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Evaluation of Fungicides against *Fusarium oxysporum* f.sp. *lycopersici* the Cause of Fusarium Wilt of Tomato

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ABSTRACT

Tomato is very significant and full of nutrition vegetable crop. It is enriched with vitamin a and C, lycopene, and protects from prostate cancer, cardiovascular diseases. Tomato wilt disease (TWD) caused by *F. oxysporum* f.sp. *lycopersici* (*Fol*) is a serious threat to tomato crop in Pakistan and spreading to those areas in which it was not previously present. The current research was planned to evaluate the fungi-toxic activity of fungicides against *F. oxysporum* in vitro and under greenhouse conditions. Four fungicides which include Carbendazim, Benomyl, Curzate and Nativo, at 500ppm, 750ppm and 1000ppm concentrations, were evaluated using food poison technique. The overall results showed that all fungicides significantly ($P < 0.05$) inhibited mycelial growth of *F. oxysporum*, however, Nativo proved to be best followed by Carbendazim, Benomyl at all concentrations. The reduction in colony growth of pathogen gradually increased by increasing the fungicide concentration. Two fungicides, Nativo and Carbendazim and their concentrations 750 and 1000 ppm, which proved to be effective in laboratory conditions, also evaluated under greenhouse conditions. Both fungicides effectively controlled TWD under greenhouse conditions. The present research revealed that Nativo and Carbendazim at 750 and 1000 ppm concentrations are effective against *Fol*, thus may be included in management strategy of TWD.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is among the world's most widely farmed and consumed vegetable crops, and it is thought to be the world's second most important vegetable after potato (Saeed *et al*., 2014). Tomatoes are being consumed as fresh fruit and salad, as well as several other products, such as tomato juice, tomato sauce, soup, and even a variety of other foods, are also made from tomatoes (Alam *et al*., 2007: Bhowmik *et al*., 2012). It is grown in 145 countries including Turkey, China, India, United States, and Egypt which are the major tomato-producing countries in the global economy It is farmed on 58.4 thousand hectares in Pakistan, yielding 0.55 million tonnes / ha per year (FAOSTAT, 2019). Sindh province is leading tomato-producing area in Pakistan, with an annual production of 153.3 thousand tons and a yield per hectare is 7.3 thousand tons. Tandojam is one of Pakistan's most important tomato-producing districts. Every year, it yields many tons of tomatoes (Nizamani *et al*., 2012). In comparison to other tomato-growing countries, this average yield is poor. The United States is the leading producer of tomato with 96.80 tons per

hectare yield, followed by China, Egypt, India, and Pakistan 59.25 t/ha, 40.96 t/ha, 24.65 t/ha, and 9.44 t/ha respectively (GOP, 2018). At distinct essential development phases from sowing to maturity, the tomato crop is extremely vulnerable to different biotic and abiotic diseases.

Adaptability in relation to different habitats and high nutritive value has made tomato more popular in recent years. Various factors are responsible for low yield and among them diseases are of most concern. Tomato crop is prone to different fungal, bacterial, nematode and viral diseases. Among the fungal diseases, tomato wilt is the worst and caused large destruction in terms of both quality and quantity the causal organism of tomato wilt is *Fusarium oxysporum*, which attacks roots and causes production losses of 30 to 40 %, in some cases losses reaching 90 % (Nirmaladev *et al*., 2016). *Fusarium* wilt disease of tomato is a most important vascular disease of tomato worldwide. The soil-borne pathogen *Fusarium* spp. infects a wide range of hosts. The disease is characterized by the falling, discoloration, wilting, and death of lower leaves (Akrami *et al*., 2015). The aerial mycelia of *Fusariumoxysporum* are round and white at first, then turn to pale pink (Nizamani *et al*., 2020).

During the last few years, it has been noticed that fusarium wilt found most abundantly in tomato growing areas due to favorable environmental conditions and currently its management is being managed excellently with the use of different fungicides (Zhang *et al*., 2003; Verma *et al*., 2010). As inappropriate use of chemicals leads to serious human health hazards, therefore, proper concentration of fungicides at proper intervals could be helpful diminishing down deadly effects of fungicides (Kankwatsa *et al*., 2003; Kirk *et al*., 2005; Ghazanfar *et al*., 2016). The main objective of this study was to evaluate the in vitro and greenhouse efficacy of different fungicides with different concentrations against fusarium wilt of tomato.

METHODS AND MATERIAL

Surveys of most tomato wilt affected districts of Province Punjab including Hafizabad, Pakpattan, Khushab, Sargodha and Faisalabad, Pakistan, was conducted in 2018-19. Samples were collected from diseased tomato plants with typical symptoms and were placed in polythene zipper bags separately, labelled properly and stored in an ice box prior to isolations (Figure 1).

Figure 1. Collection of samples on the basis of symptoms.

After the surveys, isolation of *F. oxysporum* fungus from diseased plants was carried out. Samples were cut into pieces of 1-cm^2 containing both diseased and healthy portions (Nishimura, 2007). Samples were washed in 75 % ethanol for 30 s, 0.5 % NaOCl for 1 min as well as rinsed with sterilized double distilled water (Ignjatov *et al*., 2012). These washed samples were placed in petri-plates having commercial potato dextrose agar

medium (Product No. 70139-500G, Merck, Germany) (Machado *et al*., 2002; Mathur and Kongsdal, 2003). PDA media petri-plates having leaf samples were placed in incubator at ± 25°C in dark for seven days. The isolates were then shifted to a thermostat with UV light and having 25°C temperature, for ten days (Burgess *et al*., 1994). This was done to induce pigmentation and sporulation in culture. The cultures were purified using single spore technique (Choi *et al*., 1999). First on the basis of symptomology and then on conidial characteristics of the fungus, was identified as *F. oxysporum* (Figure 2).

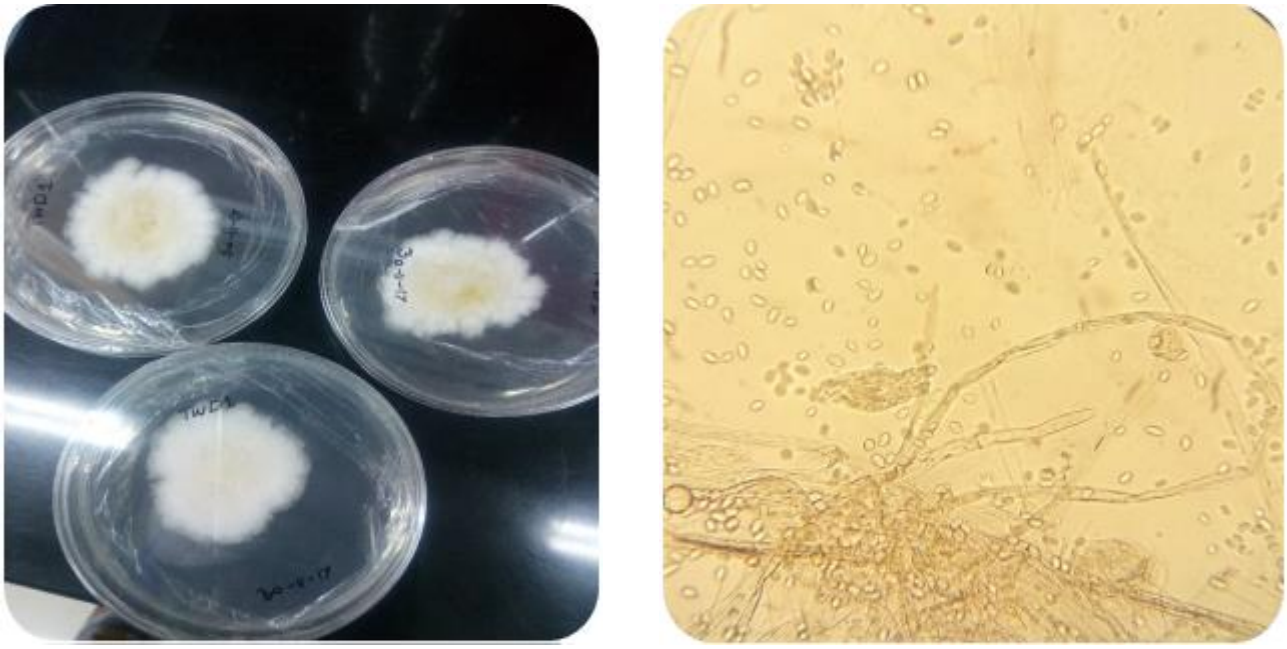

Figure 2. Characteristics of the fungus identified as *F. oxysporum* under microscope.

Evaluation of systemic fungicides against TWD in Laboratory Conditions

Efficacy of different systemic fungicides, Crabendazim, Curzate, Nativo and Benomyl, with the concentration of 500ppm, 750ppm and 1000ppm, was evaluated *in vitro* conditions against the selected isolates of TWD by poisoned food technique (Mj *et al*., 2017). For this, fungicides solutions were prepared and then added in the molten PDA media, and then the media was allowed to solidify in the petri-plates. Then, mycelial plugs of isolates of TWD were placed in the center of poisoned media plates and control plates. In control plates, fungicides solutions were not added. The rate of mycelial growth was recorded, and percent inhibition of mycelia growth over control was calculated using the following formula (Vincet, 1947).

$$\% \text{ Inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

Evaluation of systemic fungicides against the selected isolates of TWD under greenhouse conditions

On the basis of in vitro results, further experiments were conducted to check the effectiveness of fungicides under greenhouse conditions. The concentrations found best in vitro were used in greenhouse experiments and the fungicides were applied alone and in combinations. Tomato plants were inoculated with 1 x 109 conidia/mL by drenching method after transplanting seedlings in greenhouse pots (Basco *et al*., 2017). Three treatments were applied by the soil drenching method in such a way that first application of treatments was applied before inoculation of *F. oxysporum*, second treatment application was applied after one week of inoculation and third treatment application was applied after three weeks of inoculation. Disease development data was noted 7 times on weekly basis while percent disease incidence data was recorded using following formula (Lievens *et al*., 2009).

$$\text{DSI \%} = \frac{[\text{sum (class frequency} \times \text{score of rating class)}]}{[(\text{total number of plants}) \times (\text{maximal disease index})]} \times 100$$

Statistical Analysis

Recorded data was subjected to statistical analysis by using R-software. Lease significant test was used to determine the most significant treatments (Fisher, 1948).

RESULTS

Evaluation of Fungicides

The effect of four different fungicides, carbendazim (Bavistin 50 % WP), benomyl (Benlate 50 % WP), Curzate (Cymoxanil 60 %) and Nativo (Tebuconazole 50 % + Trifloxystrobin 25 %), at concentrations of 500, 750, and 1000 ppm, respectively, were evaluated *in vitro* against the growth of three isolates of *Fol* on PDA.

Efficacy of fungicides against isolates TWD-20, TWD-25 and TWD-28 of *Fol*

All fungicides significantly (P < 0.05) inhibited mycelial growth of isolate 20 of *Fol in-vitro*. Nativo showed significantly (P < 0.05) higher mycelial inhibition at all concentrations at 3rd, 6th and 9th day of colony growth followed by Carbendazim, Benomyl and Curzate (Figure 3). All fungicides showed significantly higher mycelial growth inhibition at 9th day of growth while significantly less at 3rd day of growth. At 6th and 9th day of growth, with the increase of fungicidal concentration from 750 ppm to 1000 ppm, growth inhibition increased, however, at 3rd day of growth, growth inhibition decreased with the increase of fungicidal concentration (Figure 3). All fungicides significantly (P < 0.05) inhibited mycelial growth of isolate 25 of *Fol in-vitro*. Nativo showed significantly (P < 0.05) higher mycelial inhibition at all concentrations at 6th and 9th day of colony growth followed by Carbendazim, Benomyl and Curzate (Figure 3). At 3rd day of growth, the inhibitory effect of Nativo and Benomyl was same while Carbendazim was second effective. Curzate was significantly less effective at 3rd day of growth. All fungicides showed significantly higher mycelial growth inhibition at 9th day of growth while significantly less at 3rd day of growth. At 3rd, 6th and 9th day of growth, with the increase of fungicidal concentration from 500pm to 1000 ppm, growth inhibition also increased (Figure 4). All fungicides significantly (P < 0.05) inhibited mycelial growth of isolate 28 of *Folin vitro*. Nativo showed significantly (P < 0.05) higher mycelial inhibition at all concentrations at 3rd, 6th and 9th day of colony growth followed by Carbendazim, Benomyl and Curzate (Figure 5). At 3rd day of growth, the inhibitory effect of Benomyl and Curzate was same at 500 ppm; however, was different at 750 ppm and 1000 ppm. Curzate was significantly less effective at 3rd, 6th and 9th day of growth at all concentrations. All fungicides showed significantly higher mycelial growth inhibition at 9th day of growth while significantly less at 3rd day of growth. At 3rd, 6th and 9th day of growth, with the increase of fungicidal concentration from 500pm to 1000 ppm, growth inhibition also increased (Figure 5).

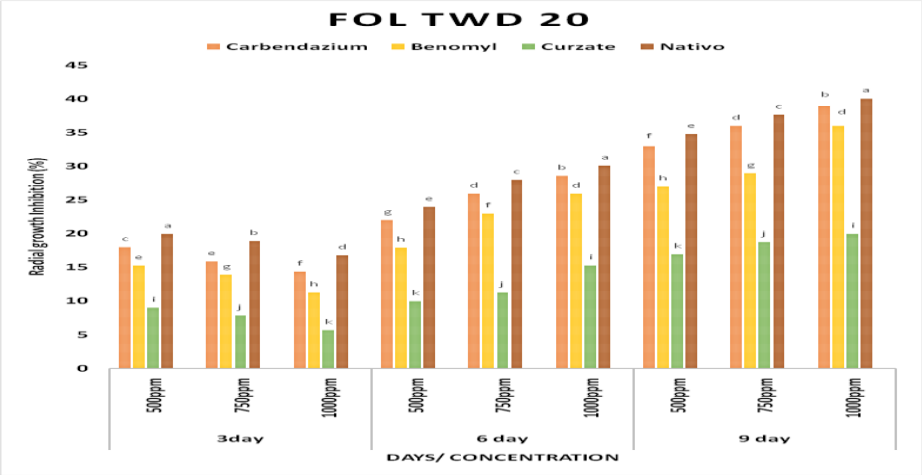

Figure 3. Effect of four fungicides at three different concentrations on mycelial growth of isolate TWD-20 of Fol*in vitro* at 3rd, 6th and 9th day of colony growth.

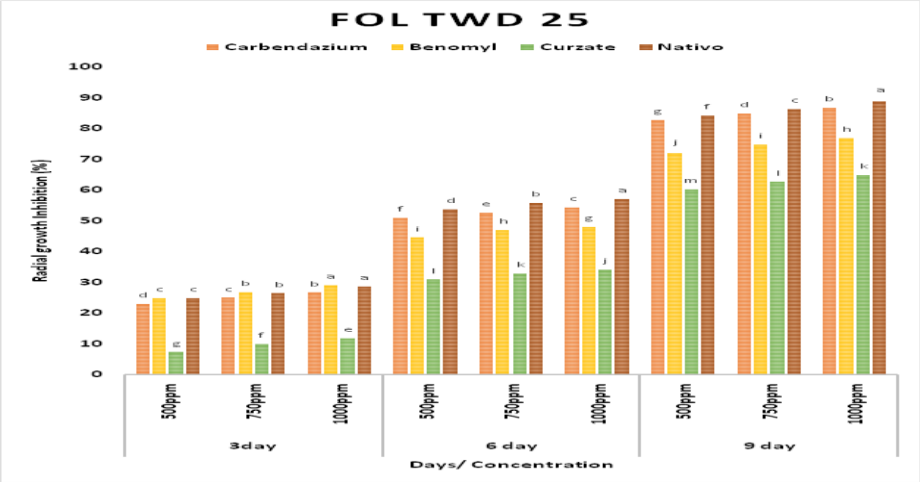

Figure 4. Effect of four fungicides at three different concentrations on mycelial growth of isolate TWD-25 of *Folin vitro* at 3rd, 6th and 9th day of colony growth.

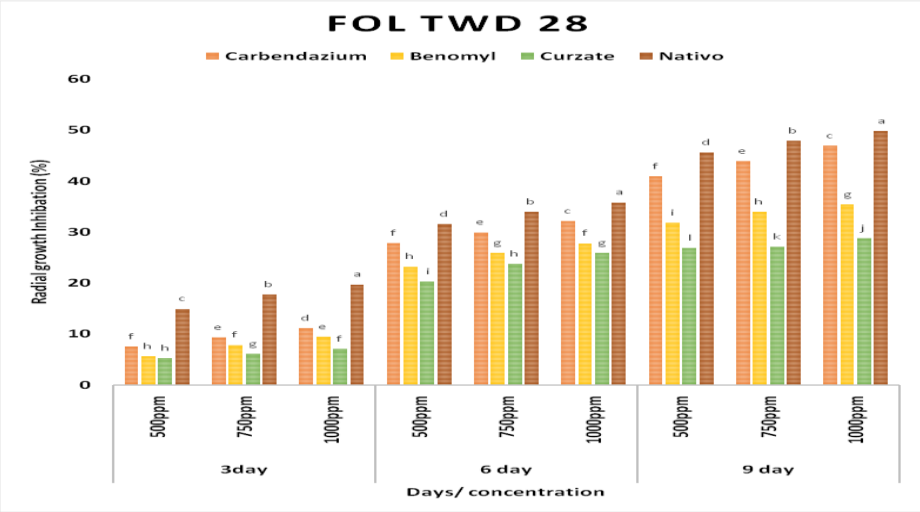

Figure 5. Effect of four fungicides at three different concentrations on mycelial growth of isolate TWD-28 of *Folin vitro* at 3rd, 6th and 9th day of colony growth.

Efficacy of fungicides against isolates TWD-20, TWD-25, TWD-28, TWD-31, TWD-33 and TWD-39 of *Fol* under greenhouse conditions

Two fungicides, Nativo and Carbendazim, and two concentrations 750 ppm and 1000 ppm, which were found effective *in vitro*, were evaluated under greenhouse conditions against five isolates. Nativo significantly ($P < 0.05$) controlled higher disease severity of TWD caused by isolate TWD-20 compared to Carbendazim and control (Figure 6). Nativo significantly controlled more disease severity at 750 ppm compared to 1000 ppm concentration and control. Similarly, Carbendazim also significantly ($P < 0.05$) controlled more disease severity at 750 ppm than 1000 ppm and control (Figure 6). After 1st, 2nd, 3rd, 4th, 5th, 6th and 7th week of inoculation of isolate TWD-20, Nativo treated plants showed disease severity 11 and 9 %, 32 and 20 %, 50 and 38 %, 59 and 41 %, 69 and 41 %, 76 and 56 %, and 69 and 45 %, at 750 ppm and 1000 ppm, respectively, compared to control (40, 50, 60, 70, 80, 90 and 100%, respectively). Similarly, after 1st, 2nd, 3rd, 4th, 5th, 6th and 7th week of inoculation of isolate TWD-20, Carbendazim treated plants showed disease severity 20 and 18 %, 36 and 23 %, 53 and 41 %, 62 and 46 %, 75 and 51 %, 84 and 61 %, and 86 and 60 %, at 750 ppm and 1000 ppm, respectively, compared to control (40, 50, 60, 70, 80, 90, 100), respectively) (Figure 6).

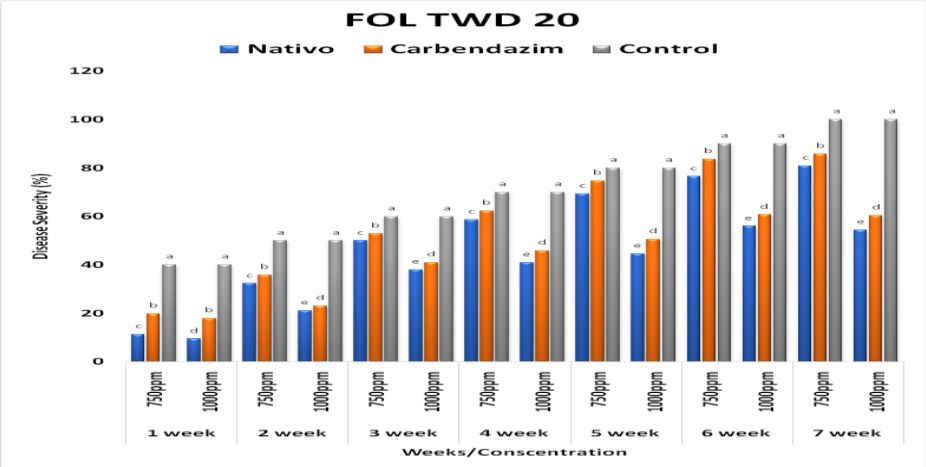

Figure 6. Effect of Nativo and Carbendazim on disease severity of isolate TWD-20 of *Fol* at 750 and 1000ppm concentrations under greenhouse conditions for the period of seven weeks.

Nativo significantly ($P < 0.05$) controlled higher disease severity of TWD caused by isolate TWD-25 compared to Carbendazim and control (Figure 6). Nativo significantly controlled more disease severity at 750 ppm compared to 1000 ppm concentration and control. Similarly, Carbendazim also significantly ($P < 0.05$) controlled more disease severity at 750 ppm than 1000 ppm and control (Figure 7). After 1st, 2nd, 3rd, 4th, 5th, 6th and 7th week of inoculation of isolate TWD-25, Nativo treated plants showed disease severity 25 and 20 %,35 and 31 %, 42 and 41 %, 52 and 51 %, 63 and 61 %, 73 and 71 %, and 85 and 81%, at 750 ppm and 1000 ppm, respectively, compared to control (40, 50, 60, 70, 80, 90 and 100%, respectively). Similarly, after 1st, 2nd, 3rd, 4th, 5th, 6th and 7th week of inoculation of isolate TWD-25, Carbendazim treated plants showed disease severity 29 and 24 %, 39 and 35 %, 47 and 45 %, 57 and 55 %, 66 and 65%, 76 and 74 %, and 89 and 84 %, at 750 ppm and 1000 ppm, respectively, compared to control (40, 50, 60, 70, 80, 90, 100%, respectively) (Figure 7).

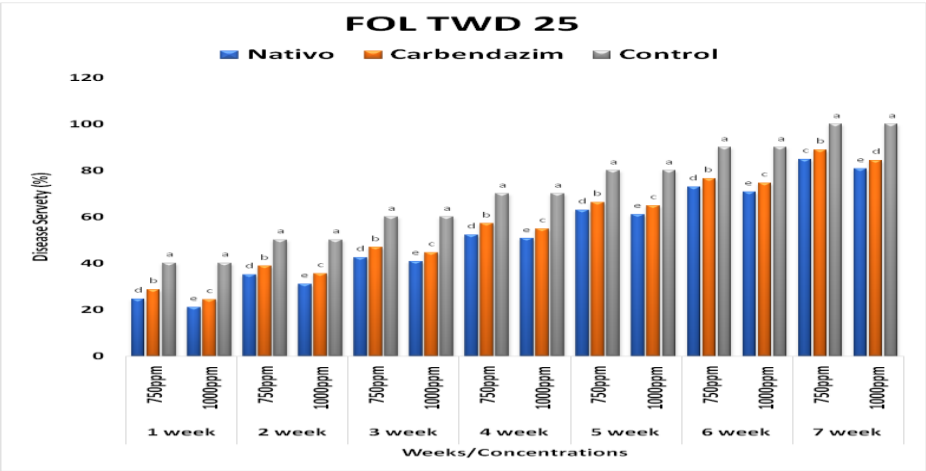

Figure 7. Effect of Nativo and Carbendazim on disease severity of isolate TWD-25 of *Fol*at 750 and 1000ppm concentrations under greenhouse conditions for seven weeks.

Nativo significantly ($P<0.05$) controlled higher disease severity of TWD caused by isolate TWD-28 compared to Carbendazim and control (Figure 8). Nativo significantly controlled more disease severity at 750 ppm while, carbendazim also significantly ($P<0.05$) prohibited more disease severity at 750 ppm than 1000 ppm and control. After isolate inoculation TWD-28, Nativo treated plants showed disease severity 13 and 10 %, 20 and 18 %, 28 and 25 %, 37 and 30 %, 45 and 35 %, 50 and 40 %, and 60 and 48 %, at 750 and 1000 ppm,

respectively, compared to control (20, 30, 40, 50, 60, 70, 80, respectively). Similarly, after 1st, 2nd, 3rd, 4th, 5th, 6th and 7th week of inoculation of isolate TWD-28, Carbendazim treated plants showed disease severity 18 and 15 %, 25 and 23 %, 35 and 29 %, 43 and 35 %, 50 and 37 %, 55 and 45 %, and 65 and 53 %, at 750 and 1000 ppm, respectively, compared to control (20, 30, 40, 50, 60, 70, 80, respectively (Figure 8).

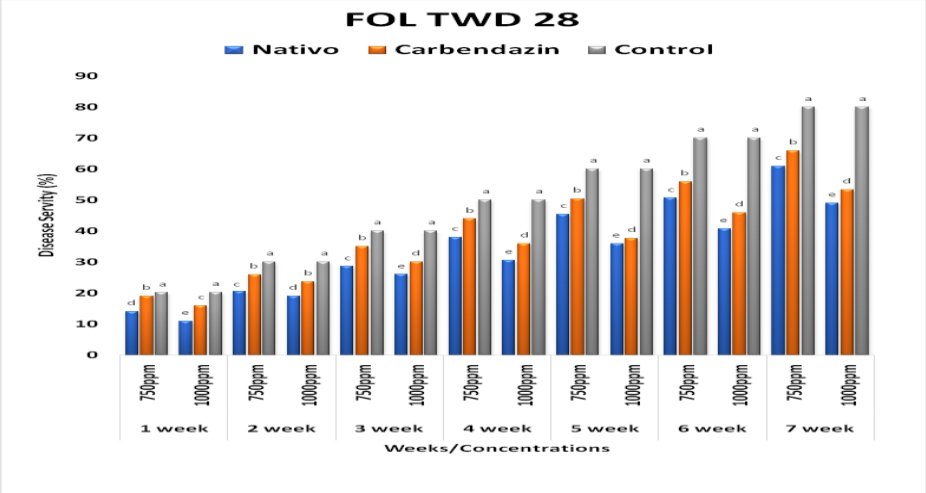

Figure 8. Effect of Nativo and Carbendazim on disease severity of isolate TWD-28 of *Fol*at 750 and 1000ppm concentrations under greenhouse conditions for seven weeks.

Nativo significantly (P<0.05) controlled higher disease severity of TWD caused by isolate TWD-31 compared to Carbendazim and control (Figure 9). Nativo significantly controlled more disease severity at 750 ppm compared to 1000 ppm concentration and control. Similarly, chemical, carbendazim considerably controlled more disease severity at 750 ppm than 1000 ppm conc. (Figure 8). After 1st, 2nd, 3rd,4th, 5th,6th and 7th week of inoculation of isolate TWD-31, Nativo treated plants showed disease severity 0.1 and 1 %, 5 and 0.8 %, 7 and 1 %, 10 and 2 %, 13 and 2 %, 18 and 3 %, and 23 and 4 %, at 750 and 1000 ppm, respectively, compared to control (5, 10, 15, 20, 25, 30 and 35 %, respectively). Similarly, after 1st, 2nd, 3rd, 4th, 5th, 6th and 7th week of inoculation of isolate TWD-31, Carbendazim treated plants showed disease severity 3 and 1 %, 7 and 1 %, 8 and 2 %, 11 and 3 %, 18 and 4 %, 21 and 5 %, and 25 and 6 %, at 750 and 1000 ppm, respectively, compared to control (5, 10, 15, 20, 25, 30 and 35 %, respectively) (Figure 9).

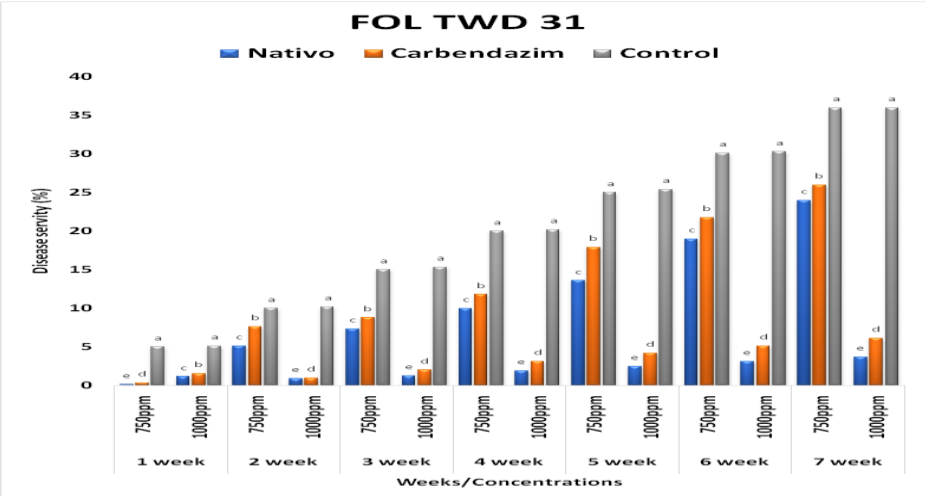

Figure 9. Effect of Nativo and Carbendazim on disease severity of isolate TWD-31 of *Fol*at 750 and 1000ppm concentrations under greenhouse conditions for seven weeks.

Nativo significantly (P<0.05) controlled higher disease severity of TWD caused by isolate TWD-33 compared to Carbendazim and control. Nativo significantly controlled more disease severity at 1000 ppm compared to 750 ppm concentration and control. Similarly, Carbendazim also significantly (P<0.05) restricted more disease severity at 1000 ppm than 750 ppm and control (Figure 10). After 1st, 2nd, 3rd, 4th, 5th,6th and 7th week of inoculation of isolate TWD-33, Nativo treated plants showed disease severity 14 and 11 %, 23 and 21 %, 30 and 28 %, 41 and 33 %, 48 and 41 %, 56 and 47 %, and 65 and 49 %, at 750 and 1000 ppm, respectively, compared to control (20, 30, 40, 50, 60, 70 and 80 %, respectively). Similarly, after 1st, 2nd, 3rd,4th, 5th,6th and 7th week of inoculation of isolate TWD-33, Carbendazim treated plants showed disease severity 19 and 16 %, 28 and 26 %, 30 and 33 %, 46 and 39 %, 53 and 46 %, 61 and 52 %, and 70 and 55 %, at 750 and 1000 ppm, respectively, compared to control (20, 30, 40, 50, 60, 70 and 80 %, respectively) (Figure 10).

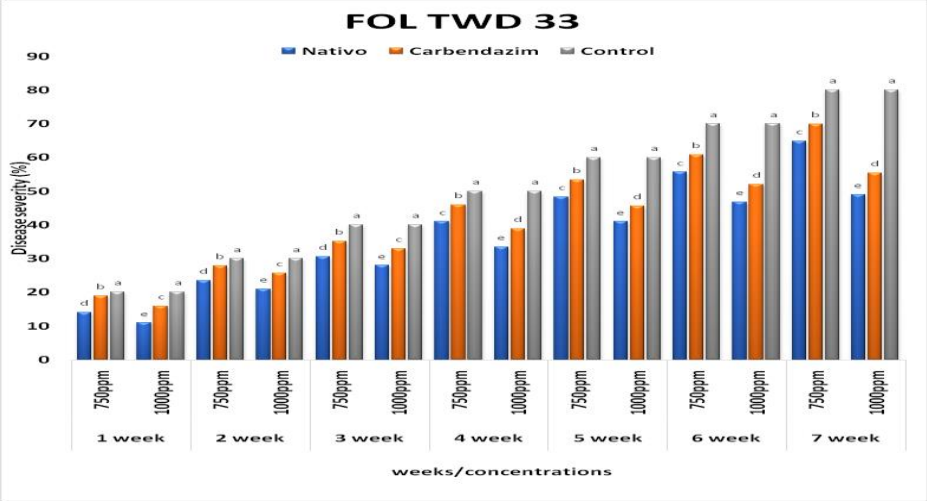

Figure 10. Effect of Nativo and Carbendazim on disease severity of isolate TWD-33 of *Fol* at 750 and 1000ppm concentration under greenhouse conditions for the period of seven weeks.

Nativo significantly (P<0.05) controlled higher disease severity of TWD caused by isolate TWD-39 compared to carbendazim and control. Nativo significantly controlled more disease severity at 1000 ppm compared to 750 ppm concentration and control. Similarly, Carbendazim also significantly (P<0.05) controlled more disease severity at 1000 ppm than 750 ppm and control (Figure 11). After 1st, 2nd, 3rd,4th, 5th,6th and 7th week of inoculation of isolate TWD-39, Nativo treated plants showed disease severity 25 and 19 %, 33 and 26 %, 40 and 33 %, 52 and 40 %, 63 and 51 %, 71 and 69 %, and 82 and 81 %, at 750 and 1000 ppm, respectively, compared to control (40, 50, 60, 70, 80, 90 and 100 %, respectively). Similarly, after 1st, 2nd, 3rd,4th, 5th,6th and 7th week of inoculation of isolate TWD-39, Carbendazim treated plants showed disease severity 29 and 22 %, 38 and 30 %, 47 and 38 %, 57 and 49 %, 66 and 58 %, 76 and 74 %, and 89 and 86 %, at 750 and 1000 ppm, respectively, compared to control (40, 50, 60, 70, 80, 90 and 100 %, respectively) (Figure 11).

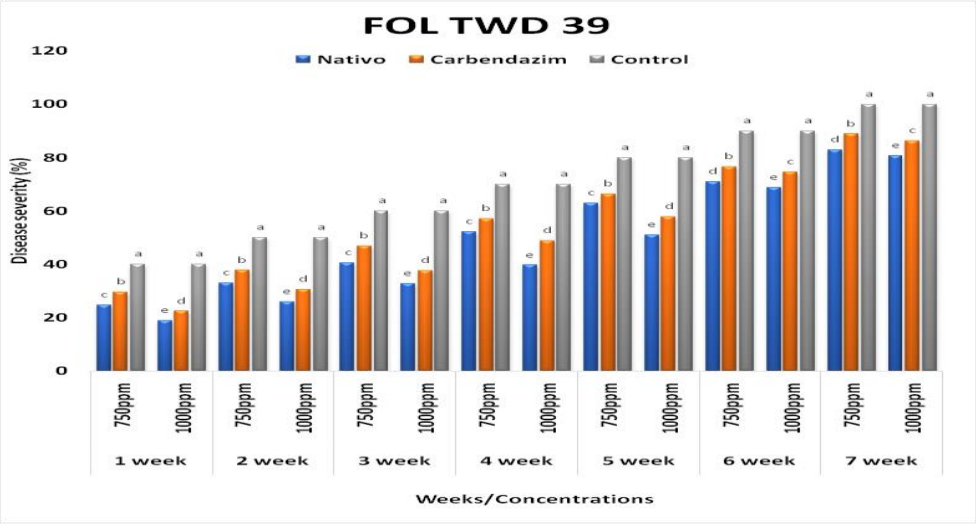

Figure 11. Effect of Nativo and Carbendazim on disease severity of isolate TWD-39 of *Fol* at 750 and 1000ppm concentrations under greenhouse conditions for the period of seven weeks.

DISCUSSION

The use of fungicides against the plant pathogen has been believed to be a rapid and potential control measure. A common strategy to mitigate losses due to *F. oxysporum* is to apply synthetic chemical fungicides, particularly those with systemic activity in the host. *In vitro* trials on PDA, the commercial fungicide Nativo was most effective in suppressing radial growth of *Fol*, followed by Carbendazim, Benomyl, and Curzate at all three tested rates. These results are in line with the previous studies in which Azoxystrobin, Benomyl, Bromuconazole, Carbendazim, Nativo, and Fludioxonil significantly minimized the growth of *Folin vitro* (McGovern, 2015). The trials in which plants in the greenhouse were inoculated with different isolates of *Fol*, Nativo and Carbendazim were the most effective synthetic fungicides. Moreover, Nativo was more effective at both rates that were tested (750 & 1000 ppm). In previous greenhouse studies, many benzimidazole (prochloraz, propiconazole, thiabendazole, carbendazim, benomyl, thiophanate, and fuberidazole) and triazole (Myclobutanil, triadimefon, difenoconazole, tebuconazole, epoxiconazole) fungicides-controlled tomato wilt to varying degrees (Macías-Sánchez, 2017).

Fungicides can boost a crop's genomic capability to produce high yield by lowering disease-related stress. Fungicides which reduce fungal growth have best penetration and therefore arise effective (Ghazanfar *et al*., 2016) however, yet fungus can acquire resistance against fungicides. Therefore, fungicides must always be administered at proper dosage and intervals (Kirk *et al*., 2015). Fungicides are used inadvertently, causing major natural concerns in addition to compromising the wellbeing of users and customers. As a result, choosing appropriate chemicals to manage disease losses is vital.

The application of fungicides can improve the genetic potential of crops and reduce the yield caused by diseases. Protective fungicides inhibit spore germination, but the pathogen can develop resistance to the appliance of fungicides. Therefore, chemicals must be applied repeatedly at appropriate doses and intervals (Krick *et al*., 2005). Fungal diseases can be controlled using chemicals that suppress or destroy the fungi that cause crop damage. Various compounds were evaluated against the causal organism of tomato wilt disease in these experiments *in vitro* and under greenhouse conditions. The focus of this research was to find appropriate fungicides to combat the *Fusarium* wilt disease effectively.

CONCLUSION

Results of present study demonstrated that Nativo and Carbendazim at 750 and 1000 ppm are effective against *Fol* both *in vitro* and under greenhouse conditions. Thus, these fungicides can be recommended to tomato farmers to control tomato wilt disease.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

Dr. Salman Ahmad and Dr. Romana Anjum conceived the

idea, facilitated, guided and supervised the surveys. Maryam Yousaf executed the field visits, took experimental data. Malik Abdul Rehman and Yasir Ali helped in data analysis. Dr. Waqas Raza re-wrote and finalized the manuscript.

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