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Research Article

Evaluation of Fungicides and Biocontrol Agents against Fusarium Wilt of Tomato

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) belonging to Solanaceae family is a chief component of daily food. It is cultivated by market gardeners as well as for kitchen gardening. It is largely produced in Punjab Sindh and KPK province in Pakistan. *Fusarium oxysporum* f. sp. *lycopersici* is the significant soil-borne pathogen causing fusarium wilt in tomato. In Pakistan, yield is reduced by 21.9% due to tomato wilting. The fusarium wilt of tomato was managed by using different fungicides and biocontrol agents. Tomato seeds were sown in trays followed by transplanting. Fungicides; Score (Difenoconazole) at dose of 0.3 ml/L, 2 g/L Topsin-M (Thiophanate methyl) and biocontrol agents (*Trichoderma* spp. and *Bacillus* sp. at concentrations of 1×10^3 and 1×10^3 spore/ml) were applied after inoculating the transplanted plants with disease inoculum. Treatments were applied after a week of tomato seedling transplant. Using disease rating scales, disease severity symptoms and disease incidence were recorded at weekly intervals. Collected data was analyzed. First symptoms of the disease were appeared after 8 days of disease inoculation. The result indicated that after application of treatments, in controlled pots T₀ showed maximum disease of 32.64% which was recorded after spraying with distilled water. Minimum disease was recorded in that plants which was sprayed by *Trichoderma* spp. (9.52%) and *Bacillus* spp. (12.41%) were found most effective against fusarium wilt of tomato. The fungicide sprays; Difenoconazole (25.43%) were least effective then biocontrol sprays. Thiophanate methyl (18.91%) was given fair result among other chemical. In present study, results of *Trichoderma* spp. and *Bacillus* spp. applications revealed that all the other three treatments were significantly superior over control in managing the Fusarium disease spread. Among all sprays, T₃ and T₄ were significantly superior over other treatments. With the exception of all treatments, that were found superior in action over T₀ (control). The overall results showed that T₃ (*Trichoderma* sp.) was most effective against fusarium wilt of tomato. Thus, use of biocontrol agents were most effective and at the same time it is environment friendly. This study will be fruitful for the farmers to manage these soil-borne pathogen that will lead to high production of tomato.

Keywords: Fusarium wilt, Tomato, Biological control, Fungicides, Sustainable agriculture.



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INTRODUCTION

Solanaceae is considered as the third most economically important family in plant kingdom after the *Fabaceae* and *Poaceae*. It includes many nutritionally and profitable cultivated crops. *Capsicum annum* (Chili), *Solanum tuberosum* (potato), and *Solanum lycopersicum* (tomato) are the most important solanaceous crops (Hussain and Abid, 2011). They can be used as a food, for medicines, as narcotics as well as ornamentals. Tomato (*Solanum lycopersicum* L.) is the vegetable belongs to family *Solanaceae* and genus *solanum*. Tomatoes are an important component of our daily diet, and they may be ingested as unprocessed raw fruit or as various sorts of processed goods (Brookie et al., 2018). Tomato is a member of the night

shade family 'Solanaceae,' which comprises over many species of flowering plants, including economically important crops such as potato, eggplant, chilli, and tobacco. In 1753, Linnaeus classified tomato as part of the large and complex genus *Solanum*, and called the species *Solanum lycopersicum* L. Phillip Miller later renamed it *Lycopersicon esculentum* Mill after transferring it to the *Lycopersicon* genus in 1768. Based on the physical characteristics of tomato pollens and other members of the family (Taylor, 1986). *Lycopersicon lycopersicum* L. Karst is the new taxonomy name for tomato. Based on genetic identification, it is known as *Lycopersicon esculentum* (Peralta and Spooner, 2001).

Tomatoes that are grown for food and their wild relatives are part of the genus *Lycopersicon* in the Solanaceae family. These plants are native to Peru and the Galapagos Islands, where they are grown for food (Smith, 1994). There have been about 7500 tomato cultivars produced so far, and they come in a wide range of color combinations, different shapes, and a variety of sizes (Sims, 1980). Tomato first grown as ornamental plants and consider to be toxic and harmful (Gentilcore, 2010). There may have been a "cherry tomato" that came from outside of South America (Rick, 1979). In the 16th century, tomatoes were brought to Europe. In the 18th century, they were bring to Asia. In the Indo-Pak subcontinent, they were introduced about 200 years ago (McCue, 1952).

Cultivars of Tomato are of two types; Determinate and indeterminate based on their general tendency towards growth. The minimal cost, flexibility of tomato production, and short time frame and impressive economic returns have enticed producers to produce the fruit all year, especially in hotter areas (Naika et al., 2005). Global tomato production and consumption have almost increased exponentially since a decade (Van de vooren et al., 1986). Due to its numerous yield, it is considered an important cash crop in many nations; United States, China, Turkey, India, and Egypt. Among all of these countries China is the one of the largest Tomato producer. In Pakistan, diverse ecological and climatic circumstances, as well as enhanced agro methods, support the year-round cultivation of high-quality tomatoes. It is grown on a total of 55258 hectares in Pakistan, with a total yield of 147572 tonnes (F and V STAT, 2018-19) Sindh (21002 ha), KPK (13225 ha), Balochistan (12128 ha), and Punjab (8903 ha) are the provinces with the highest tomato output (F and V STAT, 2018-19).

Tomato is a popular vegetable crop because of its savoury fruit, taste, and nutritional worth. They may also be found in salads, soups, ketchup, puree, and a number of other dishes. Nutrition wise Tomato rank top among other major fruits and vegetables. There is high amount of vitamin A, B and C is present in Tomato. Also other nutrients like antioxidants, carotene and potassium are widely available in it (Nonnecke, 1989).

Tomato is quite susceptible to cold. Flowering does not develop correctly in most cultivars below 17°C and above 32°C. The ideal temperature range for flowering is between 20 and 28 degrees Celsius. The favorable temperature for seeds germination is between 17 to 30°C and could not be germinate at soil temperatures of over 36°C and below 15°C. Tomato grow well in nearly every soil type. Sandy loam soil is best for every cultivar of tomato cultivation. Tomato grow best on soils with a pH ranging from 5.5 to 7.0. (Khalid, 2013).

Land preparation is required for good tomato crop production. Before transplanting of tomato seedlings into the field the soil should be mixed well with at least 12 tons of FYM per acre before a month. To prepare a decent seed bed, five to six ploughings will be required. For better water holding capacity the beds should be raised about 1.5m width (Khalid, 2013).

Transplanting is best done with seedlings that are aged 30-50 days and have 4-6 true leaves. The transplanting should be inured before transplantations by progressively exposing them to direct sunlight and water regularity. To reduce seedling transpiration, seedlings should be transplanted in the field late in the afternoon. The roots of the plants should be pushed down hard to establish a good contact with the soil particles and the plants should be water right after. Tomato seedlings should be transplanted on both sides of raised beds with 50 centimeter plant-plant gap (Khalid, 2013).

One of the most important limiting variables in tomato cultivation is disease. Diseases cause damage on the tomato crop, resulting in significant economic losses (Hanssen and Lapidot 2012). According to Jones et al., (1991) 25 fungal, 8 bacterial, 8 nematode, 12 viruses, and 4 viroid cause diseases on tomato plants, although fungi-induced diseases are the most common (Persley, 1993), causing significant infections, reducing production by 95 percent, and deteriorating natural quality of fruits and their nutrients and natural quality of fruits (Dellavalle et al., 2011). Other than post-harvest losses, the most common fungal diseases are damping off, root rot, seed rot, early blight of tomato and late blight of tomato, leaf spots and wilts are widely present in soils of most regions of Pakistan.

Several bacteria, fungi and viruses attack tomato plants throughout various phases of its development and growth. Among the diseases of tomato (*Lycopersicon esculentum* L.), Fusarium wilt is utmost important and damaging

diseases. Fungi named *Fusarium oxysporum* f. sp. *Lycopersici* is the disease causing agent of Fusarium wilt is one of the most deadly and wide spread disease around the world (Abdel-Monaim, et al., 2012; Morid et al., 2012). The spread of *Fusarium* spp. is generally influenced by weather factors such as temperature and humidity (Kosiak et al., 2004). *Fusarium* spp. can be found in a broad variety of conditions in nature. *Fusarium* species are toxic and able to cause disease among animals, individuals and especially in plants. The fungus is usually found in the subtropical areas of the world (McGovern et al., 2001). The ideal temperature for the pathogen to cause disease is fall between 25 and 30 degrees Celsius, but the disease can only be able to grow at lower soil moisture ranging from 30% to 70% with a soil pH of 5-8 (Glen et al., 2003). However, the most harm is done in areas with low moisture content. *Fusarium oxysporum* f. sp. *lycopersici* can live up to many decades in the soil (Thangavelu et al., 2003). It spreads through soil and destroy plant tissues quickly. It's too late by the time a plant displays any visible signs of disease; the plant wilts and dies.

Fusarium wilt results in 10-80% crop losses (Bharat & Sharma, 2014). The yield of infected tomato crops are being recorded is fall between 12-60% in green house and in open field of tomato (Borrero et al., 2004; Larkin and Fravel, 1998). Particularly fusarium wilt of tomato is most common in Pakistan, where it causes significant losses and has earned the recognition of a serious tomato disease in Pakistan (Tahir et al., 2012). According to the stage of tomato plant growth and its environmental conditions *F. oxysporum* causes a decrease in tomato production ranging from 15% to 85% (Singh, 2005; Kumar and Sood; 2002).

It inhibits normal physiological processes such as water transport, interacting with the roots vascular system. Thus, it damages the quantity and quality of tomatoes (Malhotra and Vashistha, 1993). The optimum temperature for this pathogen effects is 28°C (Debbi et al., 2018). The pathogen enters through root epidermis, spreads via the vascular system, and gets established in xylem of the plant. Due to pathogen colonization, the plant is water stressed due to clogging of the xylem vessels which leads to the expression of wilt symptoms (Singh et al., 2017). Soil-borne phytopathogenic fungi have systems with regulatory pathways that alter expression of host genes enabling easier recognition and subsequent infection of the host (Rep and Kistler, 2010). The pathogen attaches to the root hair surface and extends its hypha. Advances toward root cortex vascular bundles where differentiation occurs in xylem and ooze is produced displaying virulence.

The xylem inhabited by pathogens undergoes colonization, drives the disease progress, and ultimately results in wilting (Di et al., 2016). In the absence of a suitable host, the chlamydo spores go dormant and can outlast a pathogen for extended durations (Cha et al., 2016; Khan et al., 2017).

Wilted plants with yellow coloured leaves and poor or missing crop production are morphologically indicative of the disease.

Fusarium wilt causes wilted plants with yellowed leaves, resulting in a loss of 70 to 60% of fruit output (Ravindra et al., 2015). Polluted irrigation water with FOL spores and contaminated farm equipment is responsible for spreading the disease from short distances to long places. Inoculated seedlings, soil samples, seeds, cultivars with FOL are also responsible for causing from one region to another one. If a single soil is infested with FOL, the pathogen tends to stay there eternally (Animashaun et al., 2017; Prihatna et al., 2018).

In many countries, integrated disease management is one of most efficient environment friendly strategy which is not only enhancing agricultural production but also intercept environment from degradation (Waiganjo et al., 2006). Crop rotation, organic matter additions, and the use of high-residue tillage equipment are all practices that can assist to develop healthy soils. Pest and disease reduction through the use of compost products has been the subject of much research across the world. According to the findings of Recycled Organics Unit, (2006), soil borne diseases that harm seeds, roots, collars and plant tissues can be easily controlled by using composts in infected soil, this phenomenon is also known as natural biological management. Crop rotation with green manures and other cover crops are one of most beneficial method in controlling fusarium wilt of tomato, it is not only control disease but also increase the fertility of soil, destroy harmful weeds and disturb the insects cycles (Jeff, 2009).

Use of disease free resistant tomato cultivars, antagonistic microorganisms like *Trichoderma* and *Bacillus*, decontamination of farm equipment, chemicals; prochloraz and methyl bromide and cabbage and cauliflower can be used a cover crops and used in crop rotation for at least 3-6 years, these are the best IDM strategies in controlling fusarium wilt of tomato (Ajilogba et al., 2013; Dishon, 2012).

These diseases can be controlled by biological methods. There are some suppressive soils present in many regions of the world that suppress fusarium wilt and suppression is usually because of other living organisms in soil. Different biocontrol agents, such as *Bacillus* spp. *Gliocladium* spp. and *Trichoderma* spp. have been helpful for the management of fusarium wilt (Zhang et al., 1996).

MATERIALS AND METHODS

The experiment was carried out in Lath house. Two tomato varieties; Nadir and Naqeeb seeds were sown in plug trays. The tomato seedlings have been grown in In-vitro conditions. The irrigation was given regularly. The nutrients were provided to the seedlings on weekly intervals. Seven weeks old seedlings were then transplanted into clay pots. Each pot filled with soil mixed with FYM. The experiment was comprised of 2 varieties, 6 treatments including control, chemicals as well as biocontrol agents and 3 replication, each replication contained one tomato plant per pot. Before a week of transplantation of tomato seedlings, the soil was infested with pathogen; *Fusarium oxysporum* f. sp. *Lycopersici*, the pathogen was isolated, purified and prepared in Diagnostic lab. The suspension of pathogen inoculum was prepared and injected into plant roots by using syringe and also applied into plant's soil by drench. For the treatments preventive method was used for application of biocontrol agents; *Trichoderma* spp. and *Bacillus* spp. at 1×10^3 spore/mL by drenching into pot's soil before transplanting of tomato seedlings, the biocontrol agents were also prepared in Diagnostic lab and the curative method is used for the application of chemicals; Score (Difenoconazole) 0.3 ml/L and Topsin M (Thiophanate- methyl) 70% W.P were applied at 2 g/L. Disease severity was recorded from the first symptoms of fusarium wilt from each replication of each variety and highly affected leaves and stem discoloration was observed and given the status according to the disease rating scale. Disease severity data was recorded after 7 days intervals. The data of disease severity was recorded from the first symptoms of fusarium wilt from each replication of each variety and highly affected leaves and stem discoloration was observed and given the status according to the disease rating scale in which 0 presents no disease symptoms and 1,2,3,4,5 shows 0.1-10, 25, 50, 75 and 100% respectively. The disease severity percentage was calculated by using the formula written below:

$$\text{Disease Severity\%} = \frac{\text{Proportion of leaves with disease symptoms}}{\text{Total number of leaves}} \times 100$$

All the treatment data were analyzed by using statistix 8.1 software. All possible effects of treatments were analyzed by ANNOVA test under CRD design and means were compared by using Tukey HSD test at 5% significant level (Steel et al., 1997).

RESULTS

Greenhouse Assessment of Treatments Against Fusarium Wilt of Tomato

The experiment was performed in greenhouse. Sixty days old nursery were transplanted in clay pots. The clay pots contained soil infested with *Fusarium oxysporum* f. sp. *lycopersici* inoculum. Different concentrations of chemicals and biocontrol agents were applied in pots. All treatments showed significant different results except *Bacillus* spp. (T₄) and *Trichoderma* spp. + *Bacillus* spp. (T₅). There were total 6 treatments ranging from T₀– T₅ give rise to different results against disease severity. T₀ showed maximum disease of 32.64% which was highest among other treatments.

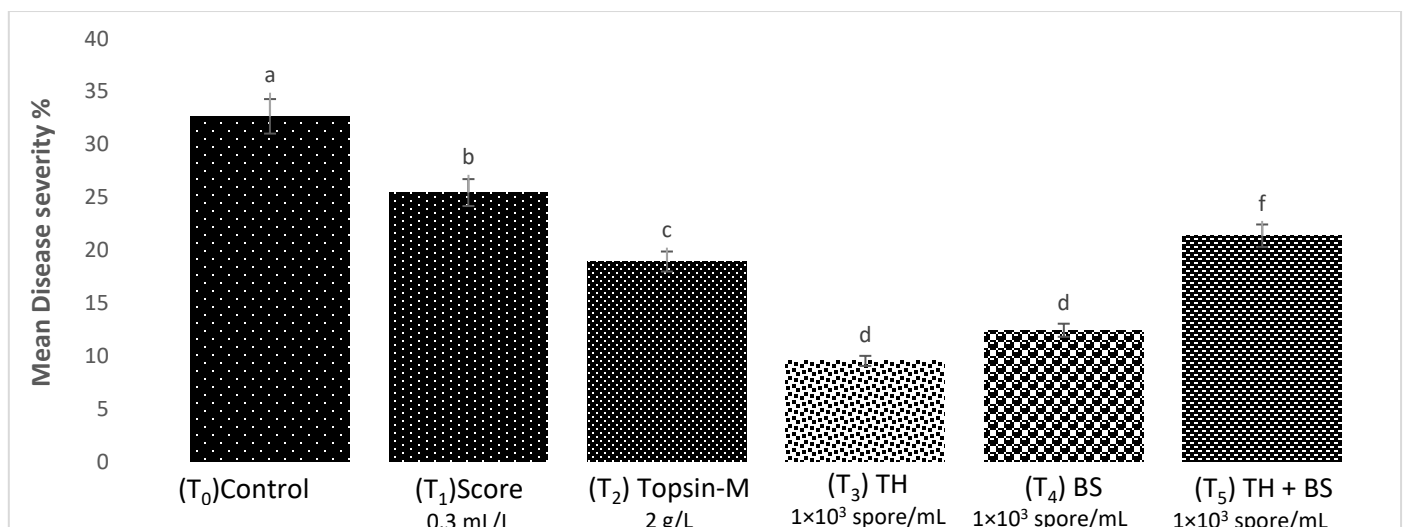


Figure 1. Greenhouse assessment of treatments against.

All the applied treatments reduced the disease severity significantly. Among treatments, T₁, T₂, T₃, T₄ and T₅ reduced the disease mean upto 25.43%, 18.91%, 9.52%, 12.41% and 21.35% respectively. The most significant results were shown by T₃, T₄, and T₅. T₃ and T₄ were concentrations of *Trichoderma* spp. and *Bacillus* sp. that caused 78% and 62% reduction in disease severity. T₅ was concentration of *Trichoderma* spp. + *Bacillus* spp. showed 56% valuable results to overcome the effect of fusarium wilt in tomato plants. T₂ and T₁ were concentrations of Topsin M (Thiophanate- methyl) and Score (Difenoconazole) caused 42.06% and 21.08% reduction in disease severity respectively. Similar results reported by (Monda, 2002, Morsy et al., 2009 Houssien et al., 2010 Al-Azawi et al., 2012 Cucu et al., 2020).

Comparison Between Varieties Against Disease Severity

Two varieties; Nadir and Naqeeb of tomato were selected and germinated in control room for 60 days and then transplanted into clay pots for experiment. Both varieties showed disease symptoms of fusarium wilt of tomato. The results of disease severity among both varieties were found significantly different from each other, Nadir was moderately susceptible and Naqeeb was more of resistant variety as compared to Nadir that’s why Nadir showed high disease severity as compared to Naqeeb.

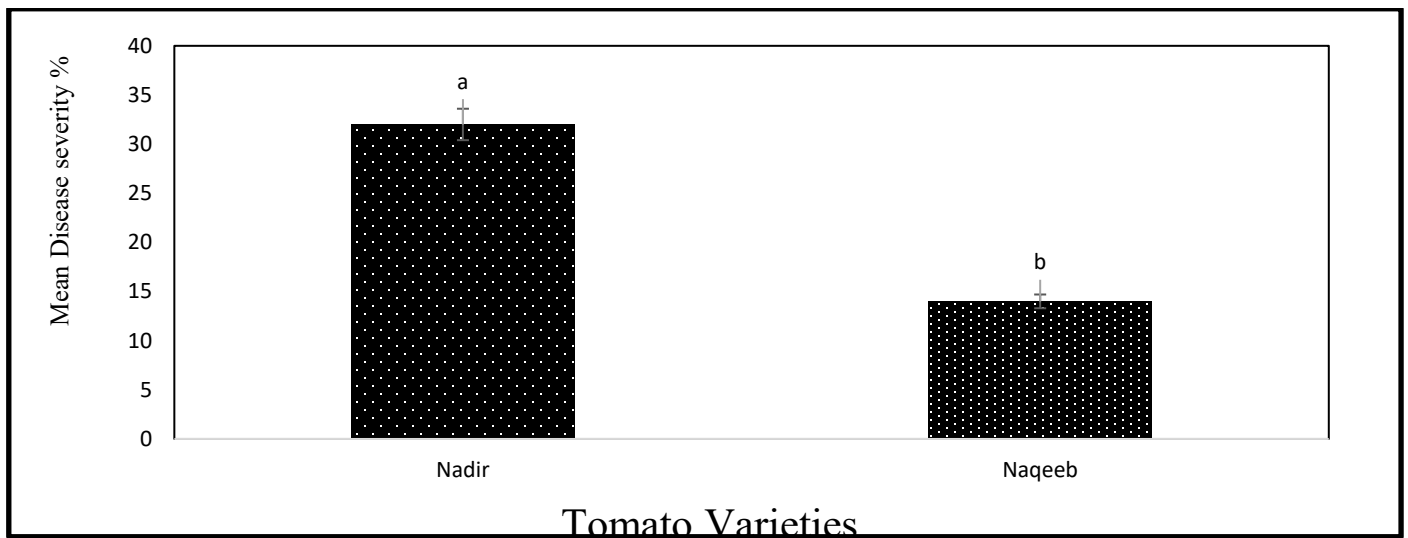


Figure 2. Comparisons of varieties against disease severity.

Comparison Test Disease Severity by Weeks

Disease symptoms were produced in all other treatments. Lowest disease was appeared at 2nd week which continued to spread and maximum disease was observed at 8th week.

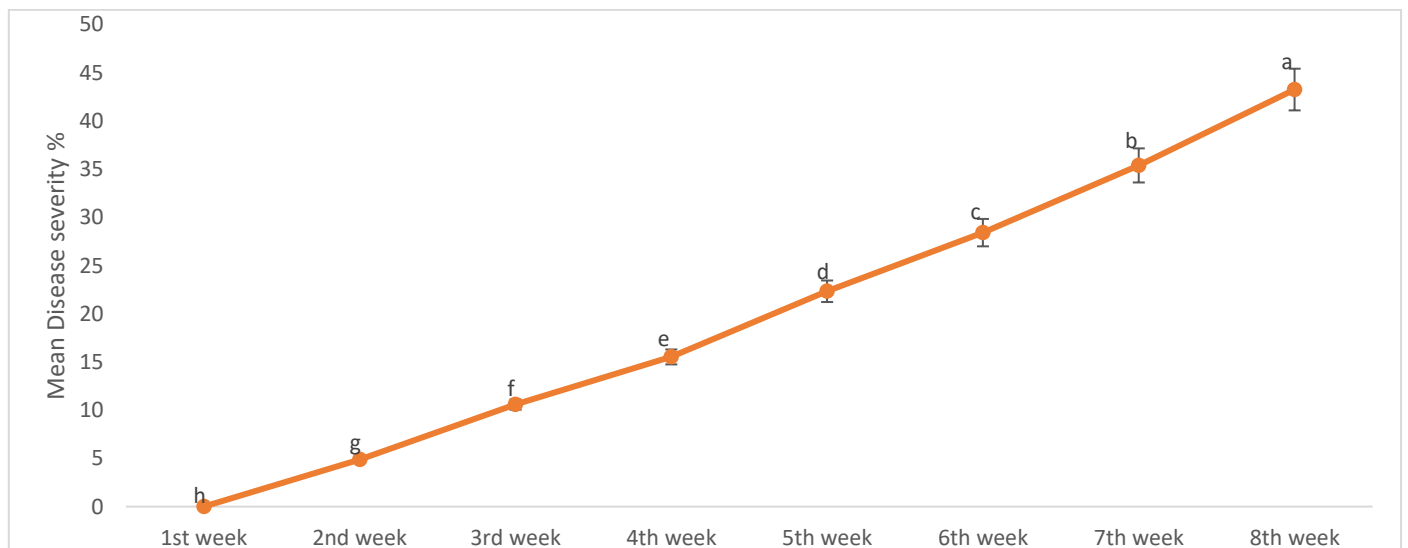


Figure 3. Comparison of disease severity by weeks.

Comparisons Test of Disease Severity Between Treatment and Variety

Results revealed that all the treatments significantly reduce the severity of the disease as compare to control. The maximum disease was recorded in Nadir (T₀). Variety A (Nadir) showed high disease severity as compared to variety B (Naqeeb) and among treatments, the most significant results were showed by T₃ (*Trichoderma* spp.) and T₄ (*Bacillus* spp.). T₂ (Topsin M) and T₁ (Score) were showed low significant results. Biocontrol agents are found to be most effective in controlling fusarium wilt in both tomato varieties.

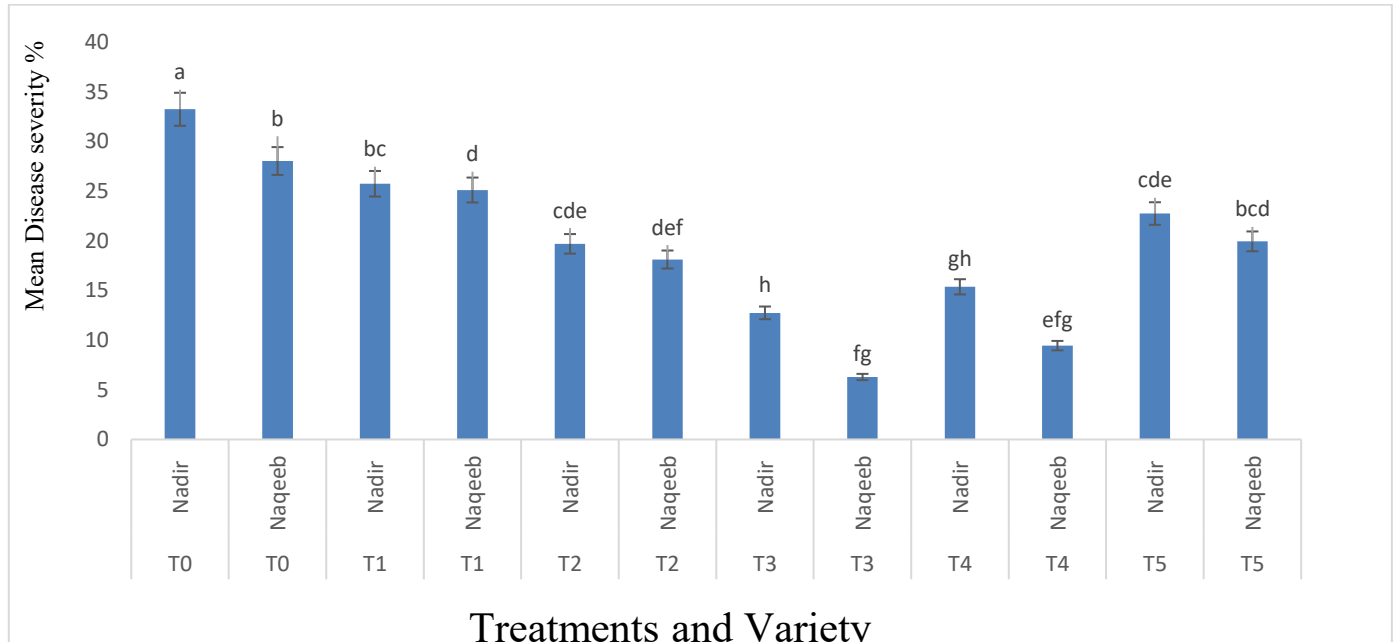


Figure 4. Comparison test of disease% in both varieties against treatments

Evaluation of Biocontrol Agents Against Fusarium wilt of Tomato

Different concentrations of *Trichoderma* spp. and *Bacillus* spp. were applied in soil of infected tomato plants. Among biocontrol agents *Trichoderma* spp. found to be the most effective against fusarium wilt of tomato as compared to *Bacillus* sp. which moderately control Fol (*Fusarium oxysporum* f. sp. *lycopersici*). The highest disease severity found in control and lowest disease severity recorded in tomato plants which were treated with *Trichoderma* spp. (Figure 5).

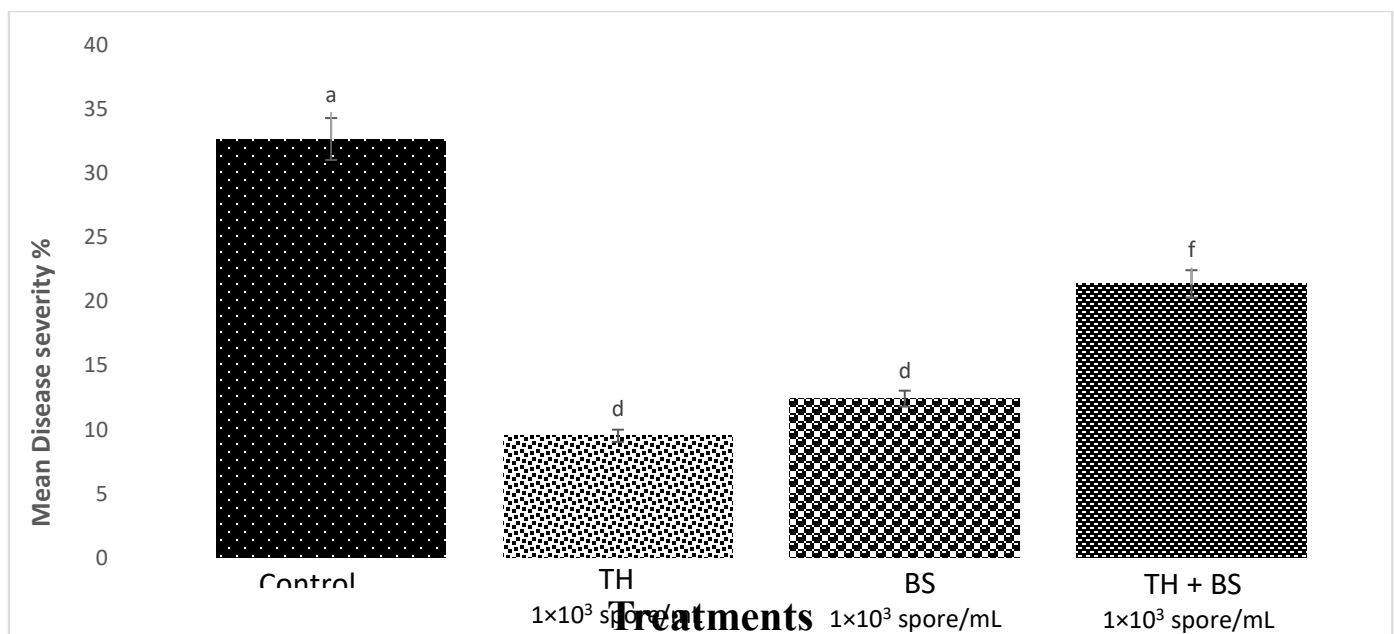


Figure 5. Comparison among Biocontrol agents.

Evaluation of Fungicides Against Fusarium Wilt

A comparison of the disease percentage in all the treatments showed that disease percentage was significantly reduced as compared to Control. The average percentages of disease percentage in all treatments were significantly different from the control. The maximum disease reduction 42.06% was observed in Topsin M (Thiophanate methyl) (Fig 1.4). Among fungicides tested, Topsin M found more effective in controlling the fusarium wilt in comparison with the Score (Difenconazole) which 21.08% in disease reduction. Similar results founded by (Patiyal et al., 2020, Sahar et al., 2013, Poussio et al., 2021, Sanap et al., 2020).

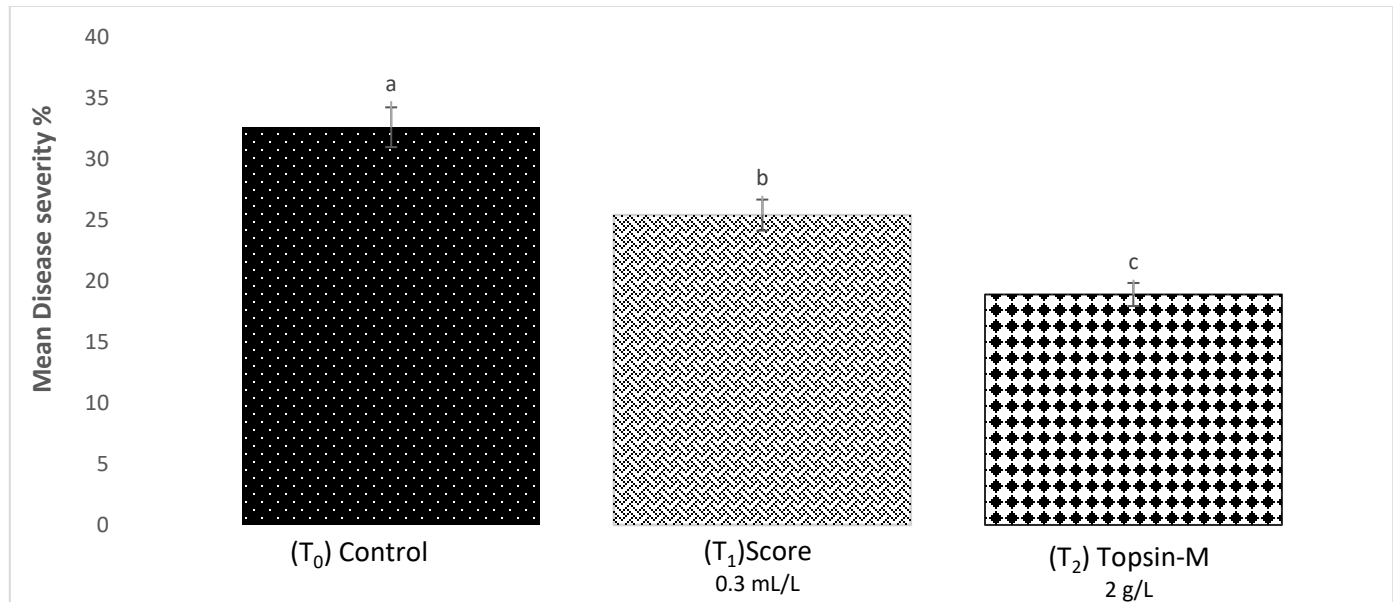


Figure 6. Comparison among the fungicides for the management of Fusarium wilt of tomato.

DISCUSSION

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetable in the world. The production of the tomato decreases due to pre and post-harvest losses. Diseases is one of the major threat to tomato production. Among other diseases, Fusarium wilt is the most destructive soil borne disease. Every year fusarium wilt cause massive global loss in the production of tomato. In Pakistan, Fusarium wilt caused 49% yield loss in tomato crop. In present study, fusarium wilt of tomato is evaluated by using integrated management strategies. Two varieties; Nadir and Naqeeb of Tomato were evaluated in Lath house. One of them (Nadir) was highly susceptible to the fusarium wilt of tomato and the second (Naqeeb) is more of a resistant variety as compare to 1st one. The trial was conducted in pots. Each pot considered as one replication and each pot contained one tomato plant. For treatments application of two different chemicals has been used. These chemicals were Score (Difenoconazole) and Topsin M (Thiophanate methyl) with concentrations of 0.3 ml/L and 2g/L respectively was applied. Both showed significantly different results. Among these chemicals, Topsin M (Thiophanate methyl) reduce disease severity by 42% and Score (Difenoconazole) with concentration of 2g/L was able to overcome the fusarium wilt with only 21%. Thus, fungicide Topsin M was more effective then Score. The chemical results were resembled with results of Sahar et al., (2013) who reported that Topsin – M was significantly effective against fusarium wilt of tomato. He evaluated four different fungicides under greenhouse conditions. He applied the Topsin-M in soil with different concentrations. The Topsin M was able reduce disease other than fungicides. Similar results were reported by Poussio et al., (2021). He evaluated six different fungicides. The plants treated artificially with fungicides found to be extremely useful in controlling disease severity of fusarium wilt of tomato. The chemical observations in line with Niknezhad et al., (2001) tested the effectiveness of fungicides including Thiphenate methyl found to be most effective in reducing fusarium wilt.

Sanap et al. (2020) conducted an experiment against fusarium wilt of tomato. They reported that *F. Oxysporum* f. sp. *lycopersici* of tomato was suppressed by spraying of Thiophenate methyl. In that experiment, three different concentrations of difenoconazole and thiophenate methyl were used and all of them inhibited the growth of *F. Oxysporum* f. sp. *lycopersici*. He found that Thiophanate methyl was found comparatively more effective of the tested *Fusarium* spp. over untreated control. Patiyal et al., (2020) evaluated different fungicides. All the fungicides found to

be successful in controlling *Fusarium oxysporum* f. sp. *lycopersici* but among these chemicals Difenconazole was found most effective against fusarium wilt. According to present study observations the disease severity was maximum in variety A (Nadir) as compare to second variety B (Naqeeb). The similar findings were observed by Al-Shebele, (2011) he evaluated five different cultivars for fusarium wilt of tomato. These varieties differed also in their susceptibility to infection. Present work in line with Harshita et al., (2019) evaluated different potential systemic fungicides against *Fusarium Oxysporum* f. sp. *lycopersici* at three different concentrations by using Poison food technique. The appropriate amount of each fungicide based on active ingredient was added in soil. Different results were obtained among them Topsin M found to significantly decrease the disease severity by 42%. Crop protection using commercial fungicides is an effective strategy for controlling numerous plant pathogenic fungus (Monda, 2002).

However, widespread use of these fungicides is now widely acknowledged to be connected with a variety of environmental concerns across the world. To reduce these hazards, fungicide usage has been subjected to stringent controls. Nonetheless, several treatments of various different fungicides may be necessary at times to ensure efficient fungal disease control. Such complex fungicide treatments can increase xenobiotic disturbances in the environment (Kariuki et al., 2020). Another troubling side effect of the industrial purpose of fungicides for crop protection is the degree of resistance by phytopathogenic fungi, which harms vital agricultural crops (Zhou et al., 2021). Because of this resistance, any protective benefits are typically short-lived and ultimately uneconomical. Several solutions are being researched to counteract or limit the development of fungus resistance and to lessen the environmental effect of fungicide use. The use of fungicides in conjunction with bio-control agents are major examples of disease resistance (Singh et al., 2008).

Results for percentage disease severity depicted highest trends in the pots of tomato plants which are treated with control and grown up to eight weeks. However, the severity rates started increasing on an interval of 2nd week that depicts the influence of aging duration as aging is an inevitable phenomenon. Researchers attributed the cause of appearing symptoms towards the presence of fusarium wilt during tomato plant growth and almost zero influence of control treatment. Findings from trial revealed that significant differences were obtained disease severity of fusarium wilt of tomato. The inoculated plants treated with the spore concentrations of *Trichoderma* sp. at 1×10^3 spore/mL (T₃) and *Bacillus* sp. at 1×10^3 spore/mL (T₄) compared to the control treatment (T₀). Though none of the spore suspensions completely prevented fusarium wilt on tomato the percentage of disease severity was low in relation to the control. All the biocontrol agents including T₃, T₄ and T₅ significantly restricted the disease mean percentage to 9.52%, 12.41% and 21.35% respectively. Whereas disease mean for control (T₀) was 32.64%. *Trichoderma* sp. at 1×10^3 spore/mL (T₃) most effectively controlled the pathogen development other than any treatment. T₃ caused 78% reduction in disease development. While *Bacillus* sp. at 1×10^3 spore/mL (T₄) was the second most effective treatment among others treatments. T₄ able to overcome the development of fusarium wilt at 62%. The last treatment was the combination of *Trichoderma* spp. + *Bacillus* spp. at 1×10^3 spore/mL (T₅) despite of showing significant results as compared to control was not much effective in controlling the pathogen development. *Trichoderma* spp. suspension was used to treat the tomato plant, which lowered the severity of the disease.

This study found that fungal antagonist of *Trichoderma* spp. has the potential to be tested further. This method might be used instead of fungicide since it is less harmful to the environment. *Trichoderma* spp. was shown to be more effective as a biocontrol agent against fusarium wilt of tomato. *Bacillus* spp. also considerably decreased the disease. The results for *Trichoderma* spp. were similar to those of Larkin and Fravel (1998), whom investigated different treatments to reduce fusarium wilt disease and discovered *Trichoderma* spp. as the most efficient biocontrol agents for fusarium wilt of tomato. *Trichoderma* spp. were known to be effective biocontrol agent in lowering pathogens that cause fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. Similar findings resembled with the results of Patiyal et al., (2020) found that *Trichoderma* spp. as a biocontrol agent induces resistance by improving the root system of the plant. *Trichoderma* spp. is appears to be most promising biocontrol agent in comparison to the others for the control of *Fusarium oxysporum* f. sp. *lycopersici*. Thus, *Trichoderma* spp. proved to be as efficient and yielded considerable outcomes. The research work was line with Rao et al (2020) checked the competency of *Trichoderma* spp. and potency of fungicides against fusarium wilt of tomato. The maximum suppression of the fusarium wilt disease was obtained by *Trichoderma* spp. Larkin and Fravel (1998) tested the efficacy of isolates obtained from the roots of tomato plants and tested known bio-control agents also against tomato fusarium wilt. In greenhouse, Tomato seedlings were transplanted into infested soil by treating with potential bio-control agents. They found *Pseudomonas fluorescens* and *Trichoderma* spp. as the most efficient biocontrol agents for Fusarium wilt.

Rao et al., (2020) checked the competency of *Trichoderma* spp. and potency of fungicides against fusarium wilt of tomato. *Trichoderma harzianum* was able to suppress overall disease of 80%. The results presented here are comparable to the results by Basco et al., (2017), who found that pots sprayed with *Trichoderma* spp. decreased the number of Fusarium wilt causal agent in the soil to about 29% after 90 days of pretreatment process. According to (Rosyidah et al., 2013) introducing *Trichoderma* spp. strains to the soil reduced the incidence of Fusarium in tomatoes significantly. *Trichoderma* spp. strains with the ability to adapt under harsh ground conditions may be responsible for pathogen decrease in the soil. *Trichoderma* spp. can colonize soils, outcompete pathogens, and control them due to this adaptability.

According to (Singh et al., 2008), *Trichoderma* spp. strains generate antibiotics such as gliotoxins and dermadine that can inhibit the growth of various fungal diseases. The current research in line with Sundaramoorthy and Balabaskar, (2013). They examined at the use of *Trichoderma* in the soil, which was proven to be effective in minimizing the prevalence of wilt (15-25%). When compared to the other isolates, ANR-1 antagonistic fungal formulation caused the least amount of wilt followed by *Trichoderma* spp. In my research, one treatment of *Trichoderma* spp. was used to regulate fusarium wilt of tomato plants, and the results were promising. 9.52% disease severity which is lowest among other treatments. As compared to control 32.64% and other treatments, *Trichoderma* spp. showed the highest percent inhibition of 78% percent, hence study concluded that application of *Trichoderma* spp. was not able to inhibit the mycelial growth of F.O.L but also enhance the growth of plant. The research work was line with (Akrami and Yousefi, 2015). He revealed that disease incidence percentage when compared to the infected controls, which had a 0% infection rate, each controlling agent considerably lowered the infection rate. There was a higher efficacy of all treatments for generating resistance; however, in Fusarium-infected areas that were then treated with *Trichoderma* spp. disease incidence percentages were dramatically lowered by using all *Trichoderma* spp. for managing infected tomato plants. As a result, it was discovered that there was greater protection against disease occurrence. In my investigation, I discovered that *Trichoderma* spp. provided superior disease control than isolated *F. Oxysporum* f. sp. *lycopersici* isolates when used alone. It was highly interacting in all three environments: root, soil, and foliar. A number of chemicals are produced or released by them, and these compounds cause localized or systemic resistance responses in plants. *Trichoderma* spp. on the other hand, demonstrated the greatest disease control performance against fusarium wilt.

The present study correlate with (Barari, 2016) studied that *Trichoderma* spp. was shown to be efficient in reducing the occurrence of wilt (between 14.75 and 20.15 %). In particular, the use of *Trichoderma* spp. was shown to have the lowest wilt incidence (14.75%) as compared to the control (which was untreated). In my experiment, i observed that the efficiency of inoculating tomato plants with *Trichoderma* spp. was found to be most useful in reducing disease and also yield enhancement. Present work in line with (Abdur Rashid Khan et al., 2020) studied that under greenhouse and field conditions, the treatment of *Bacillus* spp. and organic compost, either alone or in combination, greatly reduced disease growth. In my research I applied *Bacillus* sp. as a biocontrol agent, it showed 62% reduction in disease as compared to other fungicide sprays. *B. subtilis* evoke an antibiosis effect or induce resistance against pathogen infection. It showed 62 percent inhibition of FOL followed by *Trichoderma* spp. (78%) and Topsin-M (42%) and Score (21%), respectively.

Another group of scientists revealed the antagonistic relationship of the influence of *Bacillus* spp. to control fusarium wilt of tomato (Ajilogba et al., 2013). In my research, *Bacillus* spp. was also make antagonistic relationship with pathogen and was able to inhibit pathogen growth. Ajilogba et al., (2013) reported that *Bacillus* spp. was not only effective against reducing disease incidence but also increased almost all the growth parameters in tomatoes grown inside a greenhouse chamber. In compared to the control treatment, root and shoot dry weights increased significantly following *Bacillus* spp. treatment. The plants which are treated with biocontrol agents; *Trichoderma* spp. and *Bacillus* spp. showed the minimum activity against fusarium wilt attacks. As well as, the results from the difference in mode of action on two different cultivars showed the inborn resistivity of tomato. Similar results reported by (Sahar et al., 2013; Sanap et al., 2020; Patiyal et al., 2020, Poussio et al., 2021).

CONCLUSIONS

In the present study, fungicides and biocontrol agents were used to manage fusarium wilt of tomato. In the experiment, the soil was infested artificially by pouring suspension of *Fusarium oxysporum* f. sp. *lycopersici*. After a week treatments were applied in soil. Symptoms started to develop at day 8 in all treatments. Overall disease severity mean in inoculated plants for control was 32.64%. *Trichoderma* spp. at spore concentrations of 1×10^3 spore/mL (T_3) and *Bacillus* spp.

concentrations of 1×10^3 spore/mL at spore (T_4) were extremely effective as a biocontrol agent showed disease reduction of 78% and 62% respectively. Topsin M (Thiophanate-methyl) at dose of 2g/L (T_2) and Score (Difenoconazole) at dose of 0.3 ml/L (T_1) caused 42.06% and 21.08% reduction in disease severity respectively. Use of biocontrol agents can be considered as an alternative to fungicides as it is effective and at the same time it is environment friendly.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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