerica* and *Arthrinium* sp. gave a moderate response at higher and medium concentrations (Figure 2).

Similarly, plant mortality was significantly reduced with the applications of bio-control fungi as compared to untreated plants (control). The minimum 16.66% and 21% mortality was observed in plants treated with *T. harzianum* followed by *Neurospora* sp. 22% and 25% at higher and medium concentrations. The *N. sphaerica* and *Dermateaceae* sp. were observed as less effective in providing maximum mortality percent at minimum concentrations as compared to other antagonistic fungi (Figure 3).

The less root infection (RI%) was found in plants treated with bio-control fungi along with pathogen as compared to alone pathogen treated plant (control). The minimum RI% was observed by *T. harzianum* 18% and 22% followed by *Neurospora* sp., *C. subaffine* and *Arthrinium* sp. at higher and medium concentrations; whereas *N. sphaerica* and *Dermateaceae* sp. were found as less effective as compared to other tested antagonistic fungi. The maximum RI% was recorded in inoculated-untreated plant 77.33% (Figure 4).

The bio-control fungi did not reduce only the infection percent of the pathogen but also enhance the plant growth. The highest plant height was recorded in plants treated with *T. harzianum* 34, 31.33 and 27 cm at 10^5, 10^4 and 10^3 conidia ml^{-1} of soil followed by *Neurospora* sp., *C. subaffine* and *Arthrinium* sp. The lowest plant biomass was observed by *Dermateaceae* sp. and *N. sphaerica*. Similarly, the plant fresh weight was higher in the treated plants as compared to untreated plants. The highest plant weight (1.95 g) was observed by *T. harzianum* at a higher dose. The lowest weight (0.36 g) was observed by *Dermateaceae* sp. followed by *N. sphaerica* at the lowest dose. *C. subaffine* and *Arthrinium* were observed as moderate effective as compared to other bioagents (Table 1).

Table 1. Effect of different bio-control agents on plant height and weight of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in a pot experiment.

Treatment	Concentrations of bioagents (conidia ml $^{-1}$)					
	1000		10000		100000	
	Plant height (cm)	Plant weight (g)	Plant height (cm)	Plant weight (g)	Plant height (cm)	Plant weight (g)
Control	10.333 l	0.2033 k	10.333 l	0.2033 k	10.333 l	0.2033 k
Dermateaceae sp.	16.333 k	0.3633 j	19.333 j	0.44 ij	21.333 hi	0.55 jh
Nigrospora sphaerica	17.00 k	0.3633 j	20.00 ij	0.44 ij	22.00 ih	0.5867 jh
Arthrinium sp	19.667 j	0.4333 ij	22 .00gh	0.5133 hi	24 .00ef	0.7333 f
Chaetomium subaffine	20.333 ij	0.54 gh	23.333 fg	0.7567 f	25.333 de	0.8733 e
Neurospora sp.	23.00 fg	0.6167 g	25.667 cd	0.9167 e	31.00 b	1.1367 d
Trichoderma harzianum	27.00 c	1.35 c	31.333 b	1.5133 b	34.00 a	1.95 a
SE	0.8165	0.0425	0.8165	0.0425	0.8165	0.8165
LSD	1.6478	0.0858	1.6478	0.0858	1.6478	1.6478

Note. Mean values following the similar letter within a column are not significantly different according to the LSD (least significant difference) test at $P < 0.05$.

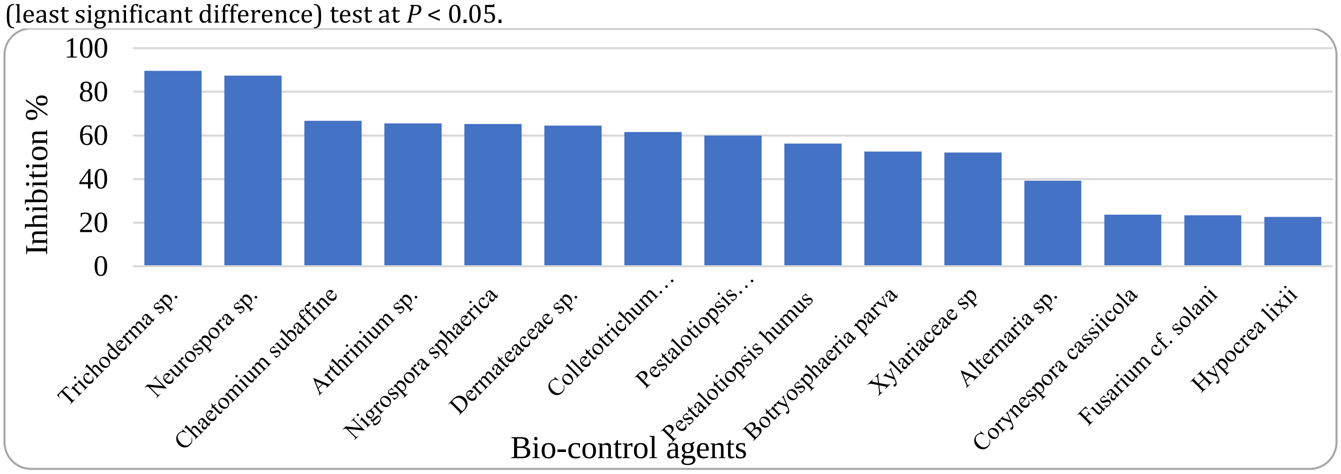

Figure 1. Effect of different bio-control agents on inhibition of radial colony growth of *Fusarium oxysporum* f. sp. *lycopersici* (FOL).

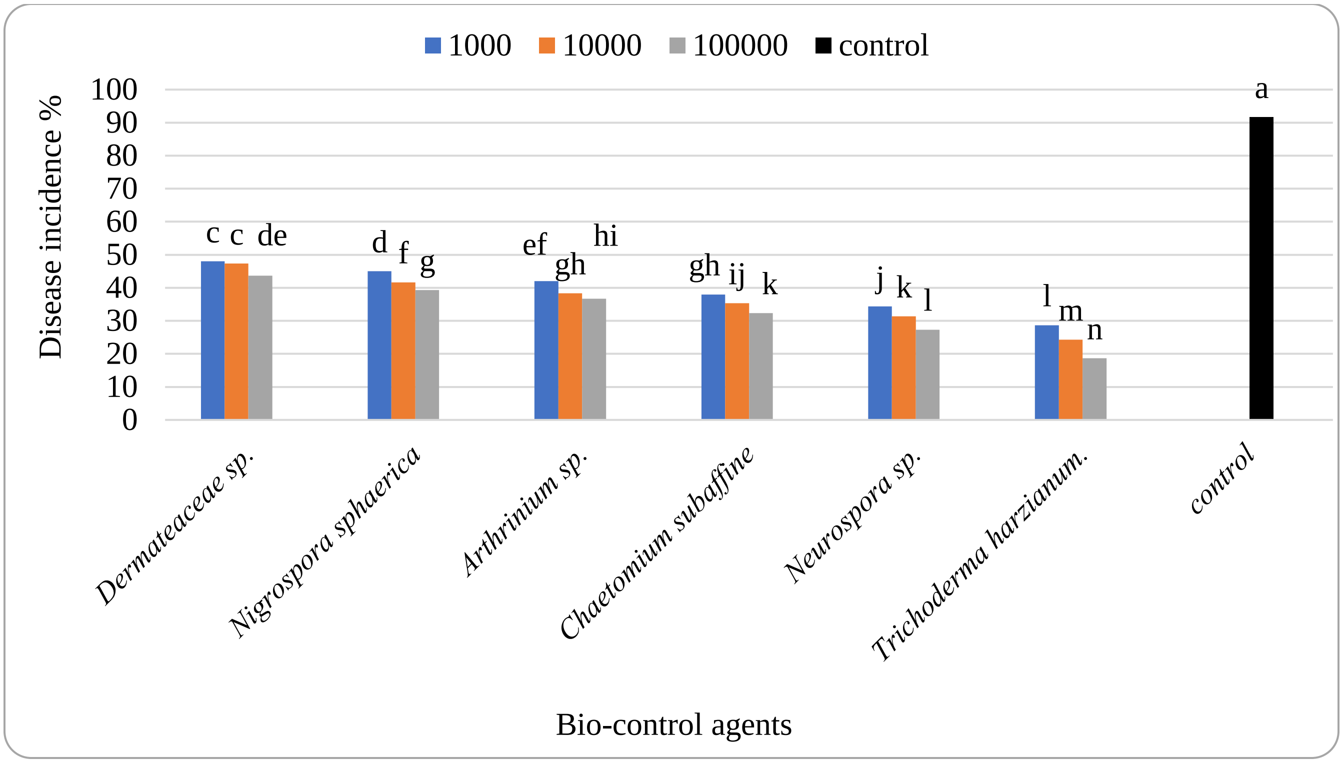

Figure 2. Effect of different bioagents on the disease incidence of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in a pot experiment

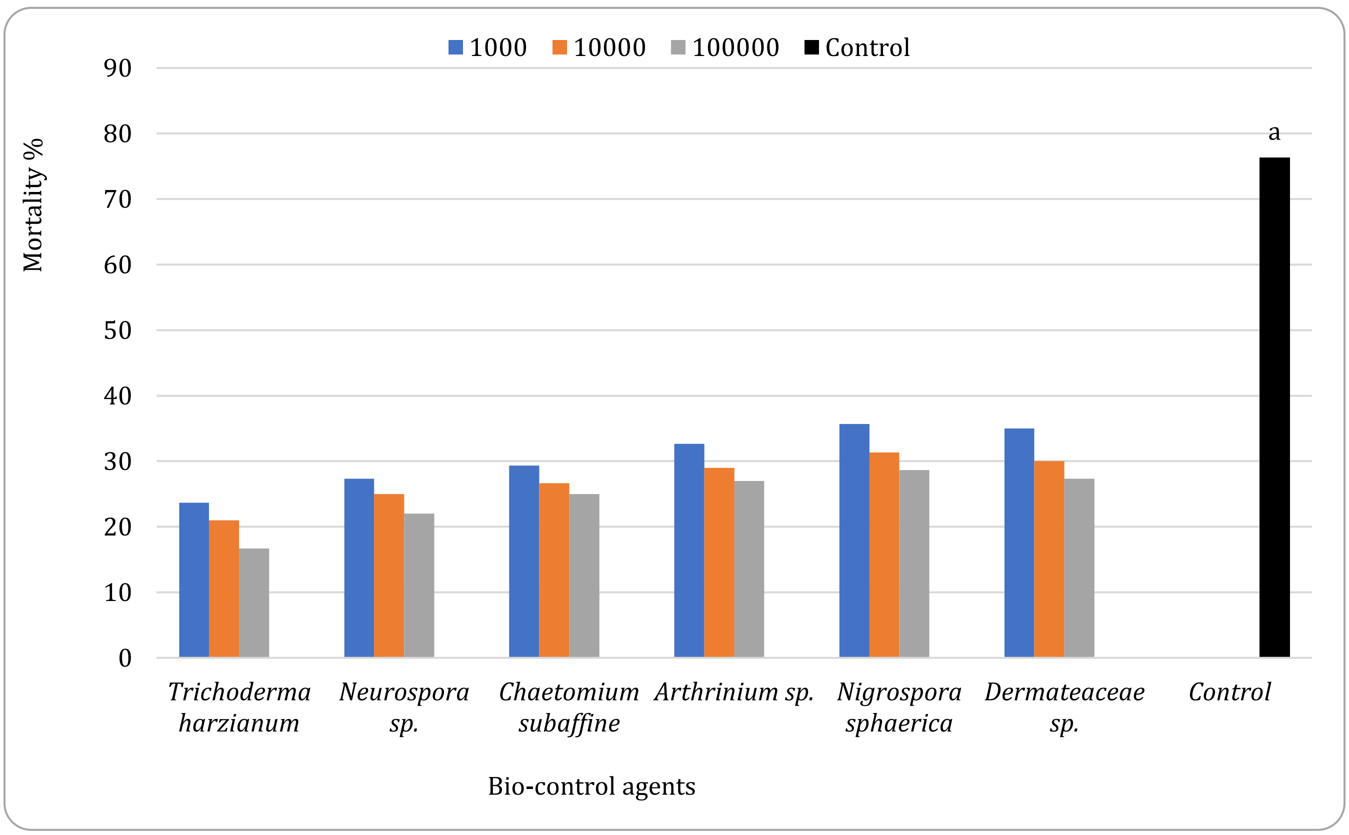

Figure 3. Effect of different bio-control agents on mortality of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in a pot experiment.

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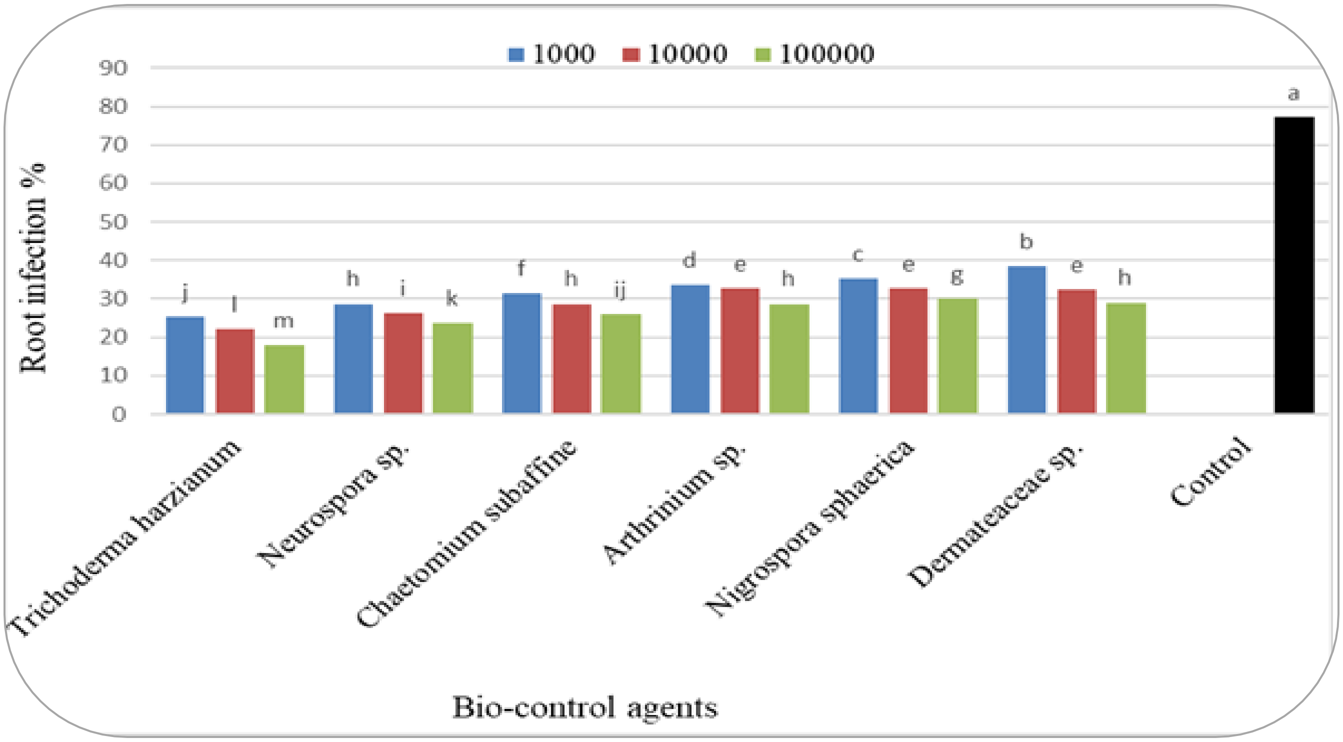

Figure 4. Effect of different bio-control agents on root infection of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in a pot experiment.

Field experiment effect: The applications of antagonistic fungi under the field conditions significantly reduced the DI% in treated plants as compared to untreated. The minimum DI% was noted in plants treated with *T. harzianum* 11% followed by *Neurospora* sp. 23%. There was no significant difference among the plants treated with *N. sphaerica*, *Dermateaceae* sp. and *C. subaffine.* The maximum DI% was recorded in untreated plants 78.33% followed by *Arthrinium* sp. 30.66% (Figure 5).

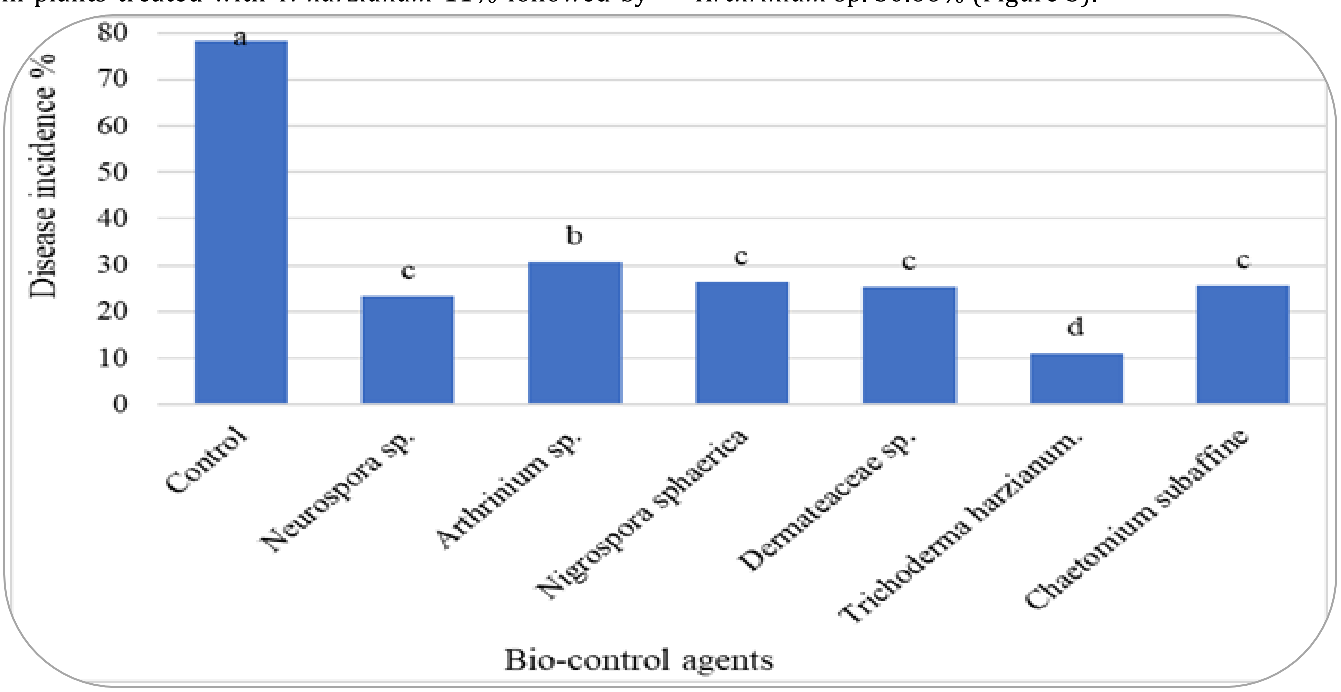

Figure 5. Effect of different bio-control agents on disease incidence of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) under field conditions.

Similarly, the bioagents had a significant impact on the morality percent of tomato plants. The lowest mortality percent was recorded in plants treated with *T. harzianum* 7.33% followed by *Neurospora* sp. 9.66%, *C. subaffine* 12.33%, *Dermateaceae* sp. 13.66%, *N. sphaerica* 15% and *Arthrinium* sp. 17%. The maximum mortality was recorded in untreated plants 67% under field conditions (Figure 6).

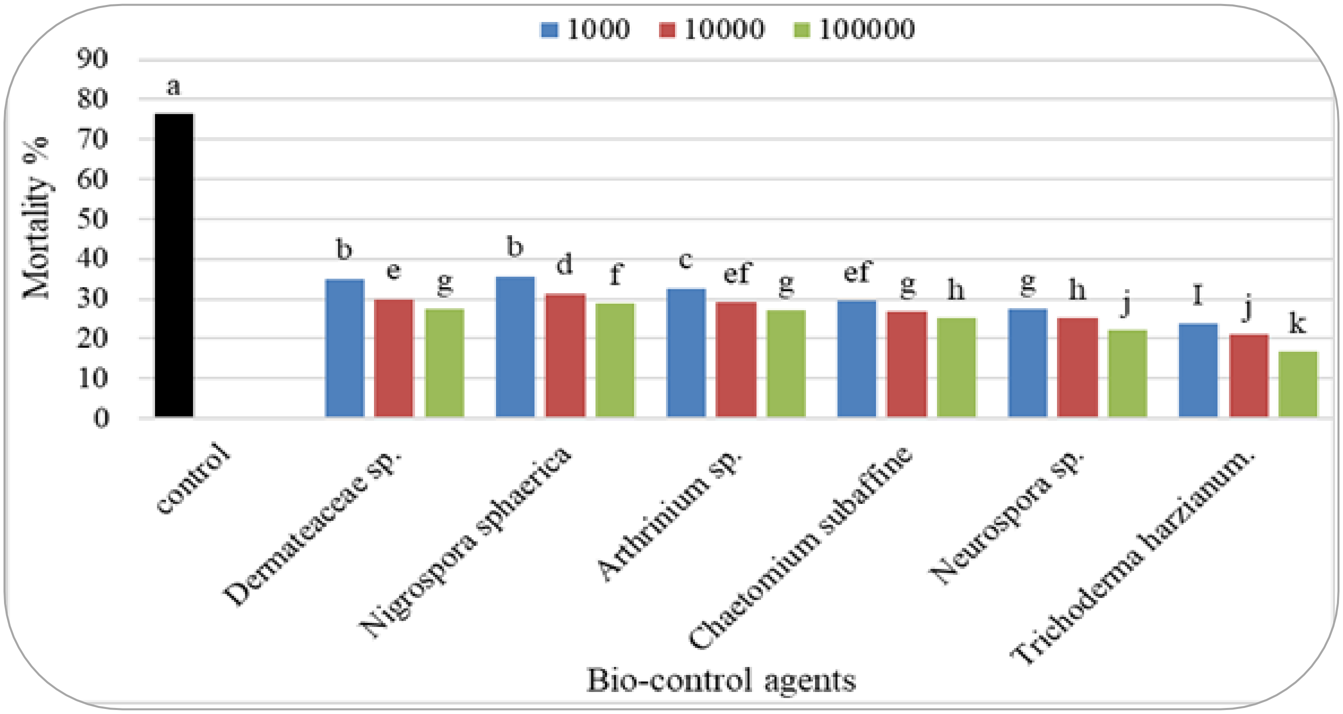

Figure 6. Effect of different bio-control agents on mortality of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) under field conditions.

The antagonistic were also found as effective in the field in a relation to plant biomass. The highest plant height was noted by *T. harzianum* (106 cm) followed by *Neurospora* sp. (98.33 cm), *Arthrinium* (96.33 cm), *N. sphaerica* (96.67 cm), *C. subaffine* and *Dermateaceae* sp. (93 cm). The minimum plant height (89.33 cm) was recorded in untreated plants (Table 2). The highest fresh weight was noted in plants treated with *T. harzianum* (607 g) followed by *Neurospora* sp. (578.33 g), *C. subaffine* (553 g), *N. sphaerica* (546.67 g), *Dermateaceae* sp. (545 g) and *Arthrinium* sp. (539.67 g), the lowest weight 477.67 g was recorded in untreated plants.

Table 2. Effect of different bio-control agents on plant height and weight of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in the field experiment.

Treatment	Plant height (cm)	Plant weight (g)
Control	89.33 d	477.67 f
Neurospora sp.	98.33 b	578.33 b
Arthrinium sp.	96.33 bc	539.67 e
Nigrospora sphaerica	95.67 bc	546.67 d
Dermateaceae sp.	93.00 c	545.00 d
Trichoderma harzianum	106.00 a	607.00 a
Chaetomium subaffine	93.33 c	553.00 c
SE	1.5516	1.1841
LSD	3.3806	2.5800

Note. Mean values following the similar letter within a column are not significantly different according to the LSD (least significant difference) test at $P < 0.05$.

Applications of tested biological fungi in the field was effective as the significantly lowermost pathogen infection percent was recorded by *T. harzianum* 19.33% followed by *Neurospora* sp. 25.33%, *C. subaffine* 29.33%, *N. sphaerica* 30.66%, *Dermateaceae* sp. 32% and *Arthrinium* sp. 33%. The highest pathogen infection 89.66% was noted in un-treated (control) plants (Figure 7).

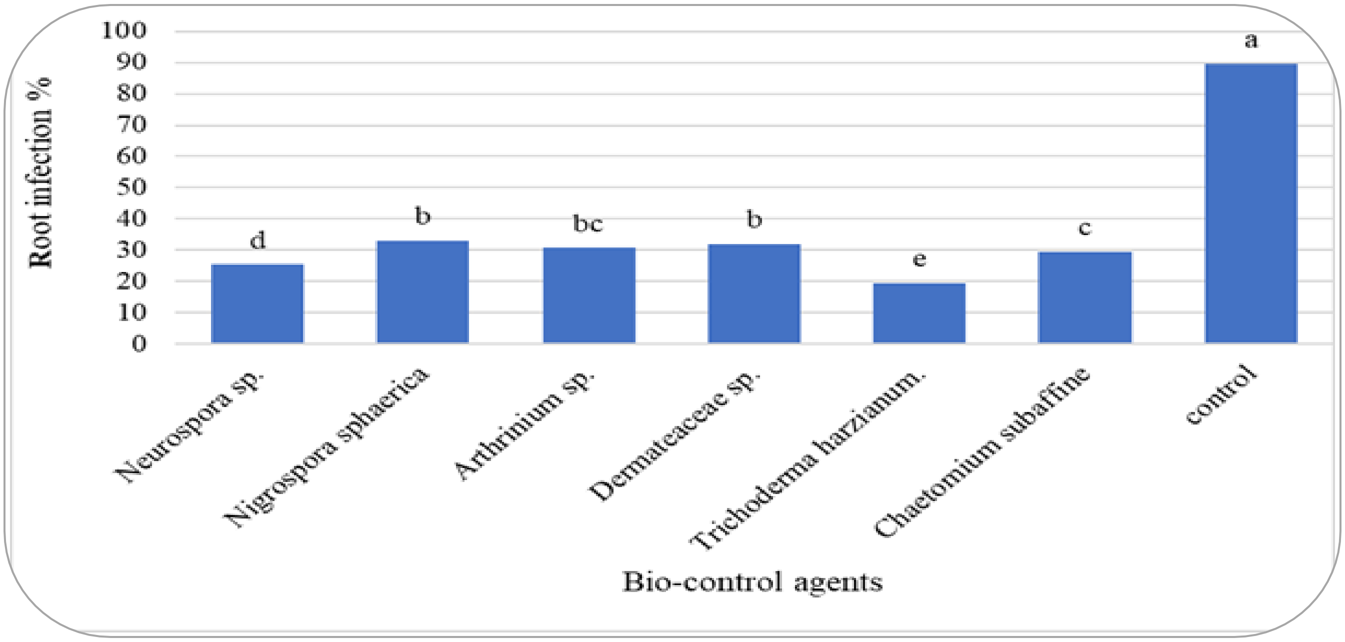

Figure 7. Effect of different bio-control agents on a root infection of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) under field conditions.

The application of antagonistic fungi also improved the yield of tomato as compared to untreated plants. Significantly, the highest fruit yield per plant was noted by *T. harzianum* (6.66 kg) followed by *Neurospora* sp. (5.66 kg), *N. sphaerica* and *Dermateaceae* sp. (5 kg). No significant difference was observed in plants treated with *Arthrinium* sp. and *C. subaffine* (4.33 kg). The lowest fruit yield 2.9 kg was recorded in untreated plants (Figure 8).

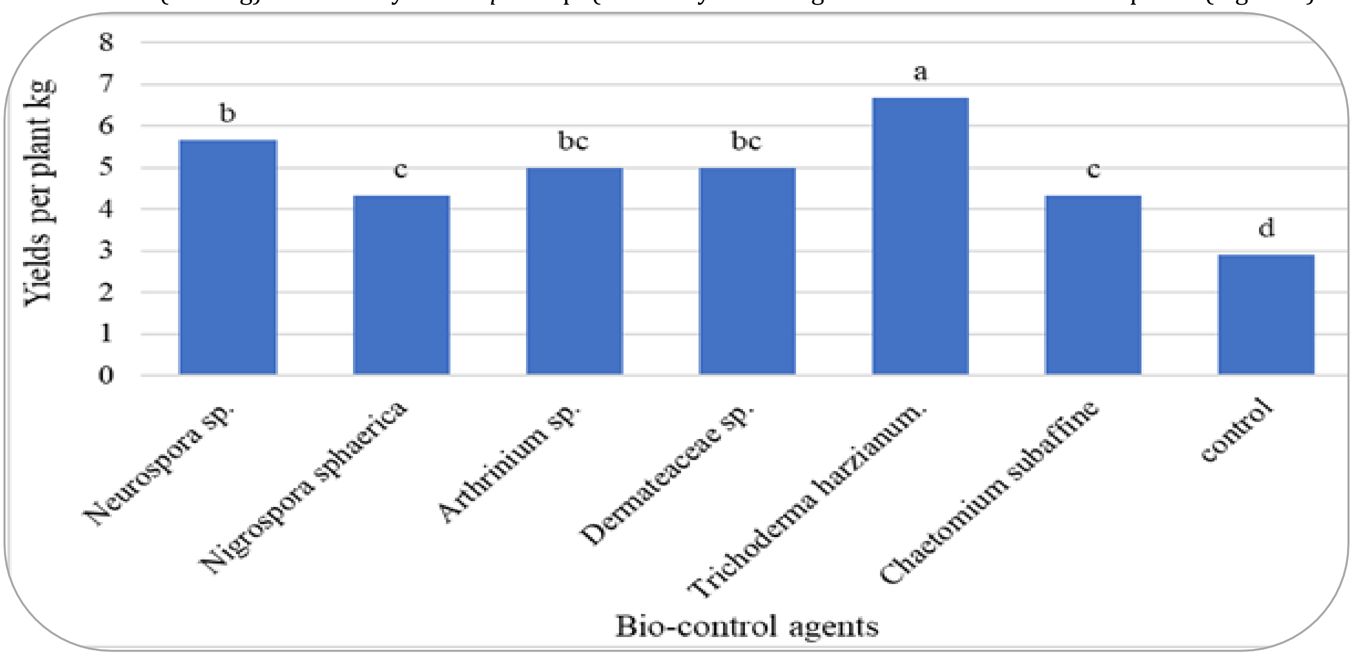

Figure 8. Effect of different bio-control agents on fruits yield per plant inoculated with *Fusarium oxysporum* f. sp. *lycopersici* (FOL) under field conditions.

DISCUSSION

Extensive use of chemical substances can produce resistance to pathogens and cause harmful effects on the atmosphere. Substitute treatments for managing plant diseases are always desirable. Fusarium wilt disease of tomato is known as the most aggressive disease generating yield losses between 30% to 90% depending upon management practice, availability of resistant cultivars and favorable climatic conditions for the growth of the associated fungus (Nirmaladevi *et al.*, 2016).

In the current study, different fungal strains such as *Trichoderma harzianum*, *Neurospora* sp., *Chaetomium subaffine, Arthrinium* sp., *Nigrospora sphaerica, Dermateaceae* sp., *Colletotrichum gloeosporioides, Pestalotiopsis mangiferae, Pestalotiopsis humus, Botryosphaeria parva, Xylariaceae* sp., *Alternaria* sp., *Corynespora cassiicola, Fusarium* cf. *solani* and *Hypocrea lixii* were tested *in vitro;* whereas, six best bioagents based on laboratory assays such as *T. harzianum*, *Neurospora* sp., *C. subaffine, Arthrinium* sp., *N. sphaerica,* and *Dermateaceae* sp., were evaluated in pot experiment as well as in filed conditions. The *T. harzianum* and *Neurospora* sp. showed maximum inhibition. No significant difference was noted among *Chaetomium subaffine, Arthrinium* sp., *N. sphaerica, Dermateaceae* sp. and *C. gloeosporioides*. The *Hypocrea lixii, Fusarium* cf. *solani, Corynespora cassiicola*, *Alternaria* sp. were found least effective, respectively. While the moderate response was noted with *Xylariaceae* sp., *Botryosphaeria parva*, *Pestalotiopsis humus* and *Pestalotiopsis mangiferae* against the FOL *in-vitro* conditions. Our results confirm Laila *et al*. (2018) who studied the antagonistic effects of *Trichoderma, Sclerotium* and *Aspergillus* on *Fusarium oxysporum* f.sp. *lycopersici* and it was found that *Trichoderma* has the highest inhibition % on FOL (82%) and *Aspergillus* the lowest (68.95%). Similarly, Javaid *et al*. (2014) evaluated the antagonistic performance of seven species of *Trichoderma* against FOL and *Macrophomina phaseolina* (Tassi) Goid. All *Trichoderma* species showed antagonistic effect against the test fungi. *T. harzianum* was found highly efficient biocontrol agent followed by *T. aureoviridi* and *T. hanatus* against both pathogens. In recent past, Alwathnani and Perveen (2012) studied the biological control of tomato fusarium wilt and it was found that *Aspergillus niger*, *Penicillium citrinum, Penicillium* sp. and *Trichoderma harzianum* could inhibit the radial colony growth of pathogens. In another study, Phong *et al.* (2015) reported the antifungal activity of *Chaetomium* to tea fusarium wilt. In their study, all *Trichoderma* showed obvious antagonistic behavior against target fungal pathogens in dual culture, resulting in a 59-74% reduction in FOL radial growth. *Trichoderma harzianum* has the best biocontrol effect, followed by *T. aureoviridi* and *T. hanatus*.

In the pot experiment, the *Trichoderma harzianum* and *Neurospora* sp. were found highly effective antagonistic fungi, provided the lowest DI% at higher, medium and lower concentrations. The *C. subaffine* and *Arthrinium* sp. were noted moderately effective followed by. *Dermateaceae* sp. and *N. sphaerica*. The mortality percent and RI% were quite lower in plants treated with *T. harzianum* and *Neurospora* sp. However, *N. sphaerica* and *Dermateaceae* sp. did not produce a good response and were found less effective.

Recently, Sultana and Ghaffar (2013) examined the capability of *Trichoderma* spp. to control the Fusarium wilt of tomato. Two native *Trichoderma* isolates *T. citrinoviride*, and *Trichoderma* sp. significantly decreased tomato fusarium wilt infection and enhanced plant biomass. Similarly, Barari (2016) conducted studies against fusarium wilt disease in laboratory and filed conditions and found that the application of *T. harzianum* (N-8) shown the least DI%. Also, tomato plants treated with *T. harzianum* (N-8) isolate) significantly promoted plant height and dry weight of tomato. The use of microorganisms to control plant pathogens is recognized as an alternative, or an additional way to decrease the practice of chemicals against plant diseases (Compant *et al*., 2005).

Biocontrol preparations of fungi, bacteria, and yeast have been applied to seed, seedlings, and planting media to reduce tomato wilt disease under greenhouse and field conditions with various degrees of success (Sabuquillo *et al*., 2006). The antagonistic fungi did not reduce the infection percent in pathogen inoculated plant but also enhance the plant growth. The plant treated with *T. harzianum, Neurospora* sp., *C. subaffine* and *Arthrinium* sp. produced higher biomass. whereas the lowest plant growth was observed by the application of *N. sphaerica* and *Dermateaceae* sp. Similar to our study, Vargas-Inciarte *et al*. (2019) tested *T. koningiopsis, T. virens*, *T. spirale* and *T. harzianum* antagonistic fungi against FOL and found *T. spirale* is highly effective in all experiments against FOL wilt. Similarly, Sultana and Ghaffar (2013) found that *T. harzianum, T. viride, Gliocladium virens, Bacillus subtilis* and *Stachybotrys atra* significantly lowered mortality and root rot infection of FOL in the seedling of bottle gourd and cucumber during laboratory and field trials. In our field experiment, we also noted that *T. harzianum* was extremely operative in lowering seedling mortality and root infection. The minimum DI was noted by *T. harzianum* treated plants followed by *N. sphaerica*. There was no significant difference among the plants treated with *Dermateaceae* sp and *C. subaffine*. The plants treated with *T. harzianum, N. sphaerica, C. subaffine* and *Arthrinium* sp. produced maximum plant biomass.

Considerably lowermost pathogen infection was recorded in plants treated with *T. harzianum* followed by *N. sphaerica, C. subaffine* and *Arthrinium* sp in field conditions. While it was highest in un-treated (control) plants followed by *Dermateaceae* sp. our results are consistent with Sundaramoorthy and Balabaskar (2013) who detected 15 *Trichoderma* antagonists from rhizosphere soil of healthy tomato from diverse terrestrial areas and used to promote the growth and yield parameters of tomato and management of fusarium wilt disease under laboratory and in field conditions. In our results, the application of antagonistic fungi also enhanced the plant biomass and yield, specifically when plants treated with *T. harzianum* followed by *N. sphaerica* and *C. subaffine.* The lowest fruit yield was noted in untreated plants followed by *Arthrinium* sp., *Dermateaceae* sp. Our results are in line with Ramezani (2014) who stated that application of *T. harzianum* biomass to the soil can effectively control the occurrence of Fusarium wilt, and ultimately promote the growth of plants with different characteristics under greenhouse conditions Similarly, Ramasamy (2020) reported that *Trichoderma* spp. control plant pathogen and improve yield and quality of the crop. Fungal species belonging to the genus *Trichoderma* act as a biological agent. Bio inoculated plant significantly enhances plant biomass. Recently Abro *et al*. (2019) studied on 30 different fungal bioagents against Fusarium wilt of cucumber *in-vitro* conditions and reported that bioagents have ability to reduce the disease severity and have potential to enhance the plant biomass of inoculated host plant.

CONCLUSION

In the current studies the fungal biocontrol agent *Trichoderma harzianum* was recorded the most effective bioagent providing the highest inhibition of radial mycelial colony growth with the lowest root infection percentage (RI%), highest plant growth and fruit yield followed by *Neurospora* sp., *Chaetomium subaffine, Arthrinium* sp., *Nigrospora sphaerica* and *Dermateaceae* sp., respectively. Interestingly, these all bioagents except *Trichoderma harzianum* and *Chaetomium* sp. have been first time recognized in the current study as potential bioagents against Fusarium wilt disease of tomato. These bioagents should also further be evaluated *in-vitro* and *in-vivo* to find out their significant effectiveness against other fungal wilt diseases in vegetable crops.

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