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INTEGRATED MANAGEMENT OF FUSARIUM WILT OF CHILLI CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *CAPSICI* THROUGH DIFFERENT MANAGEMENT APPROACHES

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Abstract

Fusarium wilt (*Fusarium oxysporum* f. sp. *capsici*) of reduces the yield of chilli annually. For management of this destructive disease, five plant extracts (*Moringa oleifera, Zingiber officinale*, *Azadirachta indica*, *Aloe vera*, and *Allium sativum*) @ 10 and 15% and four chemicals (Carbendazim, Topsin-M, Capnazol and Aliette) @ 300 and 500 ppm were evaluated by poisoned food technique. Among all treatments, *M. oleifera* and Carbendazim showed maximum reduction in mycelial growth. Furthermore, six different plant activators were evaluated under the field condition @ 0.5, 0.75 and 1% concentrations respectively. Salicylic acid showed minimum disease incidence followed by benzoic acid, citric acid K_2HPO_4, KH_2PO_4 and ascorbic acid respectively. *M. oleifera*, salicylic acid and carbendazim were evaluated alone and in combination against Fusarium wilt under field conditions. Minimum disease incidence was shown by salicylic acid + *M. oleifera* + carbendazim followed by Salicylic acid + Carbendazim, *Moringa oleifera* + Carbendazim, Carbendazim, Salicylic acid + *Moringa oleifera*, Salicylic acid and *Moringa oleifera* respectively as compared to control. It is concluded that combination of salicylic acid + *M. oleifera* + carbendazim showed minimum disease incidence under field conditions.

Key words: Plant extracts, Fungicides, Plant activators, Control, Field.

Introduction

Pakistan is fifth largest chilli exporter in the world, but the production of chilli is gradually decreasing due to several biotic and abiotic diseases (Hussain and Abid, 2011). In Pakistan, chilli crop is grown on an area of 45.7 thousand hectares with 103.7 thousand tones production (Anon., 2020-2021). It is prone to number of diseases, but Fusarium wilt (FW) caused by *Fusarium oxysporum* f. sp. *capsici* (FOC) is the most destructive one which significantly reduces the yield. Disease symptoms appear at various stages of the crop from seedlings to harvesting depending upon the environmental conditions, type of soil, availability/unavailability of nutrients and the density of inoculums. FOC penetrates through root tissues into the vascular system and rapidly colonizes in the xylem vessel (El-Kazzaz *et al.*, 2008) and host plant start producing symptoms in the form of upward, inward rolling and yellowing of leaves which leads to wilting of the whole plant (Roncero *et al*., 2003).

Scientists, researchers, and farmers used a number of strategies to control FW including crop rotation, cultivation of resistant varieties, biological control, soil solarization and chemicals (Abo-Elyousr *et al*., 2009). Among these strategies, few have deleterious effects on environment and others are expensive to use. The economical and environment friendly to manage this disease, is the use of resistant varieties (Amini, 2010). However, if the disease appears as an epidemic, then farmers have no option, except the use of fungicides because fungicides show quick response towards FW. That's why, in current study four fungicides were evaluated against FW. No doubt chemicals exhibit quick response towards disease but cause environmental pollution and have health hazard effects on human beings (Hassaan & El-Nemr, 2020). Moreover, injudicious use of chemicals is also creating resistance in pathogen. So, it is a need of hour to find out an alternate solution to manage FW. For this purpose, use of plant extracts is significant because different plants produce secondary metabolites which have inhibitory effects against plant pathogens. These plants extracts have marvellous and cost-effective application against different plant pathogens (Obongoya *et al.*, 2010; Rafiq *et al*., 2021). Moreover, phytoextracts are eco-friendly and have fewer toxic effects compared to chemicals. So, in current project different phytoextracts at different concentrations were evaluated towards FOC.

Systemic acquired resistance is also an alternative approach which can be induced in plants against different types of diseases. Systemic induced resistance plays a momentous role for the activation of signal molecules and transcriptional regulators by the application of plant defense activators (Sarwar *et al.*, 2005). It is necessary to find out new strategies to overcome the aggressive virulent strains of the pathogen (Jalali *et al*., 2006) which express biochemical and physiological variations in plants such as production of phytoalexins, lignin formation and pathogenesis related proteins after their application. That's why in current study six plant activators were evaluated against FW of chilli.

Materials and Methods

Collection of infected samples, plant and chemical material: For isolation of casual pathogen, diseased samples were collected from different areas of Faisalabad, showing typical symptoms of FW in the laboratory of Plant Pathology, University of Agriculture Faisalabad

(UAF) Six plant activators salicylic Acid ($C_7H_6O_3$), benzoic acid ($C_7H_6O_2$), citric acid ($C_6H_8O_7$), dipotassium phosphate (K_2HPO_4), potassium hydrogen phosphate (KH_2PO_4), ascorbic acid ($C_6H_8O_6$) and four fungicides Carbendazim (carbendazim), Topsin-M (thiophanate-methyl), Capnazole (hexaconazole) and Aliette (fosetyl-aluminium) were purchased from Faisalabad market. Five different plants such as Neem (*Azadirachta indica*), Aloevera (*Aloe vera*), Ginger (*Zingiber officinale*), Sohanjina (*Moringa oleifera*), and Garlic (*Allium sativum*) were collected from the different growing field area of University of Agriculture Faisalabad.

Isolation, identification and purification of pathogen: Isolation was done from infected plants showing wilt symptom. Diseased sample was cut into 4-6 mm small pieces, washed with distilled water and surface sterilized with 1% $NaOCl_2$ for 30 seconds and washed three time with sterilized distilled water and left for drying. The samples were placed on PDA media with the help of sterilized forceps and incubated at 28ºC± 2^{o}C and were observed on daily basis for colony growth. Subculturing of the pathogen was also done for purification of culture. Identification of FOC was done under light microscope, based on morphological characters of the pathogen like colony growth, color (Purple and white) and conidiophores and microconidia (Soesanto *et al.*, 2011).

***In-vitro* assessment of different plant extracts:** Above mentioned plants were brought to lab for further processing. In 50 gm of dried plant materials used at the rate of 50g plant material 200mL of sterilized distilled water was added and ground in a blender machine, after that 1g of washing powder was mixed and filtered with muslin cloth in beaker and then left for 24 hours. After that another 50ml sterilized distilled water was mixed in each beaker. For the preparation of 10 and 15% concentration 10 and 15 mL of prepared plant extracts were diluted in 90 and 85 mL sterilized distilled water. Each concentration was added separately in a flask containing semi cooled PDA media, thoroughly mixed and poured in the Petri plates poured under laminar flow (Yelmame *et al*., 2010). The pathogen was inoculated at 28±2^{o}C in each treated Petri plate including control. The data was recorded on the basis of mycelial growth percentage and compared with control. Experiment was conducted under CRD. Mycelial growth percentage was calculated using following formula (Yelmame *et al.,* 2010).

$$\text{Mycelial growth percentage (\%)} = \frac{C-T}{C} \times 100$$

C= Pathogen growth after incubation in control
T= Pathogen growth after incubation in treatments

***In-vitro* assessment of different fungicides:** Carbendazim, Topsin-M, Capnazole and Aliette were evaluated against FOC through poisoned food technique @ 300, 500 ppm in the lab. Each concentration was added separately in a flask containing semi cooled PDA media, shaked and poured in Petri plates under laminar flow chamber. The pathogen was inoculated at 28±2^{o}C in each treatment including control (Parsa *et al.*, 2013) and the data was recorded on the basis of mycelial growth percentage and compared with control, under CRD and mycelial growth percentage was calculated following Yelmame *et al.*, (2010).

***In-vivo* assessment of different plant activators:** Seeds of susceptible variety (High line) were surface sterilized by sodium hypochlorite 1% solution and were soaked in different concentration (0.5, 0.75 and 1%) of salicylic acid ($C_7H_6O_3$), benzoic acid ($C_7H_6O_2$), citric acid ($C_6H_8O_7$), dipotassium phosphate (K_2HPO_4), Potassium phosphate (KH_2PO_4), ascorbic acid ($C_6H_8O_6$) for 24 hours while for control, sterilized distilled water was used (Sarwar *et al*., 2005). After 15 days of sowing, seedlings were uprooted and washed in running water to remove clay particles form roots, then were dipped in FOC spore suspension (10^6spores/ mL) for 4 hours and transplanted in pots contained sterilized soil and placed in the greenhouse. Data regarding disease incidence was recorded with one week interval of the whole crop season and disease data was estimated under the RCBD. The disease incidence was calculated using following formula (Monaim *et al*., 2010).

$$\text{Wilt incidence (\%)} = \frac{\text{No of wilted plants}}{\text{No of total plants}} \times 100$$

Assessment of the plant activators, plant extracts and chemical against Fusarium wilt disease of chilli under field conditions: Susceptible variety of Chilli (High line) seeds, were surface sterilized by sodium hypochlorite (1% solution) and were grown in a plastic tray containing sterilized soil and kept under greenhouse condition. After 15 days of sowing (seedlings with three leaves) were uprooted and washed in running water to remove the clay particles form roots. The seedlings were dipped in *F. oxysporum* f. sp. *capsici* spore suspension (10^6 spores/mL) for 4 hours and transplanted in the field. Salicylic acid @ 1%, *M. oleifera* @ 15%, Carbendazim 1% were used alone and in combination (salicylic acid + *M. oleifera,* salicylic acid + Carbendazim, salicylic acid + *M. oleifera, M. oleifera* + carbendazim and salicylic acid + *M. oleifera* + carbendazim) and sterilized water for control. Each treatment was applied separately after one day intervals respectively through soil drenching with three replications under the RCBD. Disease incidence data were recorded after one week interval for the whole crop season.

Results

***In-vitro* assessment of plant extract against pathogen:** Out of 5 different plant extracts, minimum mycelial growth of the pathogen was shown by *M. oleifera* (1.132 cm) followed by *Z. officinale, A. indica, A. vera* and *A. sativum* (1.37, 2.18, 2.6 and 3.22cm) respectively (Fig. 1A). In interaction between treatments and their concentration (T x C), minimum mycelial growth (0.97cm) was expressed by *M. oleifera* at 15% concentration followed by 10% concentration, followed by *Z. officinale, A. indica, A. vera* and *A. sativum* compared to control (Fig. 1B) while in case of Interaction T x C x D,

minimum mycelial growth (0.49, 0.98, and 1.50cm) was expressed by *M. oleifera* after 3, 7 and 10 days, at 15% concentration followed by 10% concentration. At the same concentration, remaining treatments (*Z. officinale, A. indica, A. vera* and *A. sativum*) showed less mycelial growth compared to control (1.40, 3.92, and 7.05cm) after 3, 7 and 10 days respectively (Fig. 1C).

***In-vitro* assessment of fungicides against pathogen:** Out of 4 different fungicides minimum mycelial growth of the pathogen was expressed by Carbendazim (0.87cm) followed by Topsin-M (1.07cm), Capnazole (1.54cm) and Aliette (1.76cm) respectively (Fig. 2A). Interaction between treatment and their concentration (TxC), minimum mycelial growth (0.706cm) was recorded in Carbendazim at 500ppm followed by 300ppm concentration while at the same concentrations, remaining treatments (Topsin-M, Capnazole and Aliette) showed less mycelial growth compared to control (Fig. 2B). Interaction between T x C x D, minimum mycelial growth (0.37, 0.75, 0.99cm) was observed in Carbendazim at 500pmm concentration after 3, 7 and 10 days, followed by 300ppm concentrations (Fig. 2C).

***In-vivo* assessment of plant activators against pathogen:** Out of 6 different plant activators, salicylic acid expressed minimum disease incidence (28.039%), followed by benzoic acid, citric acid, $K2HPO_4$, $KH2PO_4$, and ascorbic acid (32.55, 41.48, 43.31, 44.47 and 46.2%) respectively (Fig. 3A). in interaction between treatments and their concentration (T x C), salicylic acid exhibited minimum disease incidence (26.65%) at 1%, followed by 0.75 and 0.5% concentrations, at the same concentrations remaining treatments expressed less disease incidence compared to control (Fig. 3B).

Assessment of the plant activators, plant extracts and chemical against *Fusarium oxysporum* f. sp. *capsici* under field condition: Salicylic acid + *Moringa oleifera* + Carbendazim expressed minimum disease incidence (13.48%) followed by Salicylic acid + Carbendazim (19.94%), *Moringa oleifera* + Carbendazim (23.23%), Carbendazim (26.06%), Salicylic acid + *Moringa oleifera* (29.87%), Salicylic acid (35.99%) and *Moringa oleifera* (45.72%) expressed disease incidence respectively as compared to control (68.72%) (Fig. 4).

Discussion

Different antifungal compound has been reported in many plant species (Singh *et al.*, 2004; Sridhar *et al.*, 2003). These metabolites naturally synthesized in plants, having antifungal activity and less phytotoxicity are safe, translocatable and decomposable (Sitara *et al*., 2008; Lee *et al.*, 2007). Plants extract used as chemical alternative, because these extracts are not harmful to man, animal and plants compared to chemicals (Asthana, *et al*., 2001; Rajput *et al*., 2018). Plant extracts were used in many compositions such as dipping solution, cakes, essential oils, spray, water solution and fumigants (Rajput e*t al*., 2011; Siripornvisal & Ngamchawee, 2011). Therefore, in current study 5 plant extracts *A. indica, A. Verai, Z. officinale, M. oleifera* and *A. sativumica* were used against FW, the causal agent of chilli wilt disease. In current study, out of 5 plant extracts, *M. oleifera* exhibited outstanding results as compared to other plant extracts. Two plants extract *A. indica* and *Z. officinale* contained antifungal compounds (Dissanayake, 2014) which prohibited *F. oxysporum* spore germination. Results of current study were also supported by the work of Dwivedi and Sangeeta, (2014), Jamil *et al.*, (2007) who evaluated various plant extracts against *F. oxysporum* and found them effective.

Use of systematic fungicide is an essential tool for the management of different soil borne diseases due to non-availability of resistant cultivars (Iqbal *et al*., 2010; Song *et al*., 2004). In the present study, 4 fungicides (Carbendazim, Capnazole, Aliette and Topsin-M) were used against FOC at two different concentrations. Among these fungicides, Carbendazim @ 500ppm showed the best results to inhibit the mycelial growth of the pathogen (Iqbal *et al.*, 2010; Bilgrami & Dube, 1976). It disturbs the ionic concentration and microtubules of fungus by chemical force strongly bind (Magnucka *et al*., 2007). The results of our study were also supported by the findings of Naik *et al*., (2007) who evaluated carbendazim, Triademifon and thiophanate @ 3000, 2000, 1000 and 500 ppm against FOC and detected that Carbendazim expressed best results followed by other fungicides. Shah *et al.*, (2014), Parsa *et al.*, (2013) and Yucel *et al*., (2007) appraised Alliet, Topsin-M, Carbendazim, Nativo and Difenconazole against *F. oxysporum* and noted that Carbendazim expressed significant reduction in mycelial growth of FOC.

Defense system of the plant can stimulate by using numerous complex courses of plant growth regulators and transduction of signal molecules. Resistant gene play a vital role defense system activation and incompatible response produce against pathogens. Plant growth regulators externally applied at a nontoxic concentration can activate the primary defense system of host plant (Jalali *et al*., 2006) and earlier susceptible plant become resistant (Elwan and El-Hamahmy, 2009). Salicylic acid is effectual hormones in plant that increase resistance and mediated changes in growth, nitrogen metabolism, photosynthesis and antioxidant defense system in the crops (Hayat *et al*., 2012). However, suitable amount of salicylic acid raises the action of resistant genes (Khan *et al*., 2003; Howard *et al*., 2000). In contemporary study, six plant activators (Salicylic acid, Benzoic acid, Citric acid, KH_2PO_4, K_2HPO_4 and Ascorbic acid) were used against wilt disease of Chilli. Among all plant activators used in current study, salicylic acid expressed minimum disease incidence. Outcomes of the current study could be validated by the findings of Ali *et al*., (2000), Sarwar *et al.*, (2005) and Hanieh *et al*., (2013) who used salicylic acid, ferric chloride, indole acetic acid, hydrogen peroxide, calcium chloride, dipotassium hydrogen orthophosphate, salicylic acid and metalaxyl express considerable results opposing Fusarium wilt. A component of ROS hydrogen peroxide is produced after Salicylic acid application, which prevents fungal spore germination because of antifungal activity and phenoxy radical development involvement during polymerization of phenol in plant cell wall (Huynh *et al*., 2001; Inbar *et al*., 1998).

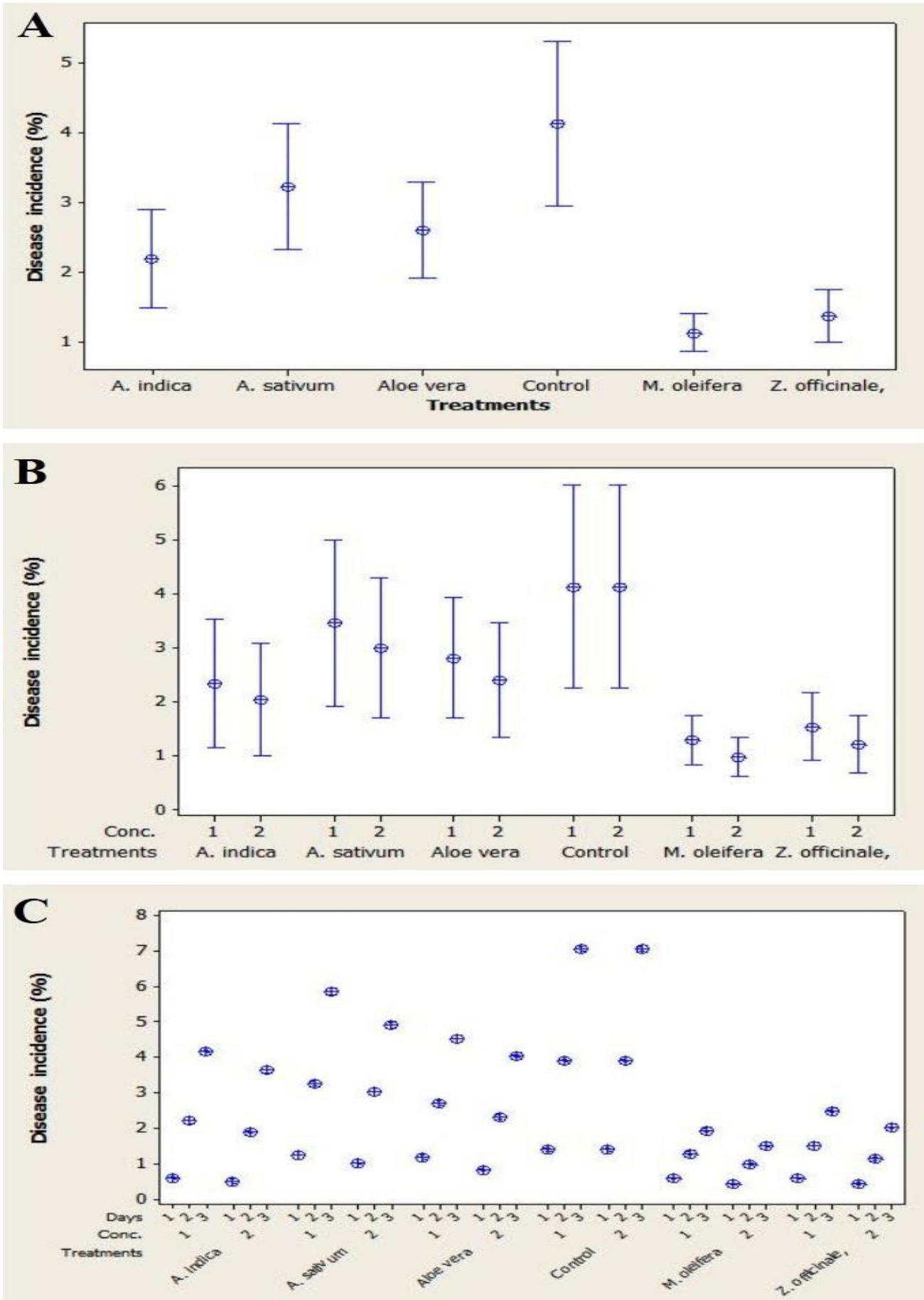

Fig. 1. *In-vitro* assessment of different plant extracts and their (A) treatment, (B) treatment and concentrations, (C) treatment, concentration and days, on the growth of *Fusarium oxysporum* f. sp. *capsici*.

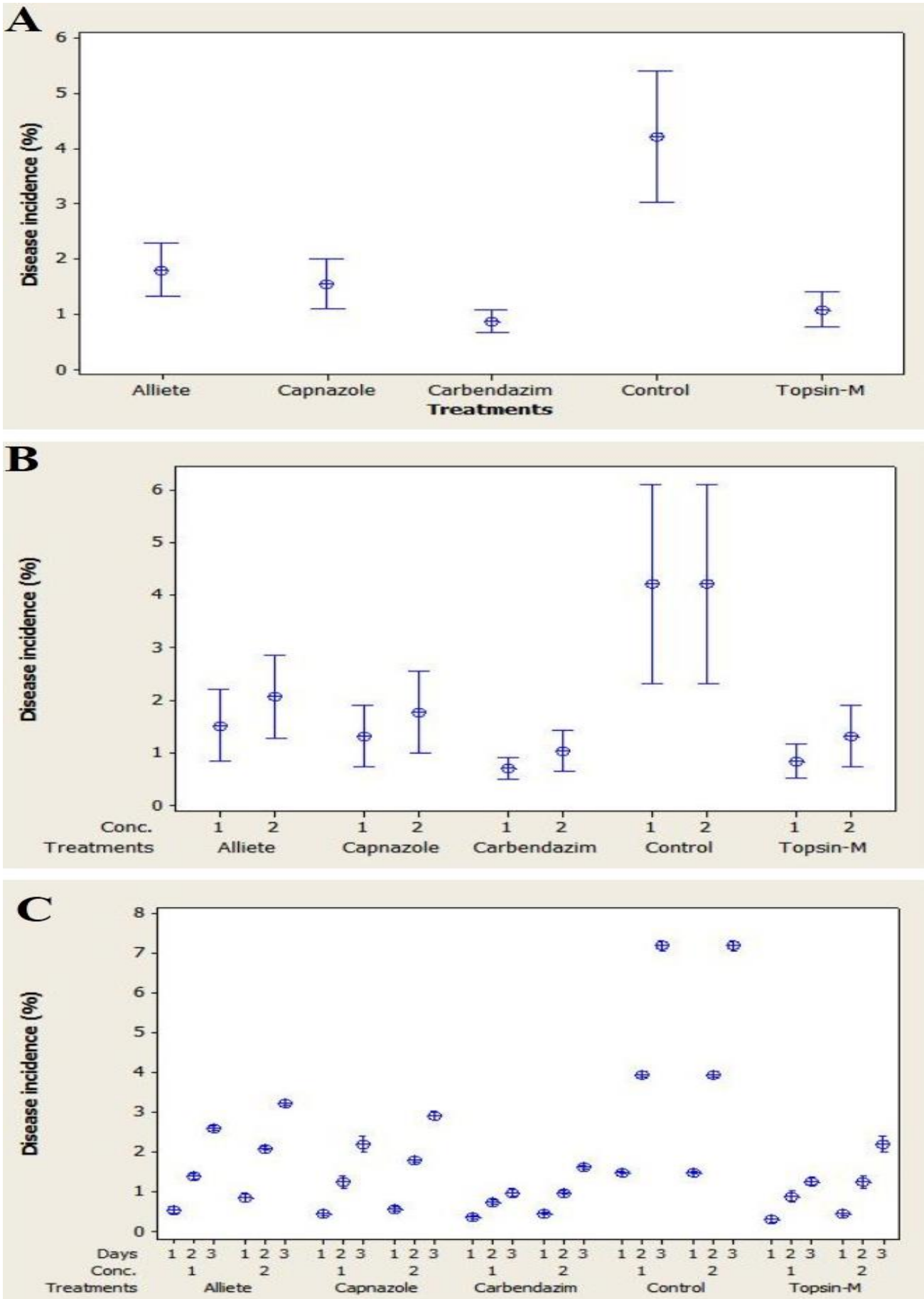

Fig. 2. *In-vitro* assessment of different fungicides and their (A) treatment, (B) treatment and concentrations, (C) treatment, concentration and days, on the growth of *Fusarium oxysporum* f. sp. *capsici*.

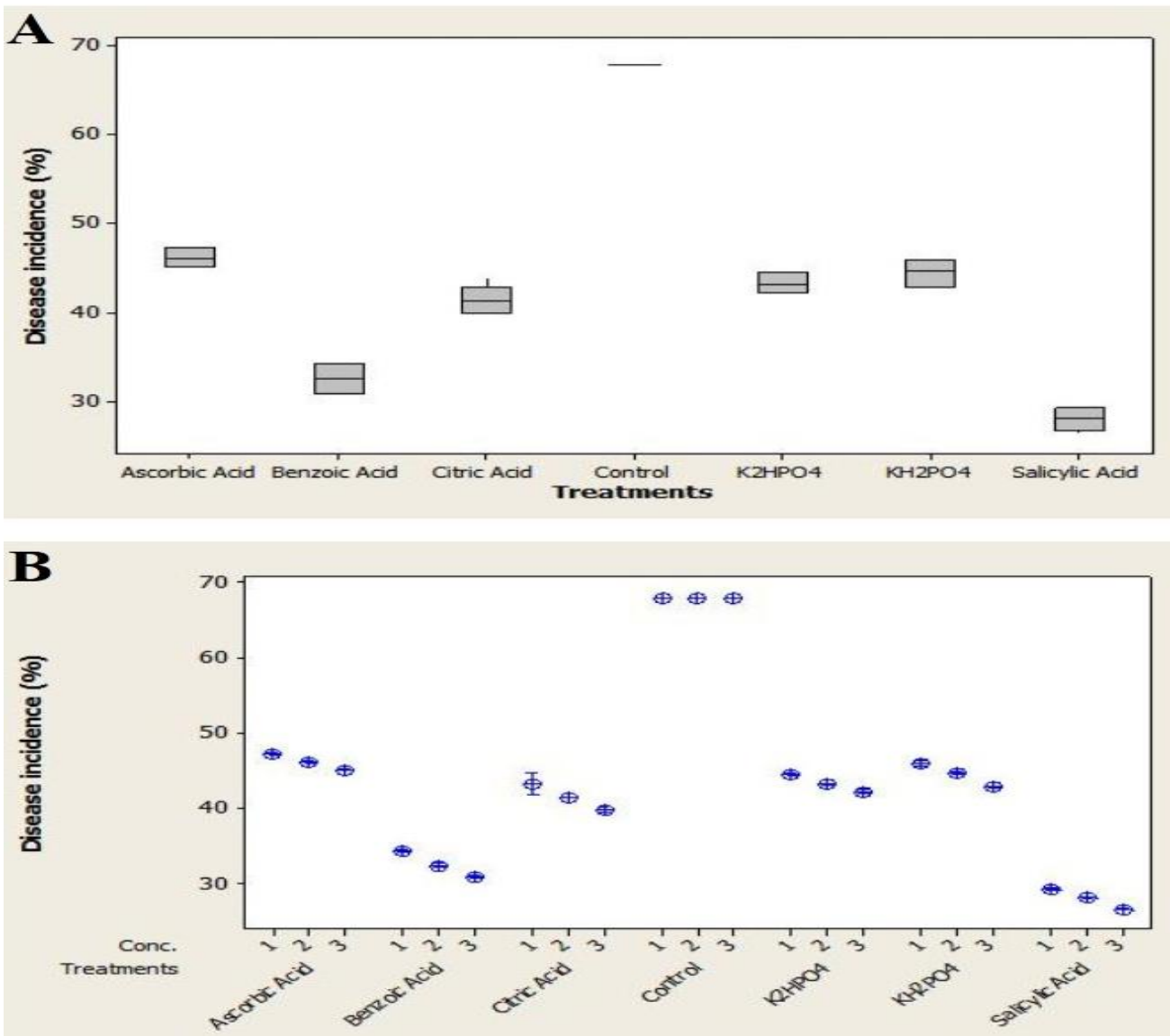

Fig. 3. *In-vivo* assessment of different plant activators and their (A) treatment, (B) treatment and concentrations, against Fusarium wilt of chilli.

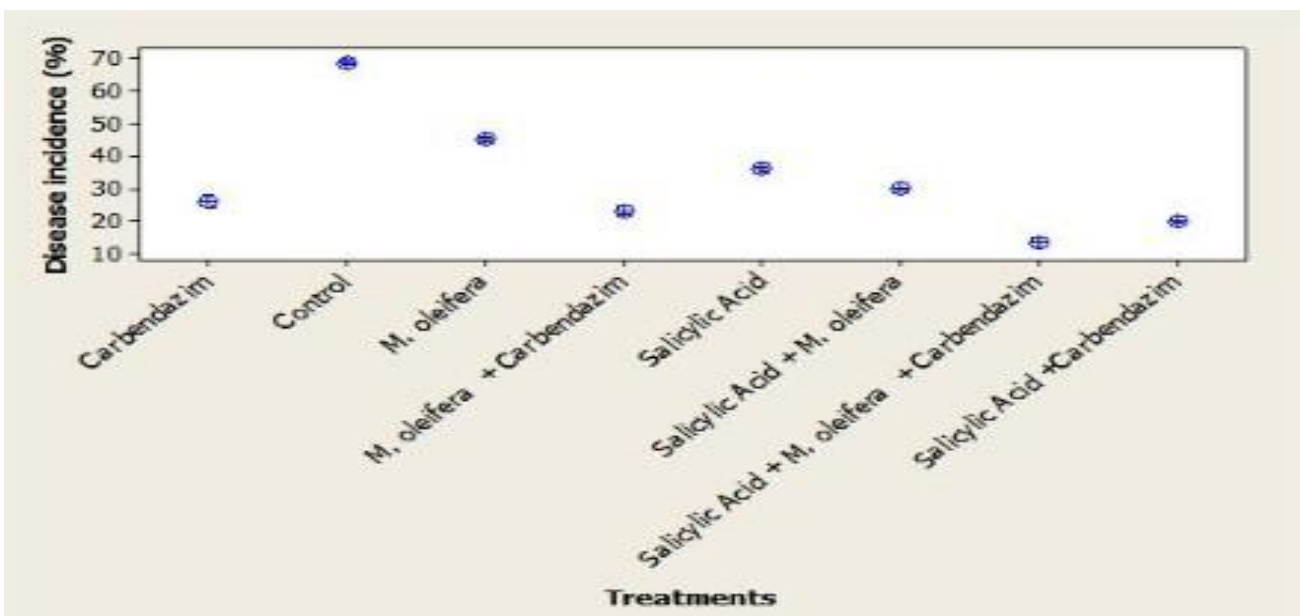

Fig. 4. Assessment of the plant activators, plant extracts and chemical against Fusarium wilt of chilli under field condition.

Conclusion

Combined application of plant extracts, fungicides and plant activators supressed the disease significantly.

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