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Research Article

Investigating the Efficacy of Diverse Biopesticides for Managing Fusarium Wilt Disease of Tomato

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Abstract

Tomatoes (*Lycopersicon esculentum L*) are considered as most useful vegetable crop and badly affected by Fusarium wilt disease on global scale including Pakistan. Its management with synthetic chemicals is highly suitable but farmers are reported multiple health hazards cases by judicious use of chemical and careless handling of chemicals, therefore a proven safe approach was implemented to treat this notorious causal agent by developing eco-friendly manner through implementation of biopesticides. In this study the aqueous extract of neem leaves and neem seeds were shown to be the most effective followed by garlic, thorn apple and eucalyptus showed lowest disease incidence percentage (%)., additionally a lowest root infection percentage and lowest plant biomass was seen in tomato plants. Moreover, in comparison of biological control agents *Neuspora sp*., *Chaetomium subaffine*, *Arthrinium sp*. and *T.harzianum*. *T.harzianum* was determined as most effective in terms of least disease percentage at high and medium doses. However: high, medium and low doses, of *Neurospora* sp., *Arthrinium* sp, and *C. subaffine* reported larger plant biomass and lower root infection percentage, Respectively, *Nigrospora sphaerica* and *Dermateaceae* sp. outlined the lowest response., In this study *N. sphaerica*, *Neurospora* sp., *Arthrinium* sp., and *Dermateaceae* sp. were identified first time as promising biopesticides against tomato Fusarium wilt disease. Through this research it i strongly suggested that *T.harzianum* and *Neurospora sp*., neem leaves and seed aqueous extracts, can be used as promising biopesticides to restrict tomato Fusarium wilt disease of tomato for eco-friendly management.

Keywords: *Biopesticides, Fusarium wilt, Management, Tomato.*

Introduction

After potatoes being the popular snack of every region, tomatoes are considered as most economical vegetable in the world, initiated in Andes from South America and are now cultivated in multiple regions altogether worldwide (Saavedra *et al.*, 2016). 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, 2019). It contains 95.3% water, 0.07% niacin and calcium, vitamins A, C and E and many nutrients such as Na, K, Fe and antioxidants, especially lycopene and salicylate.those all play an essential role in human life including adult male and female health (Domínguez *et al.*, 2020).

Tomatoes are widely grown in almost 144 countries around the world. The leading countries in tomato production globally include China, the United States, India, Turkey, Egypt, and Pakistan. In Pakistan, tomato holds significant importance as a vegetable crop. It is grown on an extensive area of 55. 5000 hectares, resulting in a production of 561 thousand tons. The average yield per hectare stands at 10. 5 thousand tons. (FAO, 2019). The Sindh Province in Pakistan is a major region known for tomato cultivation. With a total land area of 21,000 hectares dedicated to tomato farming, the province yields an impressive production of 153,200 metric tons of tomatoes. This results in a commendable yield of 7,290 metric tons per hectare. The yield of this particular country is significantly lower when compared to other countries like the USA, Turkey, China, Egypt, and India. To provide some examples, the USA has a yield of 98. 4 tons per hectare, Turkey produces 70. 5 tons per hectare, China produces 57. 4 tons per hectare and Egypt produces 38. 6 tons per hectare (FAO, 2019). Numerous biological and abiotic diseases contribute to reduced crop yields. This susceptibility arises from the crop's vulnerability to over 200 diseases caused by various pathogens, including fungi, nematodes, and bacteria (Singh *et al.*, 2017). Fusarium Wilt, Early Blight, Anthracnose, Bacterial Wilt, Bacterial Canker, Tomato Spotted Wilt, and Verticillium Wilt are all diseases from which tomato plant are mostly effective. (Van Esse *et al.*, 2020). Fusarium Wilt disease is a fungal infection that affects tomato crops entire world. It is caused by a soil-dwelling fungus called *F. oxysporum*. This disease can cause significant reductions in crop yield, as reported by (Carmona *et al.*, 2020). The fungus mainly attacks the roots of the plants and can cause severe damage, resulting in significant production losses. Under constructive climatic conditions that promote the growth of the fungus, these losses can range from 30-40% and can even escalate to as high as 80%. (Nirmaladevi *et al.*, 2016).

Fusarium Wilt disease poses a significant threat to tomato plants, particularly in regions with warm climates. This disease thrives when the soil temperature reaches approximately 28°C, making it a major concern for tomato cultivation (Nirmaladevi *et al.*, 2016). The disease is characterized by the occurrence of drooping, yellowing, wilting, and dying symptoms that consistently affect a specific part of the plant. (Maurya *et al.*, 2019). Managing Fusarium Wilt is a significant challenge, and yearly different strategies have been suggested for addressing this popular disease, with the primary method of control being the use of fungicides. According to (Patiyal *et al.*, 2020) in his recent study conducted, the efficacy of six antifungal chemicals combating Fusarium-Wilt disease in tomatoes was assessed using in vitro conditions. Among them, the Custodia-Fusarium fungicide demonstrated promising potential effective management. The overuse of numerous fungicides harms the environment and enhances the defiance of the causal agents' population. Chemicals to be used not only affect tomato healthy dietary substances but also affect soil superiority and output of soil (Singh *et al.*, 2017). Chemical fungicides are no longer regarded as environmentally friendly due to their lasting impact on the environment. As a result, the utilization of natural plant material is seen as a viable substitute for synthetic fungicides (Anil Kumar and Raj Kumar, 2015).

Continuous utilization of chemical products can lead to environmental pollution, specifically in terms of soil and water contamination. However, the negative impact can be mitigated through the implementation of diverse biopesticide methods. The utilization of biopesticides offers several advantages, such as long-lasting effectiveness, cost efficiency, environmental friendliness, and resistance against disease-causing organisms. (Verma *et al.*, 2018) and (Kala *et al.*, 2016) conducted studies focusing on the management of wilt disease in chickpeas by employing Fusarium oxysporum f. sp. *ciceri.* The results of the study were very positive and the potential results against the wilt disease were very positive. The advanced, environmentally friendly management of the fusarium-wilt of tomato by the use of different fungal and bacterial endophytes is expected to be environment-safe and highly effective protection measure (Cotes *et al.*, 2016; Al-Mekhlafi *et al.*, 2019). Another potential disease control strategy is the use of fungal biological agents for the control of tomato Fusarium wilt (Mohammed and Toama, 2019). Various studies deal with biological control of fungal diseases through the use of non-pathogenic fungal biological agents in the field and in green house (Bubici *et al.*, 2019). Alternative approaches for the control of diseases have been studied, with the emphasis on the use of various biopesticide compounds from plants. According to different reports, some of the biopesticides used are highly effective antimicrobials against food and storage grain moulds, foliar pathogens, and soil-borne fungi Phyto-pathogens (Hassan, 2020).The objective of this research was to investigate the the efficacy of biopesticide for eco-friendly management of tomato Fusarium Wilt disease with the aim of study was to reduce the financial loss of tomato production. Based on the above information, it was proposed to use water-soluble plant extracts, as well as some promising biological control agents, for the eco-friendly management of the *Fusarium oxysporum* f. sp. *lycopersici* (FOL).

Methodology

The disease-causing pathogen was isolated from a specimen exhibiting indications of Fusarium wilt disease. The isolation process was conducted on potato dextrose agar (PDA) medium, utilizing the tissue isolation technique (Agrios, 2005). The roots, stem, and branches of the tomato plant affected by disease were extracted and cut into small pieces of about 1 to 2. cm in length. Subsequently, the plant parts were washed with running water, subjected to surface sterilization for 30 seconds in a solution containing 0.5% mercuric chloride, and then rinsed twice with sterilized distilled water to remove the mercuric chloride solution. The pieces were dried on filter paper. These pieces were placed in the centre 90 mm petri plates containing Potato Dextrose Agar (PDA) These plates were then incubated at a temperature of 25 ±2°C for a period of 7 days to facilitate the growth of the targeted pathogen. The isolated fungus was identified using taxonomic keys (Booth, 1971; Nelson, 1983).

Preparation of aqueous plant extracts involved several steps. First, thorn apple (*Datura stramonium*), garlic (*Allium sativum*), neem (*Azadirachta indica*), and eucalyptus (*Eucalyptus globulus*) leaves were thoroughly cleaned with tap water. Then, they were surface sterilized using a 0.5 percent sodium hypochlorite solution and washed multiple times with sterilized distilled water. Afterward, the plant materials were left to air dry. To create the extracts, 20 ml of sterile water and 20 grams of plant material were carefully combined. The mixture was then grinded using a pestle and mortar. All the chosen plant components were combined to form a 100 percent w/v stock solution. This stock solution was filtered using muslin cloth and Whatman filter paper No. 1. The resulting extracts were centrifuged for five minutes at 10,000 rpm. The supernatant was then sterilized for ten minutes at 40 °C and stored in a freezer for future use (Jagannathan and Narasimhan, 1988; Anil Kumar and Raj Kumar, 2015).

The source of fungal bioagents. Six biological agents were purified in potato dextrose agar (PDA) medium after being imported from the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences No. 1, Beijing 100 101, which were China: *Trichoderma harzianum*, *Neurospora* sp., *Chaetomium subaffine*, *Arthrinium* sp., *Dermateaceae* sp., and *Nigrospora sphaerica*. These studies were conducted at Sindh Agriculture University Tando Jam, Sindh, Pakistan, from June to October of 2016 and 2017.

The uses of aqueous plant extracts and bioagents in pot experiment. Surface sterilization with 5 percent commercial bleach was performed on the seed of the frequently planted local tomato variety "Desi local" for two minutes, and then the seed was cleaned with sterile water. Each clay pot (20 cm in diameter) had two kg of sterilized soil that was used to nurture ten seedlings per pot. A suspension of the test pathogen containing 10^5 conidia was intentionally added to the soil to infect it. A little layer of dirt was applied to these seedlings. It was a greenhouse where the pots were stored. After seven days of seeding, the chosen aqueous plant extracts (4, 6 and 8 percent) and fungus bioagents (10^3, 10^4 and 10^5 conidia ml) were soaked into the clay pots (Dubey *et al.*, 2007). Five replications were used in the complete randomized design (CRD) experiment. When necessary, irrigation water was put to earthen pots. After 15 days after seeding, the percentage of disease incidence (DI%) and the percentage of plant death were noted. After 40 days, the plants were uprooted to measure the proportion of infected roots and the plant biomass (plant height plus weight). The following formula was used to determine the proportion of infected roots, disease incidence, and plant mortality:

$$\text{Mortality } \% = \frac{\text{Number of dead plants}}{\text{Total number of plants under obserevation}} \text{ x } 100$$

$$\text{Disease incidence } \% = \frac{\text{Number of infected plants}}{\text{Total number of plant}} \text{ x } 100$$

$$\text{Root infection } \% = \frac{\text{Number of root pieces colonized by the fungus}}{\text{Total number of pieces studied}} \text{ x } 100$$

Experiment design. The experiments were set up using a completely randomized design (CRD) with four replicates for each treatment. The experiments were conducted twice over a span of two years.

Statistical analysis of the data was carried out using software Statistix, version 8.1 (Analytical software, USA). The treatment effect was assessed using analysis of variance (ANOVA). To determine the average separation, the least significant difference (LSD) test was employed. A significance level of $P < 0.\ 5$ was utilized to identify any significant differences between the treatments.

Results

Isolation and Identifications of *Fusarium oxysporum* f.sp. *lycopersici*

The pathogen was isolated and purified throughout the isolation procedure using a 90 mm Petri plate with sterilized Potato dextrose agar (PDA) media. According to Booth's descriptions of the colony morphology and the morphological traits of macro and microconidia, the isolated pathogen was identified (Booth, 1971). The *F. oxysporum* f.sp. *lycopersici* colony mycelial growth covers a 90 mm Petri dish in approximately seven days and has white aerial mycelium with pink undergrowth on the bottom surface (Fig. 1a). The Micro conidia developed singly, were septation-free, and were oval to reniform (Fig. 1b). Micro conidia had a size range of 7.54 to16.20 μm and 2.5-6-4.0 μm. The macro conidia displayed an oval shape with pointed ends, typically having three septa (Fig. 1c). The size of the macro conidia was 28 to 44 μm in length and 3 to-5 μm in width. The observed morphological characteristics of the isolated fungus from tomato plants suggest its identification as *Fusarium oxysporum* f. p. *lycopersic*i.

The effect of different aqueous plant extracts

The testes plants with neem leave biopeptide at 8 and 6 percentage concentration showed low mortality percentage (21 and 26), followed by those treated with garlic extracts for 28.66 and neem seeds 27.66 at 08 percent concentrations. The thon apple and eucalyptus extracts were shown to be less effective at their highest concentrations than other extracts, but they were found to be somewhat effective at lower doses. The untreated (control) plant showed the highest rate of plant death (93.66) (Figure 2). Less disease attack was seen in plants treated with extracts of neem seed and neem leaf extracts at 8% concentrations showed the low ID% (23.66 and 25.33). At all tested concentrations, the neem leaf extract was shown to be the most successful. In comparison to other extracts, the thorn apple and eucalyptus extracts were lesser efficient and caused the highest disease incidence percentage at 4 and 6 percent concentrations (Figure 3).

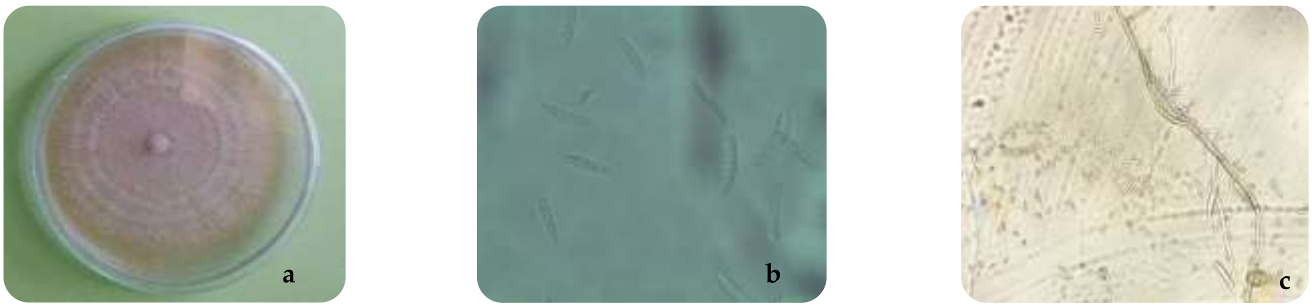

Figure 1. *Fusarium oxysporum* f.sp. *lycopersici* morphological characteristics (a) the colony mycelial growth, (b) the macro conidia and (c) the micro conidia.

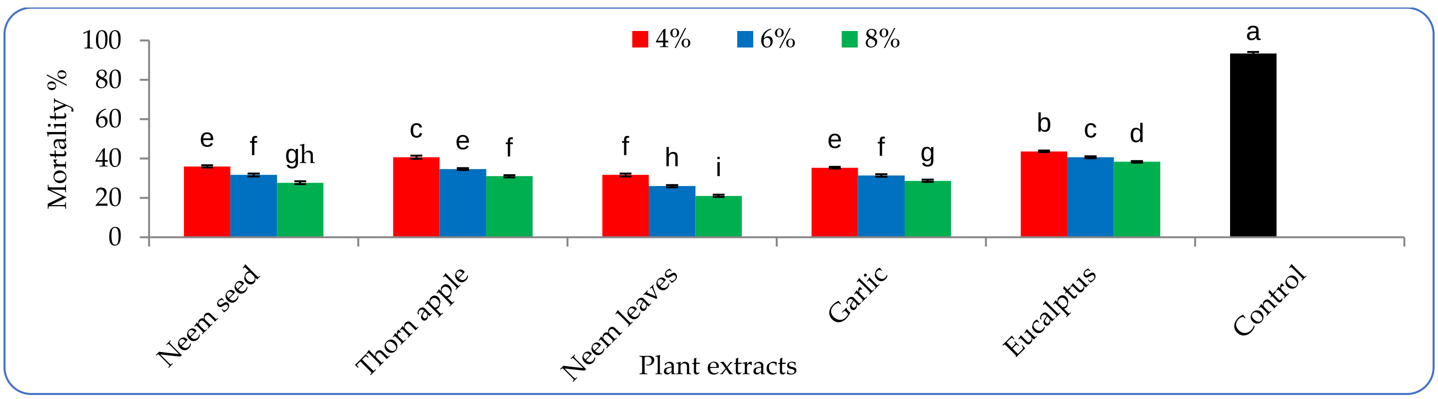

Figure 2. Impact of various plant extracts on mortality percentage of tomato plants inoculated with *F. oxysporum* f. sp. *Lycopersici*. Bars following the similar letters are indicating non-significant difference in accordance to LSD (least significant difference) test at $P < 0.05$.

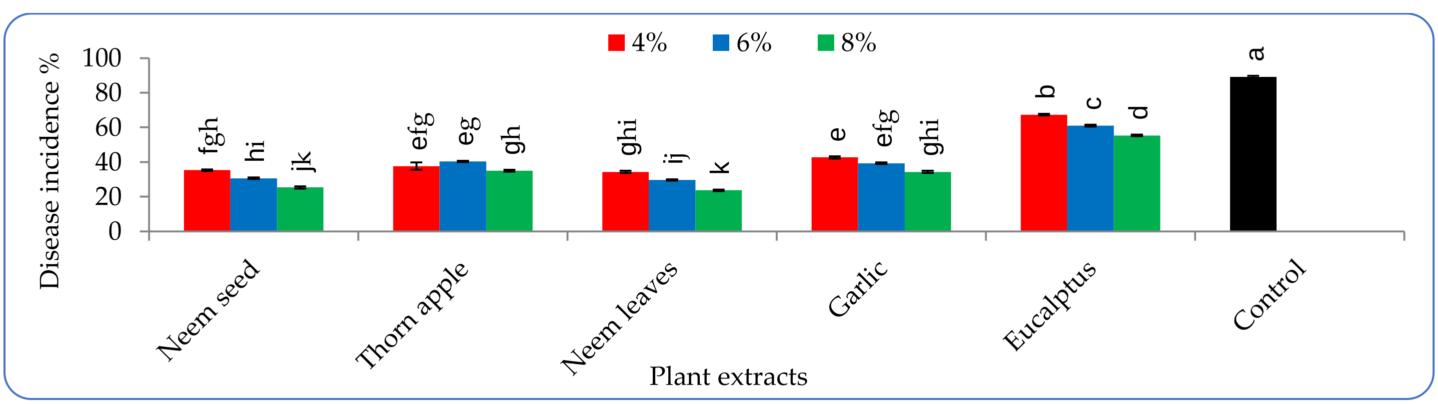

Figure 3. Impact of various plant extracts on disease incidence percentage of tomato plants inoculated with *F. oxysporum* f. sp. *Lycopersici*. Bars following the similar letters are indicating non-significant difference in accordance to LSD (least

significant difference) test at $P < 0.05$

Generally, larger concentrations of plant extracts were shown to be more effective than lower and medium dosages at promoting plant growth. The highest plant heights (28.33 and 27.66 cm) were seen in plants treated with 8 percent concentrations of neem leaf and seed extracts. Higher doses of the eucalyptus, thorn apple, and garlic extracts were also successful. In a pot experiment, the medium 6 percent doses of all tested extracts were also effective against the tomato fusarium wilt disease. The treated plants at 6% doses had plant heights between (21 and 24 cm). The plants that had been exposed to eucalyptus extracts at 4 percent concentration had the shortest plant height (18.66 cm) (Figure 4).

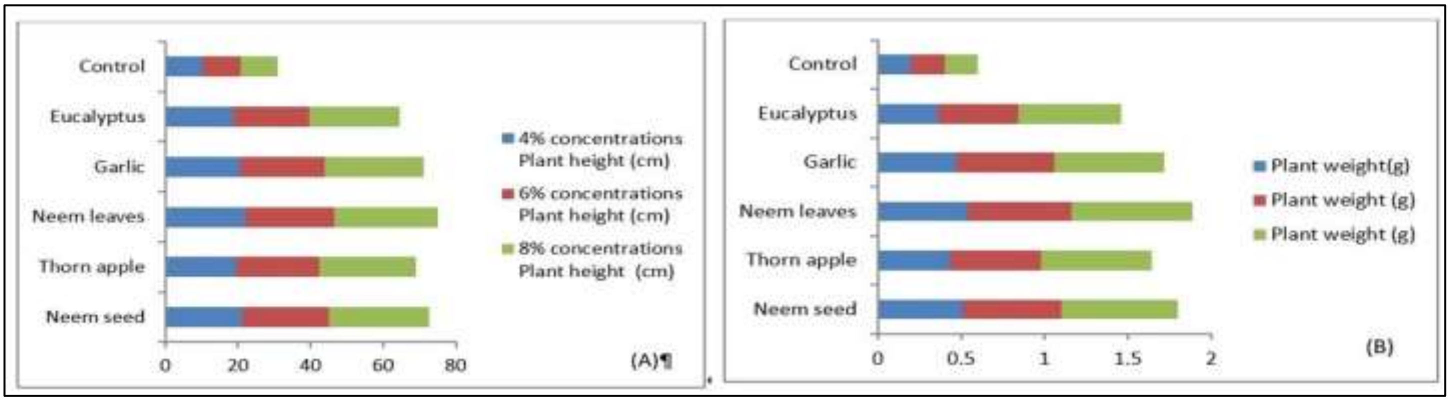

Figure 4. (A&B) Effect of different plant extracts on plant height in cm (a) and Plant weight in gram (b) of plant inoculated with F. *oxysporum* f. sp. *lycopersici*. The LSD (least significant difference) test shows that values following the identical letter within a column are not significantly different at $P < 0.05$.

In case of weight of plants, the maximum plant weight (0.73 and 0.70 g) was noted in plant treated with neem leaves and neem seed extracts at 8 percent concentrations whereas the lowest weight of plant was noted in plants treated with eucalyptus extracts at 4 percent concentrations (Fig 4. b). Additionally, the percentage of plants with root infections was measured was shown to be greatest (97%) in untreated plants. In general, the inoculated plant did not develop any root infection even at the highest doses of all tested extracts. Interestingly, even at concentrations of 6 percent, neem leaves, require seeds, and thorn apple extracts did not cause root infection. However, eucalyptus observed the highest root infection percentage (38) between the treatments (Figure 5).

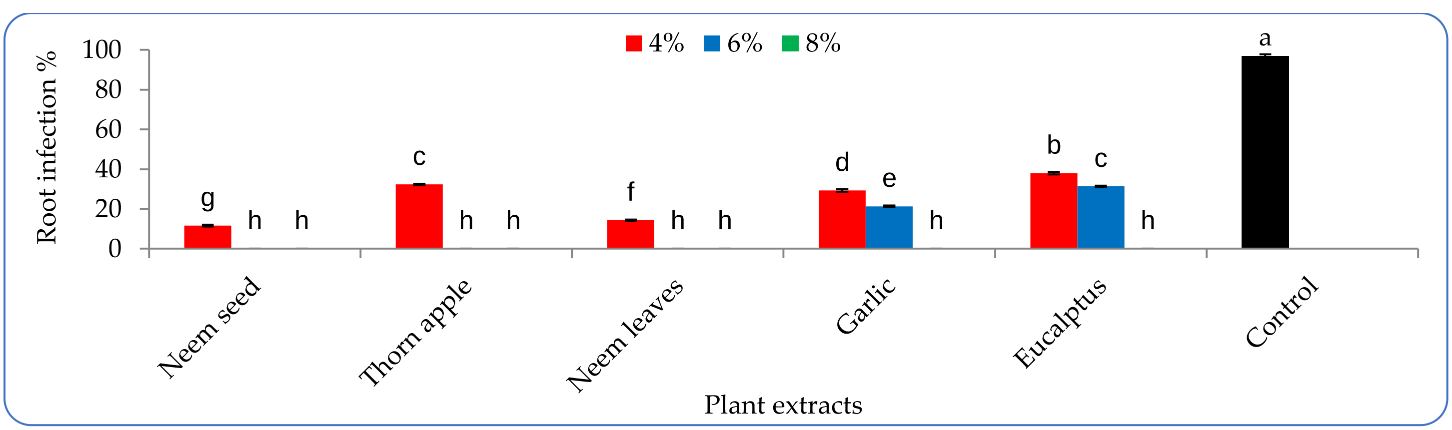

Figure 5. Impact of various plant extracts on root infection of tomato plants inoculated with *F. oxysporum* f. sp. *Lycopersici*. Bars following the identical letters are indicating nonsignificant difference in accordance to LSD (least significant difference) test at $P < 0.05$.

The impact of various fungal biocontrol agents

Tomato plants with disease incidence percentage were significantly reduced when bioagents were applied, as compared to the control. The untreated plants had a higher percentage of disease incidence (DI%), but this decreased as the concentrations of bioagents increased. Moreover, the lowest disease incidence percent of 18.6 and 24.3 at higher and medium concentrations (10^4 and 10^5 conidia g-1 of soil) were found to be highly effective in *T. harzianum.* However, *Neurospora* sp. had been observed as second most effective fungal antagonist, in comparison to *Dermateaceae* sp. *Arthrinium* sp. and *C. subaffine* were found to be lesser effective followed to rest, while *Arthrinium* sp. and *N. sphaerica* showed a moderate response at high and medium concentration (Figure 6).

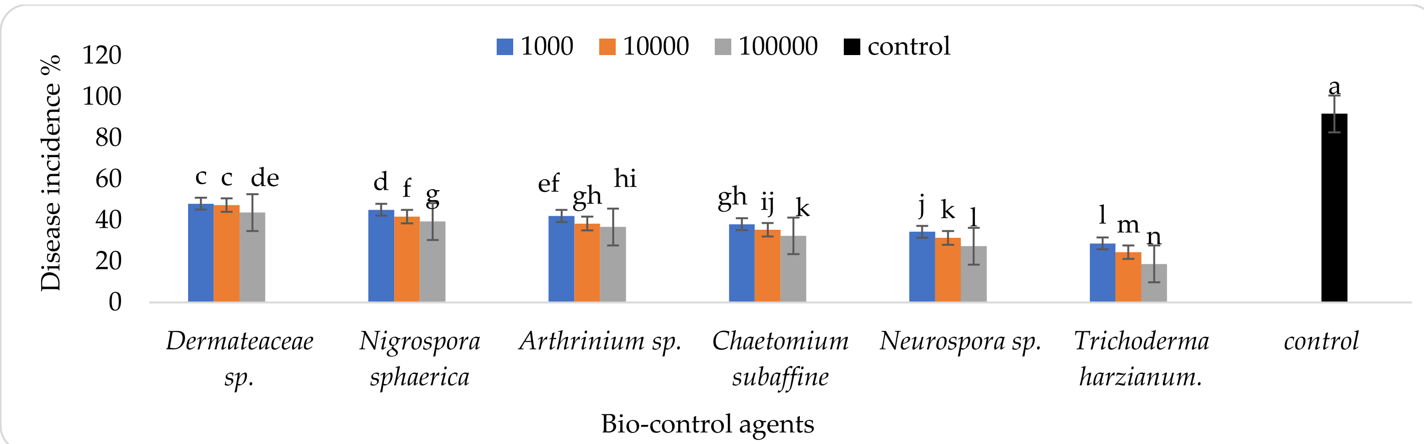

Figure 6. Impact of various bio-control agents on disease incidence % inoculated with *Fusarium oxysporum* f.sp. *lycopersici*. Bars following the identical letters are indicating nonsignificant difference in accordance to LSD (least significant difference) test at $P < 0.05$.

Comparatively to untreated plants (control), plant mortality was dramatically decreased when bio bioagents were applied. Treated plants with *Neurospora* sp. showed with 22% and 25% at high and medium doses but plants treated with *T.haziamum*, showed minimum 16.66% and 21% mortality In comparison to other bioagents, *Dermateaceae* sp. and *N. sphaerica* had showed lesser effectiveness in providing maximum mortality percent at low doses (Figure 7).

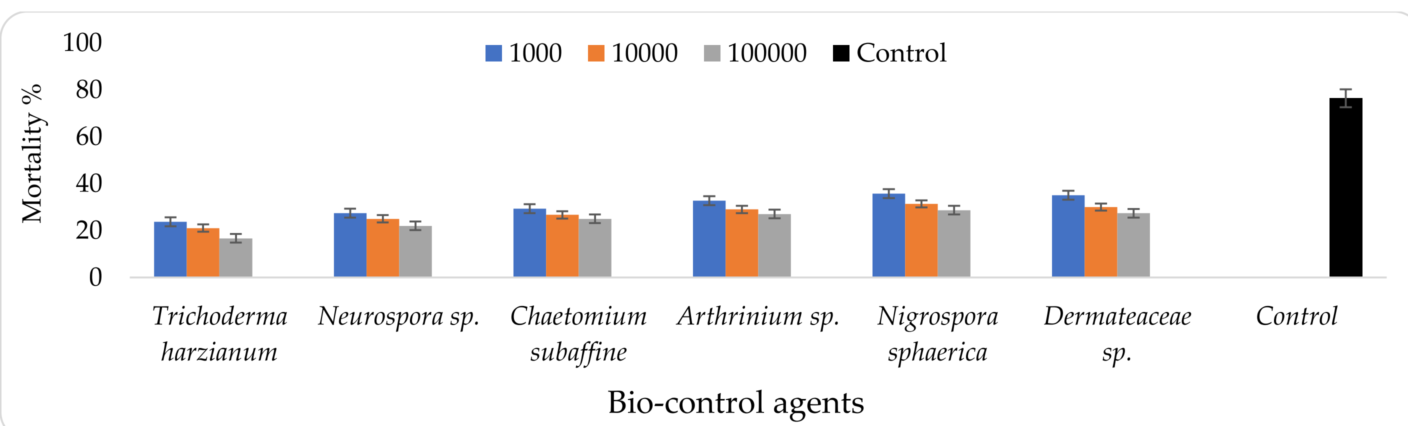

Figure 7. Impact of bio-control agents on mortality% inoculated with *Fusarium oxysporum* f. sp. *Lycopersici.* Bars following the similar letters are indicating nonsignificant difference in accordance to LSD (least significant difference) test at $P < 0.05$.

Compared to plants treated with the pathogen alone (control), plants treated with bio-agents, and the pathogen had decreased root infection (RI%). At higher and medium concentrations *C. subaffine*, *Arthrinium* sp., and *Neurospora* sp., showed lower RI%, whereas *Dermateaceae* sp. and *N. sphaerica* had shown lesser effectiveness to other studied bioagents. The plant that was infected but not treated had the highest RI% (77.33%) (Figure 8).

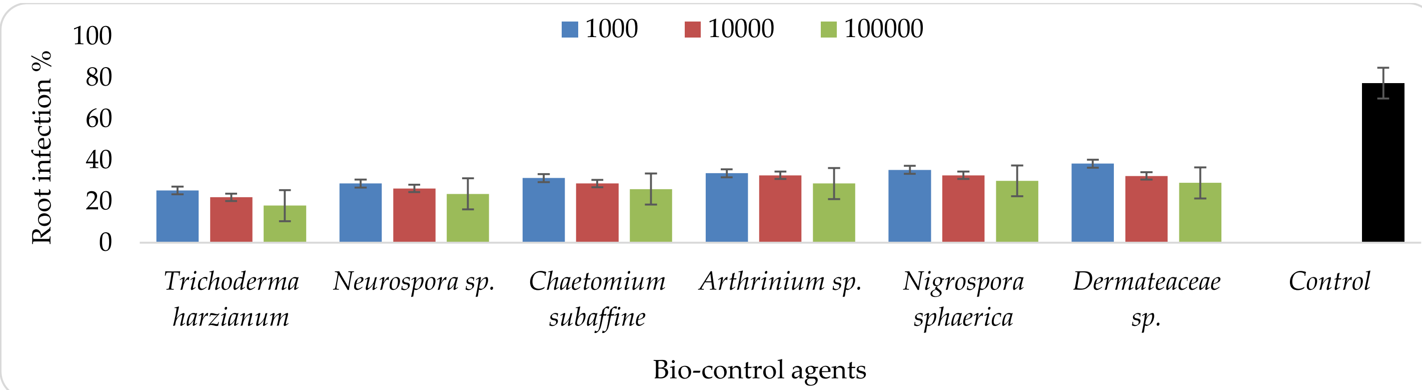

Figure 8. Impact of various bio-control agents on root infection % inoculated with *Fusarium oxysporum* f.sp. *lycopersici.* Bars following the identical letters are indicating nonsignificant difference accordance to LSD (least significant difference) test at $P < 0.05$.

The bioagents improved plant development in addition to lowering the pathogen infection rate. Treated plants with *T. harzianum* got the maximum plant heights (34, 31.33, and 27 cm) at concentrations of 10^3, 10^4, and 10^5 conidia ml^{-1} followed by *C. subaffine*., *Arthrinium* sp. and *N. sphaerica*. *N. sphaerica* and *Dermateaceae* sp. found the lowest plant biomass. In a similarly, treated plants had higher plant weights than untreated plants. At a larger dosage, *T. harzianum* recorded the largest plant weight (1.95 g). *Dermateaceae* sp. recorded the lowest weight (0.36 g), which was followed by *N. sphaerica* at the lowest dosage. When compared, *C. subaffine* and *Arthrinium* sp. had shown to be somewhat effective. (Figure 9).

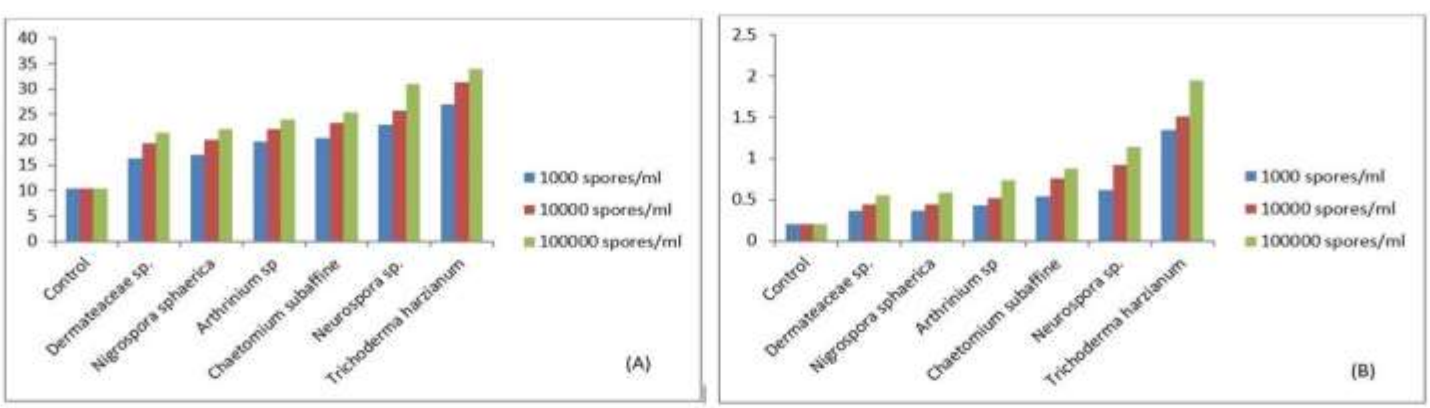

Figure 9 (A & B). Effect of different bio-control agents on plant height in cm (a) and Plant weight in gram (b) inoculated with *Fusarium oxysporum* f.sp. *lycopersici.*

Utilizing chemicals often can result in disease resistance and have negative impacts on the environment. It is usually preferable to use alternative therapies to cure plant illnesses. The most destructive disease affecting tomatoes is fusarium wilt, which causes production losses of about 30% to 90%, depending on managing techniques, the availability of resistant cultivars, and favourable climatic conditions for the establishment of the associated fungus (Nirmaladevi *et al.*, 2016).

The results revealed that using aqueous plant extracts at higher concentrations reduced the percentage of plant death. Neem leaf and seed extracts did a remarkable job of lowering plant disease mortality and disease incidence rates. Garlic and thorn apple extracts were discovered to have a mediocre level of disease-attacking efficiency. Plants treated with aqueous extracts of neem leaves, neem seeds, garlic, and thorn apple extracts showed improved plant height. Our findings are consistent with those of (Hanaa *et al.*, 2011) who investigated fusarium wilt disease in tomato seedlings and discovered that the administration of aqueous neem and willow extracts resulted in the lowest disease incidence and the maximum plant growth. The use of plant extracts enhances the height of tomato plants, according to (Etaware *et al.*, 2019) and (Elgubbi *et al.*, 2019). When several aqueous extracts' effects on tomato plants were examined, it was shown that the extracts can increase growth percentage, vegetative growth, and yield. According to (Etaware *et al.*, 2019) the application of botanicals eradicated disease symptoms. Extract-treated tomato plants displayed an increase in plant height.

The usage of bioagents was found to have a beneficial impact on tomato plant biomass and the decrease of disease. The antagonistic fungus *Trichoderma harzianum* and *Neurospora* sp., which gave the diminished Disease incidence percentage at higher, medium, and lower concentrations, were shown to be very efficient. *Dermateaceae* sp., *N. sphaerica*, *C. subaffine*, and *Arthrinium* sp. were shown to be efficient. The mortality percentage and RI% of plants treated with *T. harzianum* and *Neurospora sp*. were significantly reduced. It was discovered that N. sphaerica and *Dermateaceae* sp. were less effective and failed to generate an adequate reaction.

(Vargas-Inciarte *et al.*, 2019) recently investigated *Trichoderma* species' capacity to prevent tomato Fusarium wilt. *T. citrinoviride* and *Trichoderma* sp., two native *Trichoderma* isolates, dramatically reduced tomato fusarium wilt infection while increasing plant biomass. Similarly, *T. harzianum* (N-8) application showed the least DI% in experiments against fusarium wilt disease done by (Barari, 2016) in both lab and field settings. Additionally, *T. harzianum* (N-8) isolation greatly increased tomato plant height and dry weight. It is acknowledged that using microorganisms to manage plant pathogens is an extra or alternative method to reducing the usage of chemicals to treat plant diseases (Köhl *et al.*, 2019).

Fungus, bacteria, and yeast biocontrol preparations have been utilized in greenhouse and field settings to mitigate tomato wilt disease. These preparations have been applied to seeds, seedlings, and planting media with varying levels of effectiveness (Sabuquillo *et al.*, 2006). The introduction of antagonistic fungus to pathogen-inoculated plants not only promoted plant growth but also reduced the incidence of infection. Notably, *T. harzianum*, *Neurospora* sp. *C. subaffine*, and *Arthrinium* sp. led to higher plant biomass, while *N. sphaerica* and *Dermateaceae* sp. treatment resulted in the lowest plant growth. (Vargas-Inciarte *et al.*, 2019) reported similar success with *T. spirale* in combatting FOL wilt, examining antagonistic fungi such as *T. koningiopsis*, *T. virens*, *T. spirale*, and *T. harzianum*. (Sultana and Ghaffar, 2013) also discovered the effectiveness of *T. harzianum*, *T. viride*, *Gliocladium virens*, *Bacillus subtilis*, and *Stachybotrys atra* in reducing mortality and root rot infection caused by FOL in bottle gourd and cucumber seedlings during laboratory and field experiments. Our own field trial revealed the significant reduction of seedling mortality and root infection through the use of T. harzianum. Plants treated with *T. harzianum* exhibited the lowest Disease Index (DI), followed by *N. sphaerica*. There were no discernible differences between the plants treated with Dermateaceae sp. and C. subaffine. The highest plant biomass was observed in plants treated with *T. harzianum*, *N. sphaerica*, *C. subaffine*, and *Arthrinium* sp.

Conclusion

The effect of the different biopesticides was investigated in pot assay against Fusarium wilt disease of tomato. The aqueous extracts of neem leaves and neem seed were shown to be highly effective. Following garlic, thorn apple, and eucalyptus, this produced the lowest disease incidence, root infection percentage, and highest plant growth of tomato plant .In compassion to all tested bioagents, *Neurospora* sp., *T. harzianum*, *Chaetomium subaffine*, *Arthrinium* sp. and *Chaetomium subaffine*. Exhibited notable efficacy as biopesticides, *these* particular bioagents demonstrated the lowest mortality rates, with maximal plant biomass and minimum root infection in tomato plant. In comparison to neem leaves and need seed extracts *T. harzianum* was reported highly effective which provided diminished mortality percent and disease incidence with highest plant biomass of tomato plant growth among all tested biopesticides.The usage of biopesticides has been seen as a secure alternative to synthetic fungicides as a means of disease management. The findings of this study showed promise in the fight against eco-friendly control of tomato fusarium wilt disease.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally in the manuscript.

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