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Assessment of synthetic fungicides against wilt of chilli caused by *Fusarium oxysporum* f. sp. *capsica*

Zuniara Akash[1], Nasir Ahmed Rajput[1,*], Muhammad Atiq[1] and Aman Ullah Malik[2]

[1]Department of Plant Pathology, University of Agriculture, Faisalabad 38000, Pakistan; [2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad 38000, Pakistan
Corresponding author's e-mail: nasirrajput81@gmail.com; nasir.ahmed@uaf.edu.pk

Chilli is one of the most important spices used in the word. It is attacked by fusarium wilt and causes huge losses. To manage this diseases, synthetic fungicides were evaluated under *in vivo* and *in vitro* conditions. Under *in vitro* conditions, ten fungicides (Electus, Nativo, Zandar, Bloom, Evito, Novice, Nanok, Kasumin and Meishile) were evaluated at three concentrations (300, 500,700 ppm) under Complete Randomized Design (CRD) by using poisoned food technique. Among all fungicides, minimum fungal growth was expressed by electus (6.656mm) followed by Meishile, Nanok, Bavestin, Kasumin, Nativo, Bloom, Novice, Zandar and Novito (9.485,16.204, 18.667, 18.874, 21.367,22.596, 22.796,31.659, 33.470 mm) as compared to control. Similar results were obtained in the interaction between treatments and their concentrations and treatments and days while under greenhouse Meishile alone exhibited minimum incidence of fusarium wilt (16.33%) as compared to Electus, Electus + Meishile and control (22.844, 20.044 and 47.00 %). Similar trend was also noted in T×C and T×D but in field conditions under Randomized Complete Block Design (RCBD), combination of Electus + Meishile expressed minimum incidence of fusarium wilt (21.9%) as compared to Meishile, Electus and control (23.467, 29,489 and 57.5 %) Corresponding results were noted in case of interaction between treatment and concentration and treatment and days. Results of the current study are helpful for integrated management of fusarium wilt of chilli. The antifungal activity of ten fungicides to counter *Fusarium oxysporum* f. sp. *capsici* (FOC) were assessed *in-vitro* and *in-vivo* conditions. *In-vitro* evaluation of fungicides by poisoned food technique showed that Electus was the most effective in hindering mycelial growth (6.65 mm), followed by Meishile (9.48 mm) at 500 ppm concentration. In green house experiments Meishile alone found to be most effective in reducing disease incidence (16.33 %) as compared to Electus (22.84 %) and a combination of Electus +Meishile (20.04 %). *In-vivo* a combination of Electus + Meishile proved the most effective for management of disease in comparison to individual treatments at 500 ppm concentration. In addition, identification of effective fungicides would enable consolidation of different chemical constituents that are required to formulate integrated disease management products. Our findings will be helpful for the future studies on the integrated management of Fusarium wilt pathogen.
Keywords: Complete randomized design, *r*andomized complete block design, poisoned food technique, disease incidence.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an economically important crop of Pakistan which belongs to *Solanaceae* family. It is native plant species of Mexico and South America. Portuguese familiarized hot Chilli in Indo-Pak subcontinent during 1585 (Hussain *et al.*, 2011). Chilli is harvested in 126 countries of the world and covers almost 1.8 million hectares. China is the world's largest producer of chilli, accounting 50% of global production followed by Mexico (7%) and Turkey (7%) (Tesfaw, 2013). In 2016-17, worldwide chilli pepper production and area under cultivation was increased upto 25% and 35% respectively with total production of 34.5 MT (FAO, 2017). While major producers of chilli was China, India, Indonesia, Mexico and Spain which produces 17.5 MT, 1.4 MT, 2.0 MT, 2.7 MT, 1.1 MT of fresh pepper respectively (FAO, 2016). Agriculture sector contributes 20% in the GDP of Pakistan. Vegetables are the major part of agriculture within the country which is subject to low yields due to biotic and abiotic challenges (Rehman *et al.*, 2015; Nawaz *et al.*, 2016). In Pakistan chilli is produced on an area of 14.81 thousand ha, with the yield of 65.1 million tonnes which accounts 3.20 percent of agricultural GDP of the country (FAO, 2017).

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Globally, chilli is used as spice due to its pungent taste which is developed by a chemical compound known as capsaicin (Mann, 2011). Medically, it is very effective against asthma, sore throat, blood regulation, cancer, sinus infection, varicose veins, liver congestion, anorexia and hemorrhoids. It also facilitates in digestion. Commercially, it is widely used in cosmetic and beverage industry (Gantait *et al.,* 2012; Yousaf *et al.,* 2017). It is rich source of fibers and essential micronutrients (Madbouly and Abdelbacki, 2017). Niacin, thiamine, betaine, choline, folic acid, pantothenic acid, ascorbic acid, Folate, and beta-carotene are also found in it. It has essential amount of fat, carbohydrate, protein and fiber (Baenas *et al.,* 2019). Annually, chilli crop suffers almost 8-10% yield losses due to different diseases (Ekundayo *et al.,* 2011). Fusarium wilt is one of the most dangerous diseases of chilli which is caused by a soil borne fungus *Fusarium oxysporum.* Fusarium wilt of chilli was initially recorded in New Mexico, where this disease affected 43 percent of the total chilli plants. Crop losses due to this disease are 25% in western countries and 50% in third-world countries (Devika Rani *et al.,* 2007). This disease has routinely resulted in annual crop losses between 10% and 80% (Vanitha *et al.,* 2009). It has been revealed that three biological forms of FOC can persist for more than eleven years while being morphologically unchanged, and that 50% of Fusarium species classified toxigenic can do so (Limón *et al.,* 2010; Wiemann *et al.,* 2013; Niehaus *et al.,* 2014; Naeem *et al.,* 2020). Fusarium wilt infection begins through roots, and hyphae penetrate in the vascular system of the plant. The pathogen hydrolytic enzymes and mycotoxins promote cellular demise in the stem and roots. Due to this blockage, transportation of water and minerals stops which leads towards yellowing of leaves. Characteristics symptoms of disease include discoloration of vascular system, upward & inward rolling, chlorosis and necrosis (Pavlovkin *et al.,* 2004; Wu *et al.,* 2009; Ul Haq *et al.,* 2020). Fusarium wilt of Chilli has caused 15-20% yield losses in dry areas during the last few years in Pakistan (Siddiqui *et al.,* 2007). Globally Fusarium wilt with has 98% disease incidence and decreased the revenue with $65300.00 million whereas, in Pakistan the recorded disease occurrence is 21.9% in vegetables and 16.6% in hot pepper which ultimately decreased the yield with 90.5 to 115.5 thousand tons from 1999 to 2005 (Hussain *et al.,* 2011).

Different management strategies including crop rotation, cultural practices, biological control, cultivation of resistant varieties, soil solarization and use of synthetic chemicals have been practiced against fusarium wilt of chilli (Tackie *et al.,* 2009). When sudden appearance of disease takes place in field, the farmers have no option except the use of synthetic fungicides because fungicides give quick response against disease. Chemical management is an imperative means for the control of different plant diseases (Iqbal and Mukhtar, 2020; Mahr, 2021). Fungicides like Carbendazim, Benomyl, Topsin-M, Difenoconazole, Nativo, and Alliete used to fight against the Fusarium wilt pathogen (Bashir *et al.,* 2018; Anjum *et al.,* 2020). Currently, fungicides such as Carbendazim, Topsin-M, Capnazol and Aliette were used against FOC (Muhammad *et al.,* 2022). Farmers in Pakistan are more concerned with improving output, yield and economic return than with the negative effects of these fungicides (Sitara and Hasan 2011). Therefore, the aim of this investigation was to evaluate synthetic fungicides which exhibit rapidity in action against FOC and to evaluate the potential application of some most effective fungicides to control the disease in greenhouse and natural environment (Ul Haq and Ijaz 2020).

MATERIALS AND METHODS

***Isolation, identification and purification of fungal pathogen*:** Based on visual observation, the plants showing typical symptoms (discolored vascular system) were collected from the vicinity of Faisalabad and carried in the laboratory for isolation. Root samples were washed thoroughly with tap water to remove surface soil then cut into small pieces (2-3mm). These pieces were washed with 1% $HgCl_2$ and then distilled water. Then portions of diseased roots incubated in Potato Dextrose Agar (PDA) at 25°C for 48-78 hours. When fungal growth starts to appear, the culture was purified by single hyphal tip technique. Identification of fungus was done with the help of compound microscope based on morphological features. FOC was recognized using morphological features such as white and purple mycelium and micro-conidia on a tiny conidiophore under a stereomicroscope (Soesanto *et al.,* 2011).

***Pathogenicity test*:** Pathogenicity of *FOC* was performed on Chilli seedlings (28 days old) grown in earthen pots (30 Cm diameter). One kg of sterilized sandy soil was infected with inoculum in the earthen pots. For the pathogenicity test, five pots with two chilli seedlings/pot were used. Treatments without the tested fungus were set up as a control. Two healthy Chilli seedlings with height of 4–6 cm were sown per pot and placed under green-house conditions. The wilting symptoms were observed on chilli plants. To complete Koch's hypothesis, re-isolation was performed on artificially diseased plants (Ignjatov *et al.,* 2015).

***In-vitro assessment of fungicides against FOC*:** Ten fungicides at three different concentrations (300, 500 and 700 ppm) were evaluated by using poisoned food technique *in-vitro* (Table 1). All concentrations were made by mixing an adequate amount of stock solution of every fungicide in flask along with PDA medium. Petri plates with 9 cm diameter were poured with amended PDA medium under laminar flow chamber. Three replications of each treatment were maintained. Every petri plate was inoculated with pathogen culture (8 days old) while control contain only PDA media (Ahmad *et al.,* 2019). After wrapping and labeling Petri plates

Table 1. Fungicides with their active ingredients used in this study.

Sr.	Trade Name	Active Ingredient	Formulation	Company
1	Electus	Azoxystrobin+Difenoconazole+Dimethomorph	51% WDG	Kanzo Ag
2	Nativo	Tebuconazole+Trifloxystrobin	75% WG	Bayer Crop Science
3	Zandar	Pyraclostrobin+Dimethomorph	18.7% WG	Bravo Crop Science
4	Bloom	Myclobutanil	25% EC	Kanzo Ag
5	Evito	Fluoxastrobin	480 SC	Arysta Lifescience
6	Novice	Azoxystrobin+Difenoconazole	32.5% SC	Sayban International
7	Nanok	Azoxystrobin+Flutriafol	25% SC	Swat Agro Chemicals
8	Kasumin	Kasugamycin	2% SL	Arysta Lifescience
9	Meishile	Azoxystrobin+Difenoconazole	40% SC	Sun Crop Pesticides
10	Bavistin	Carbendazim	50% DF	Swat Agro Chemicals

were incubated at ±25°C for ten days and the data of mycelial growth was recorded at 4, 7,10 days.

***Fungicides evaluation under greenhouse conditions against Fusarium wilt disease of chilli*:** Chilli plants were grown in the earthen pots and placed in the greenhouse of the Department of Plant Pathology, University of Agriculture, Faisalabad. Artificially inoculated chilli plants with FOC suspension were treated with two best performed fungicides selected in lab experiments (Electus and Meishile) alone and their combination (Electus + Meishile). Chilli plants sprayed with distilled water served as a control treatment. All treatments repeated thrice for the confirmation of results. Disease incidence was observed according to (Bashir *et al.*, 2018).

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of observed plants}} \times 100$$

***Fungicides evaluation under field conditions against Fusarium wilt disease of chilli*:** Under field conditions, three treatments such as Electus, Meishile and their combination (Electus+Meishile) with three replications were used in this study. For this purpose, 12 rows (20 chilli plants/row) were grown and artificially inoculated with FOC at the Research Area of Department of Plant Pathology, University of Agriculture, Faisalabad. Treatments were applied by using soil drenching and spray method. The control plants were treated with distilled water. Disease incidence was recorded for 25 days with 5 days of interval.

***Statistical analysis*:** The obtained results were statistically analyzed using statistical software SAS/STAT. All means values of statistical analysis were compared with a Fisher's least significant difference (LSD) test at $p \leq 0.05$ (Steel and Torrie, 1986).

RESULTS

***In-vitro assessment of fungicides on mycelial growth of FOC*:** Ten different fungicides viz. Electus (51% WDG), Nativo (75% WG), Zandar (6% WG), Meishile (40%), Bloom (25% EC), Evito (480 SC), Novice (32.5% SC), Kasumin (2% SL), Nanok (25%SC) and Bavistin (50% DF) were evaluated at 300, 500 and 700 ppm concentrations against FOC. Results in revealed that all selected fungicides drastically reduced the mycelial growth of FOC *in vitro*. The lowermost growth of test pathogen was recorded with Electus (6.65 mm) and Meishile (9.48 mm) followed by Nanok (16.20 mm), Bavistin (18.66 mm), Kasumin (18.87 mm), Nativo (21.36 mm), Bloom (22.59 mm), Novice (22.79 mm), Zander (31.65 mm) and Evito (33.47mm) in comparison to control (59.08 mm) at 500 ppm concentration (Figure 1).

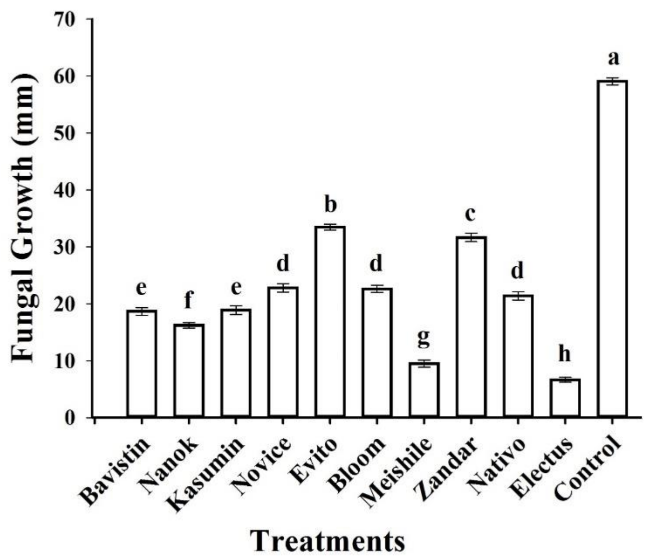

Figure 1. Impact of fungicides on mycelial growth of FOC under lab conditions. FOC mycelial growth of inhibition induced by different fungicides. Ten different fungicides were evaluated at 500 ppm concentrations against FOC. Petri plates were incubated at ±25°C for ten days and the data of mycelial growth was recorded at 10 days. Bars indicate the standard deviation. Mean with different letters at the upper side of the columns indicate significant differences (P<0.05, Least Significant Difference LSD test).

Three different concentrations of fungicides (300, 500 and 700 ppm) and day intervals (4^{th}, 7^{th} and 10^{th} day) were further tested *in vitro*. Electus and Meishile were found to be most promising and effective at 700 ppm with 6.31 mm and 8.74 mm mycelial growth, respectively (Figure 2).

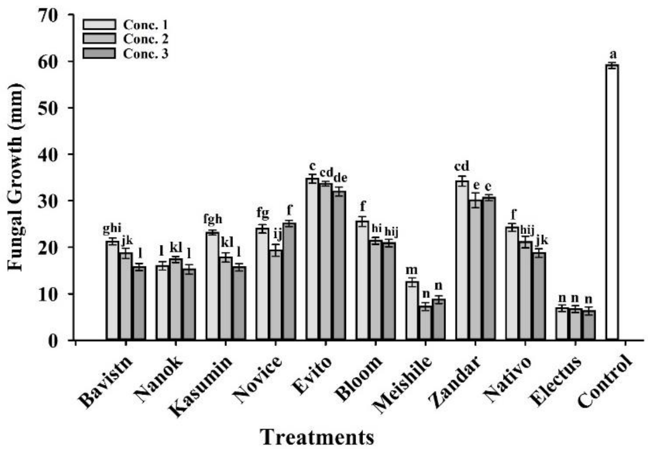

Figure 2. Impact of interactions between fungicides and their concentrations on mycelial growth of FOC under lab conditions. Ten fungicides at three different concentrations (300, 500 and 700 ppm) were evaluated by using poisoned food technique *in-vitro*. All selected fungicides significantly reduced the mycelial growth of FOC *in vitro*. The lowest growth of test pathogen was recorded with Electus at 700 ppm concentration. Bars indicate the standard deviation. Mean with different letters at the upper side of the columns indicate significant differences (P<0.05, Least Significant Difference LSD test).

Likewise, at 4th day of time interval both Electus and Meishile were found to be most suppressive fungicides with lowest fungal growth (6.23 and 7.81 mm, respectively) (Figure 3).

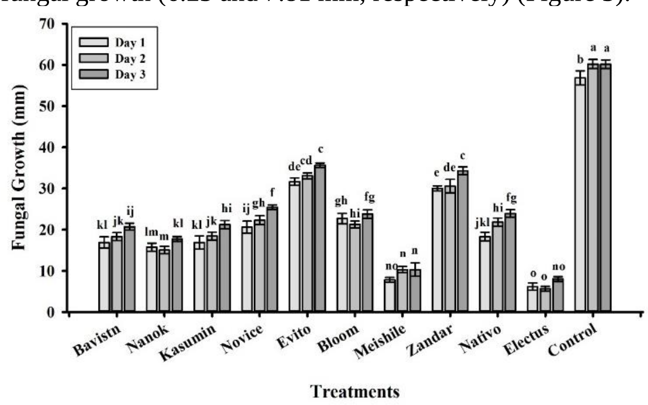

Figure 3. Impact of interactions between fungicides and time interval on mycelial growth of FOC under lab conditions. Petri plates were incubated at ±25°C for ten days and the data of mycelial growth was recorded at 4th, 7th and 10th day. The different concentrations of fungicides (300 ppm, 500 ppm and 700 ppm) and day intervals (4th, 7th and 10th day) were further tested *in vitro*. At the 4th day of time interval both Electus and Meishile were found to be most suppressive fungicides with lowest fungal growth at 700 ppm. Bars indicate the standard deviation. Mean with different letters at the upper side of the columns indicate significant differences (P<0.05, Least Significant Difference LSD test).

***Assessment of fungicides against Fusarium wilt of chilli caused by FOC under greenhouse conditions*:** The most effective fungicides *in-vitro* Electus and Meishile at three concentrations (300, 500 and 700 ppm) were tested for their efficacy against Fusarium wilt of chilli under greenhouse conditions. Both chemicals were used separately and in combination (Electus +Meishile) to assess their effectiveness. Data showed (Figure 4) that the fungicide Meishile significantly reduce disease incidence (16.33 %) followed by Electus + Meishile (20.04 %) and Electus (22.84%) as compared to control (47%) at 500 ppm. Fungicide concentrations significantly reduced the disease incidence on chilli plants. By increasing concentration up to 700 ppm, maximum disease reduction was observed with Meishile (12.43%) as compared to control (47%) treatment (Figure 5).

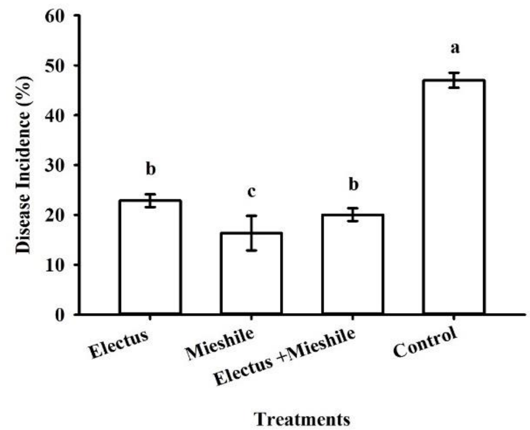

Figure 4. Impact of fungicides on disease incidence under greenhouse conditions. The disease incidence percentage was recorded in chilli plant pots. Artificially inoculated chilli plants with FOC suspension were treated with two best performed fungicides selected in lab experiments (Electus and Meishile) alone and their combination (Electus + Meishile). Meishile significantly reduce disease incidence followed by Electus +Meishile and Electus as compared to control at 700 ppm concentration. Bars indicate the standard deviation. Mean with different letters at the upper side of the columns indicate significant differences (P<0.05, Least Significant Difference LSD test).

***In-vivo assessment of fungicides against Fusarium wilt disease of chilli caused by FOC*:** Presented data in (Figure 6) revealed that combination of fungicide Electus + Meishile was found to be most effective with least disease incidence (21.90%) followed by Meishile (23.46%) and Electus (29.48%), respectively with comparison to control (57.5%) at 500 ppm in the field experiment. Electus + Meishile showed outmost disease reduction (21.46% @ 500 ppm) and (21.56% @ 300 ppm) but by increasing concentration up to 700 ppm the disease incidence was increased (22.66 %) (Figure 7). Meishile was most effective with minimum disease incidence (20.66 %) at concentration 700 ppm.

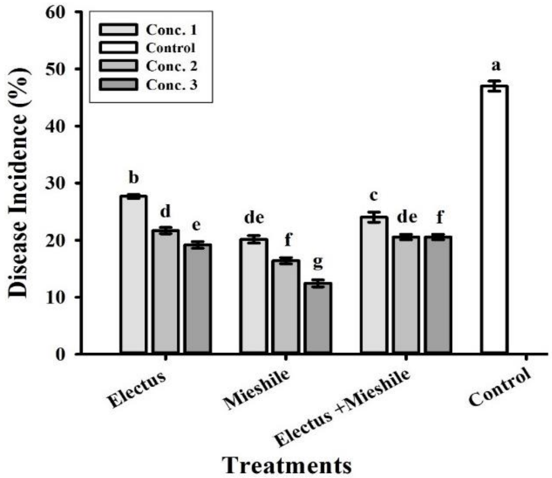

Figure 5. Impact of interactions between fungicides and their concentrations on disease incidence under greenhouse conditions. The disease incidence percentage was recorded in chilli plant pots with three concentrations (300, 500 and 700 ppm) against Fusarium wilt of chilli under greenhouse conditions. Fungicide concentrations significantly reduced the disease incidence on chilli plants. Maximum disease reduction was observed with Meishile as compared to control at 700 ppm concentration. Bars indicate the standard deviation. Mean with different letters at the upper side of the columns indicate significant differences (P<0.05, Least Significant Difference LSD test).

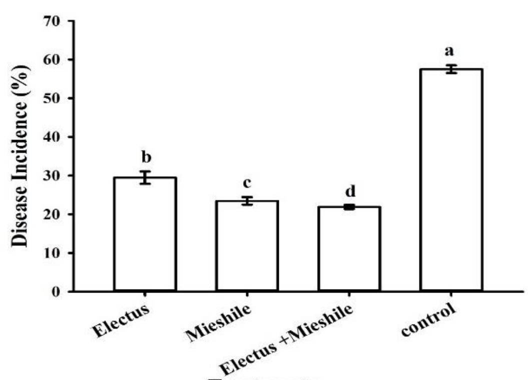

Figure 6. Impact of fungicides on disease incidence under field conditions. Disease incidence was recorded for 25 days with 5 days of interval under field conditions with three treatments such as Electus, Mieshile and their combination Electus + Mieshile with three replications. Twelve rows (20 chilli plants/row) were grown and artificially inoculated with FOC at the Research Area of UAF. Electus + Mieshile was found to be most effective with least disease incidence followed by Mieshile and Electus, respectively with comparison to control at 700 ppm in the field experiment. Bars indicate the standard deviation. Mean with different letters at the upper side of the columns indicate significant differences (P<0.05, Least Significant Difference LSD test).

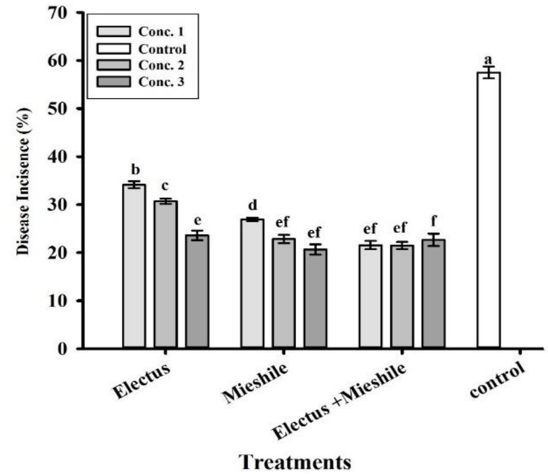

Figure 7. Impact of interactions between fungicides and their concentrations on disease incidence under field conditions. Disease incidence was recorded for 25 days with 5 days of interval under field conditions with three treatments such as Electus, Mieshile and their combination Electus + Mieshile with three replications. Twelve rows (20 chilli plants/row) were grown and artificially inoculated with FOC at the Research Area of UAF. Mieshile was most effective with minimum disease incidence at concentration 700 ppm. Bars indicate the standard deviation. Mean with different letters at the upper side of the columns indicate significant differences (P<0.05, Least Significant Difference LSD test).

DISCUSSION

Synthetic fungicides are commonly used to counter different fungal pathogens viz., *Colletotrichum*, *Rhizoctonia solani, Phytophthora*, *Verticillium, Fusarium, Botrytis cinerea*, and *Aletrnaira* (Maitlo *et al.,* 2014; Chapin *et al.,* 2006). Keeping in view the prominence of yield losses of chilli in Pakistan due to Fusarium wilt disease, *in-vitro* and *in-vivo* studies were conducted for the control of the Fusarium wilt through synthetic fungicides. Results revealed that all fungicides used in this study pointedly decreased the mycelial growth of tested pathogen. Among all, Electus found most effective in reducing the mycelial growth followed by Meishile. Whereas, all remaining fungicides, Bavistin, Kasumin, Nativo, Bloom, Novice, Nanok, Zander and Evito were found less effective as compared to control under *in vitro* conditions. The results of this study supported by Rukhsana *et al.,* (2010) who evaluated four fungicides (capton, topsin, vitavax and bayleton) against seven fungal strains (*Aspergillus niger, F. oxysporum, Dreschleratetramera, Alternaria alternate* and *Rhizopus*sp.

isolates) where Bayleton and Topsin were most effective against tested pathogens.

Efficacy of Electus and Meishile increased as the concentration increased from 300 to 700 ppm and both fungicides gave maximum effectiveness at highest concentration 700 ppm. Our findings of current study are supported by Singh *et al.* (2010) who reported that mancozeb at 200 ppm concentration was most effective against *FOC*. It was reported that Alliette, Benlate and Carbandazim completely inhibit the mycelial growth of Fusarium wilt pathogen at 100 ppm (Suitrana *et al.,* 2013). Most of the studies also concluded that the increase in concentration of chemical to be directly proportionate to the increase in effectiveness of chemical (Rafique *et al.,* 2016). Contrary to expectation, the Meishile significantly reduce disease incidence followed by Electus, Electus + Meishile as compared to control treatment in green house experiments.

In the field experiments, Electus + Meishile in combination were found to be out most effective followed by Meishile and Electus at 500 ppm. Whereas the disease incidence was increased with the increased concentration of Electus + Meishile up to 700 ppm. Only Meishile was found most effective at 700 ppm under *in-vivo* conditions. Our findings to some extent supported by Sitara *et al.*, (2011) where Metalexyl + Mancozeb, Derosol, Copper Oxychlorite and Antracol were found ineffective at 0.5% concentration while Mancozeb, Aliette and Ridomil Gold were showed best results at same dose. It was reported that fungicide Aliette exhibited better results in comparison to Mancozeb and Thiophonate methyl at same concentration level (Sitara *et al.,* 2007).

***Conclusion*:** *In vitro* results revealed that all ten fungicides reduced mycelium growth of FOC. Overall, Electus and Meishile were found the most effective treatment for the control of *Fusarium* wilt pathogen under *in-vitro* and *in-vivo* investigation. We believe that our finding will be as a starting point for the discovery of new most effective antifungal agents against FOC.

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***Authors Contributions statement*:** ZA and NAR have conceived and planned the study. ZA wrote the paper with input from all the authors. MA and AUM helped to improve the draft.

***Conflict of interest*:** The authors declare that there is no conflict of interest.

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