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EVALUATION OF FUNGICIDES AND BIOPESTICIDES FOR THE CONTROL OF *FUSARIUM* WILTOF TOMATO

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Abstract

Fusarium wilt is highly destructive soil borne pathogen in tomato. Current study was carried out to evaluate commercially available fungicides and bio-fungicides *in-vitro* and *in-vivo*, for their efficacy against *Fusarium oxysporum* f.sp. *lycopersici*. Firstly four fungicides were evaluated under laboratory conditions. Three promising fungicides, two biopesticides and *Trichoderma harzianum* were further applied both in greenhouse and field experiments. During *in-vitro* studies PDA amended with fungicides with different treatments @ 1% almost completely inhibited the growth of *Fol* with varying degree of success whereas Nativo being the most effective treatment with 98% reduction in growth as compared to control. Nativo significantly reduced the disease incidence (32.75 %) at concentration of 1%. While Poly-beta-hydroxyl-butyric-acid effectively promoted the tomato growth. Maximum reduction in disease (30.14 %) was expressed by Nativo followed by Teagro (25.06 %) under field conditions. Nativo was found to be the most effective fungicide for management of *Fol* both *In vitro* and *In vivo*.Further field evaluations of the fungicides are required.

Key words: *Fusarium oxysporum* f.sp. *lycopersici*, Pathogenicity, Antagonism, Efficacy, Tomato.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important highly nutritive *Solanaceous* crop. It is believed to be originated in west coast of South America and being cultivated all over the world (Rice *et al.*, 1987; Smith, 1994; Reis *et al.*, 2005). In Pakistan, annual production of tomato is 599.7 thousand tons with total cultivated area of 63.2 thousand hectares (Anon., 2015). Tomato crop is threatened to many biotic and abiotic factors resulting in yield losses. Around the world, 25% reduction in tomato production have been reported due to *Fusarium* wilt (*Fusarium oxysporum* f.sp *lycopersici* (Sacc.) W.C. Snyder & H. N. Hans) (*Fol*). *Fol* is one of the severe problems in tomato around the world (Fravel *et al*., 2005; Walker, 1971). In Pakistan, 49.5% yield reduction due to *Fol* have been reported by FAO, 2015. According to Armstrong and Armstrong, 1981, yellowing, lower leaves drooping, vascular system browning and dark brown streaks of stem are the characteristic symptoms used for identification of this disease. The stem discoloration is sometimes also very prominent in petiole scar.

Several management strategies such as biological control, crop rotation, sanitation and judicious use of fungicides have been reported to manage the *Fusarium* wilt (Sahar *et al*, 2013). Resistance can be important for vegetables due to self-protection by resistance genes that impart tolerance to disease (Amini & Sidovich, 2010). Although use of cultivars with genes carrying moderate to high resistance significantly resulting in decreases in pathogen population levels in rhizosphere but different fungicides and biological control agents have been regarded as quick control measure. In recent past the *Fusarium* wilt has already been controlled at low doses of suitable fungicides (Kumar *et al.*, 2001). Different biocontrol agents compete with fungal pathogens for space and nutrition and perform activities antagonistic to pathogens. Another way of biocontrol activity is through production of certain chemical toxic to pathogen and antidotes which will inhibit the growth and development of fungal pathogens (Agrios, 2005).

Current study was carried out for evaluation of biopesticides and fungicides with the objectives; to find an environment friendly management technique and to compare the efficacy of biocontrol agent, biopesticides and fungicides for the management of *Fol* disease of tomato both *In vitro* and *In vivo*.

Materials and Methods

Collection of samples, cultural media and chemical used: By using the methods described by Amini & Sidovich (2010), plants with characteristics symptoms of *Fol* were collected from field and stored in refrigerator. Potato dextrose agar (PDA) was used to culture fungi. Teagro (bacterial based biofungicides/ bactericide), Nativo, Antracol, Cordate and Ridomil Gold and *Trichoderma harzianum* ANR-1 were used to check their efficacy against *Fol*. Teagro was obtained from novozyme Inc. USA. Nativo, Antracol, Cordate and Ridomil Gold were purchased from local market. The active ingredients and their recommended doses are summarized in Table 1.

Table 1. Fungicides and their active ingredients

Sr.#	Treatments	Active ingredients	Recommended Doses/ acre
T1	Nativo 75% WG	Tebuconazole + Trifloxystrobin	65g
T2	Cordate 4WP	Kasogamysin	300g
T3	Ridomil Gold 68WP	Metalaxl + Mancozeb	3.5kg
T4	Antracol 70WP	Propineb	500g

Sample preparation for identification of *Fol*: 3-mm roots and stem samples were surface sterilized with 1% NaOCl for 2 min. with two alternative washing in ddH_2O. Then samples were blotted dry and shifted onto PDA plates. Plates were incubated at 27± 2°C for 7 days. After sub-culturing, *Fusarium* isolated were purified by single spore technique. Two media (Carnation Leaf Agar (CLA) and PDA) were used for the morphological identification of *Fusarium* species. The description of species was based on the observation of Nelson *et al.* (1983) and on characteristic described in *Fusarium* Laboratory Manual (Leslie & Summerell, 2006).

Organic matter: Soil sample was mixed with 10 mL 1.0 N $K_2Cr_2O_7$ and 20 mL conc. H_2SO_4solution for 30 min. After that 200 mL of ddH_2O and 10 mL of H_3PO_4 was added and allowed to cool. 10 to 15 drops of $C_{12}H_{11}N$ indicator were added in it and placed on magnetic stirrer bar. The solution was titrated against 0.5 M $(NH_4)_2$ $Fe(SO_4)_2$•6H2O solution until the color changed from violet-blue to green (Walkley, 1974). In pots 0.76% organic matter was calculated.

Pathogenicity test: For pathogenicity test protocol described by Rajput *et al.* (2008) and Khanzada *et al.* (2004) was followed with little modifications. Briefly, 20mL of spore suspension of 7 days old *Fusarium* spp. @ 1 x 10^6 spores/mL was sprayed on surface sterilized roots and stem of tomato. Plants sprayed with 20mL ddH_2O served as control. There were 5 replications and experiment was repeated 3 times with completely randomized design (CRD). The plant were kept at 27±2°C in growth chamber until disease symptoms appeared. Then re-isolation on PDA was done to confirm the association of *Fol* with wilt of tomato. Disease rating was based on a scale of 0-5 as described by Benyon *et al.* (1996).

In vitro* evaluation of fungicides against *Fol: Systemic and non-systemic fungicides viz., Nativo, Antracol, Cordate and Ridomil Gold were evaluated for their efficacy on mycelial growth of *Fol* by food poisoning technique (Grover & Moore, 1962). For this PDA plates amended by different concentration of each fungicide were prepared. The concentration used were 250µg/L, 500µg/L and 750µg/L. After the plates were solidified, 5 mm diameter mycelial discs from 10-days-old culture were aseptically placed in the center of plates. Plate amended by ddH_2O served as control. There were 5 replications with 3 repeats. After incubation at 27 ± 2°C for 7 days, colony diameter was measures. The percent inhibition in growth was calculated by using following formula:

$$\text{Mycelial growth inhibition (\%)} = [(dc-dt)/ dc] \times 100(\%).$$

where dc and dt represents the average diameter of fungal colony in control and treated, respectively.

Evaluation of fungicides, bactericide and biocontrol agent against *Fol* of tomato under greenhouse conditions: Three fungicides (Nativo, Antracol, Cordate), two biopesticides {Teagro and Poly-beta-hydroxyl-butyric-acid (PBHBA)} and *T. harzianum* ANR-1 (Purchased from Ayyub Agricultural Research Institute, Faisalabad, Punjab, Pakistan) were used for *In vivo* study. Pots experiment was performed under greenhouse condition. Sterilized soil containing 0.76% organic matter was used in pots. Teagro is standardized at 10^{10} CFU/g and recommended for controlling the soil borne pathogens. Nativo, Antracol and Cordate were also used against *Fol*. The concentrations used for each treatment was 1%. Soil drenching technique was used three times with the intervals of 7 days. Disease incidence was recorded three times after 7, 14 and 21 days of interval. Plants without antagonism was served as control. Data was recorded before each application and subjected to statistical analysis.

***In vivo* evaluation of bactericide and fungicides for the management of *Fol*:** Three different fungicides (Nativo, Antracol and Cordate) and bactericide (Teagro) were used against *Fol*. Meanwhile, the wheat straw after proper washing and draining was inoculated with *Fol* @ 1× 10^7 spores/mL. When *Fol* completely covered the wheat straw, it was evenly distributed in field (Temperature 26±3°C). Tomato seedlings of 30 days old were transplanted after the establishment of pathogen. After 20 days of transplantation, the concentrations prepared for each treatment based of pervious greenhouse experiment were 0.25, 0.75 and 1.25%. The chemicals were properly mixed with water in tubs. Three times soil drenching was done after 7, 14 and 21 days of interval. Plants without antagonism was served as control. Disease incidence and plant height were recorded before each application and subjected to statistical analysis.

Statistical analysis: Analysis of variance (ANOVA) was used to analyze the data in the SAS software (version 8.0.) Mean separations were performed by Least Significant Difference test (LSD).Differences at $P = 0.05$ were considered as significant.

Results and Discussion

Fungal identification: *F. oxysporum* colonies were observed that shows reddish purple color surrounded by pinkish white aerial mycelium. Under microscope, mycelium looked like sickle shape, crescent moon shape, both ends are pointed and there 3 to 4 septations. So it was confirmed that the isolated fungi was *Fol* (Fig. 1).

Pathogenicity test: After one month of inoculation, plants inoculated with *Fol* showed typical symptoms of the disease, whereas, no such symptoms were observed on plants without *Fol*. Non-inoculated plants showed no wilting. Plants inoculated with *Fol* showed wilting. In control plants, no vascular browning was observed, whereas in plants inoculated with *Fol* browning of vascular tissues was prominent. Similarly, wilting and death of leaves at the branch apices was only observed where plants were inoculated with *Fol.*

From pathogenicity test, it is confirmed that *Fol* was commonly isolated from roots and rotten stem of tomatoes which confirm the association of *Fol* to stem and root rot of tomato. Highest disease rating of *Fol* was found on stem and roots. From the results it is confirmed that *Fusarium* species are pathogens of tomatoes. *F. oxysporum*, *F. solani* and *F. proliferatum* three *Fusarium* species have been reported in various countries that are pathogenic to several crops and these species are also associated with root rot of *Cymbidium* orchid and root rot

of moth orchid (*Phalaenopsis* sp. in Australia (Lee *et al.*, 2002; Benyon *et al.*, 1996; Kim *et al.*, 2002). Yellow spot disease in *Cymbidium* was also reported in Japan by another specie *F. proliferatum* (Ichikawa & Aoki, 2000). In Korea, leaf spot was caused by *Cymbidium hybrid* (Kim *et al*., 2002). Burnett, 1975 reported the *F. solani* as root pathogen on orchids.

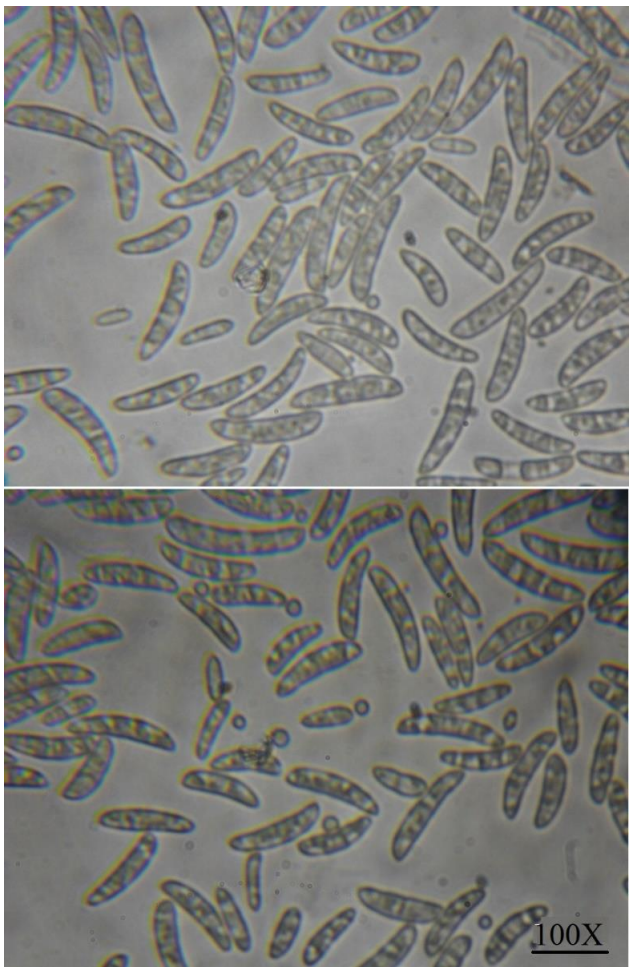

Fig. 1. Identification of *Fusarium* spp. under compound microscope.

In vitro* evaluation of fungicides against *Fol: Four different fungicides viz., Nativo, Antracol, Cordate and Ridomil Gold were evaluated against *Fol* by food poison technique. Three concentrations viz., 250, 500 and 750 ppm were prepared for each treatment. Among all Nativo was most effective to reduce the mycelial growth, followed by Ridomil Gold and Antracol whereas Cordate was least effective (Fig. 2). Results revealed that the effect of fungicides in reducing mycelial growth varied but increased after increasing dose rate. The Nativo showed relatively better results (0.53cm) in reduction of mycelial growth at 750ppm over control followed by Ridomil Gold, Antracol and Cordate (1.44cm, 2.37cm and 2.73cm respectively) as compared to control (3.43cm) at 750ppm (Fig. 2). Similar results were obtained in case of effect of incubation period on reduction of mycelial growth (Fig. 3). Again Nativo was found best in reducing the mycelial growth as compare to other fungicides and control.Banyal *et al.* (2008); Patel *et al.* (2005); Singh *et al.* (1997); Kalra & Sokhi (1985); Daradhiyar (1980) and Sommer (1982) reported the similar finding on other fungi. Mycelial growth *F. oxysporium* was completely inhibited by fungicides (Sharma & Jain, 2006; Khan *et al*., 2004). Aroosa *et al.* (2012) also reported that nativo with tibuconazole as active ingredient was most effective as compared to all other fungicides.

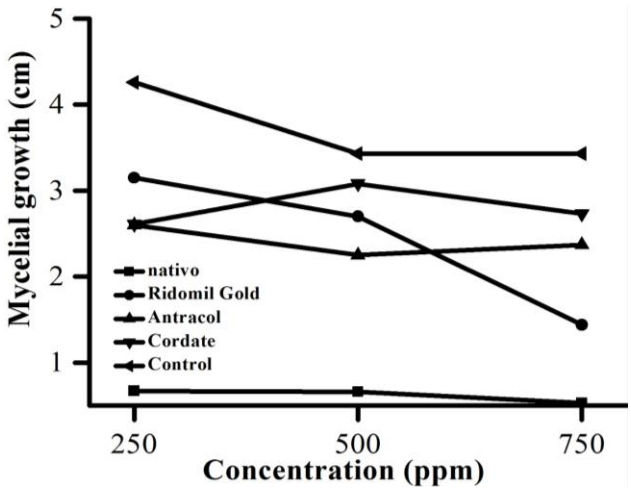

Fig. 2. Comparison with different concentrations of treatments on mycelial growth of *Fol.*

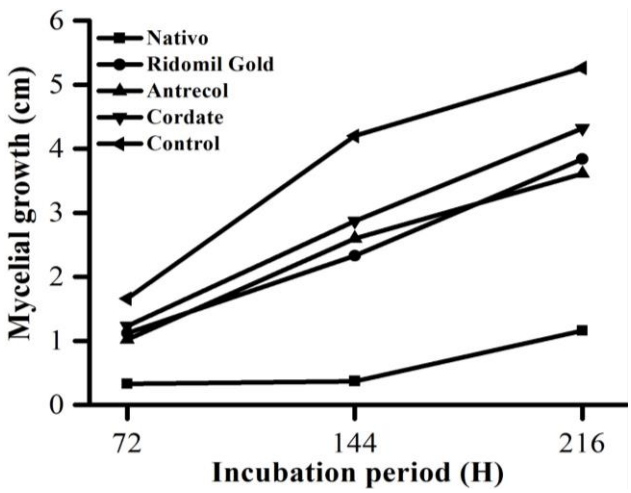

Fig. 3. Interaction effect of treatments and incubation periods (T x D) on mycelia growth of *Fol.*

Evaluation of biopesticides and commercially available chemicals against *Fol* of tomato under greenhouse conditions: Pots was filled with infested soil then 30 days old seedlings were transplanted. Plants were irrigated when needed. After three weeks of transplantation soil drenching was done. In this experiment different treatments were Nativo, Antracol, Cordate and Teagro were used against *Fol*. Results of current study are summarized in Figs. 4 and 5. Results showed that Teagro significantly (P = 0.05) reduced the disease among all treatments with disease severity after 1st, 2nd and 3rd application of 42.06%, 30.01% and 23.07% respectively (Fig. 4). In this regard, Saleh (1997) proved that *B. subtilis* has great potential to inhibit the rhizospheric microbes and it has antagonistic effect against the pathogenic fungi. Biocontrol is a safe technique for controlling the plant pathogens. However to coop the recent trend in agriculture more work is required. Density

of inoculum of pathogens and incidence of disease can be minimize by using the biocontrol agents for longer duration (Harris *et al.*, 1994; Berger *et al.*, 1996). It was concluded that application of *Bacillus subtilis* showed relatively better results as compare to fungicides in field trials especially. Maximum plant height was observed by PBHBA (21.0cm, 23.66cm and 30.33cm) after 1st, 2nd and 3rd application (Fig. 5). Bio-agents also act as growth promoters such as enhance the plant height and play a good role in minimizing the disease (Sundaramoorthy & Balabaskar, 2014). Significant effect on tomato plant height (25.0 cm) was observed when they were treated with *T. harzianum* ANR-1 isolate (Fig. 5).

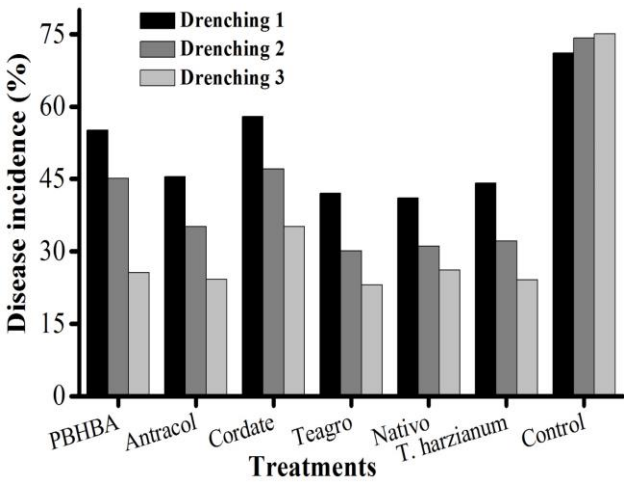

Fig. 4. Effect of different treatment applied as drenching in pots on disease incidence of *Fol* on tomato under greenhouse conditions.

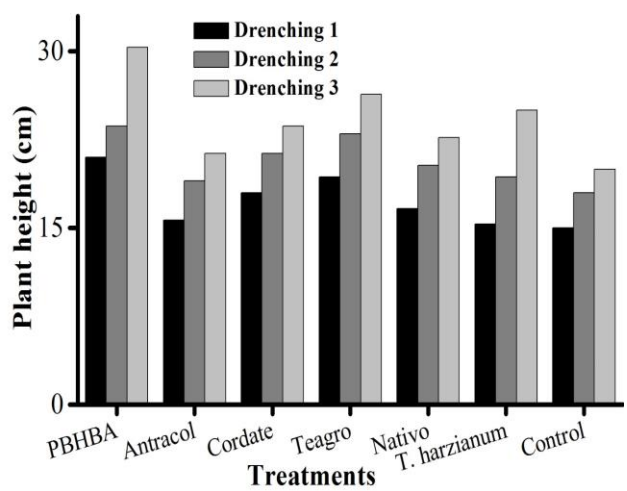

Fig. 5. Plant growth promoting effect of different treatments applied through soil drenching against *Fol* under greenhouse conditions.

Management of Fusarium wilt of tomato through biopesticides and commercially available chemicals *in-vivo* conditions: Land was prepared and 30 days old seedling was transplanted in field. Nativo was most effective under laboratory followed by Cordate, Antracol and Teagro hence further evaluated through soil drenching in field. Three doses (0.25, 0.5% and 1%) was prepared of each treatment and drenched in soil. Soil drenching was done three times and data was recorded after 7, 14 and 21 days. Significant inhibition in the spore germination was observed by different concentrations of fungicides and biopesticides shown in results (Figs. 6, 7, and 8). After third soil drenching maximum inhibition in the spore germination was found at highest concentration as compared to control which showed least inhibition in spore germination.Results indicated that after 1st, 2nd and 3rd application of Nativo exhibited minimum disease incidence at 1.25% concentration (15.13%, 12.27% and 11.09%) (Figs. 6, 7 and 8). By using the biopesticides against *Fusarium* spp. and against seventeen other different fungi, similar results were observed by Bowers and Locke (2000) and Misra & Dixit (1976). Extracts of garlic bulbs and *Bignonia* leaves have antifungal activity against various plant pathogenic species like *F. pallidoroseum*, as they can efficiently inhibit the mycelial growth (Sivaprakasan & Jacob, 1994; Arya *et al.*, 1995). Similar results of plant extracts against other plants pathogenic fungi were observed by Anwar & Khan (2001); Karade & Sawant (1999) and Datar (1999). Our results are similar with Tequida *et al.* (2002); Bhat (2002); Bansal & Gupta (2000) and Bashir (2001).

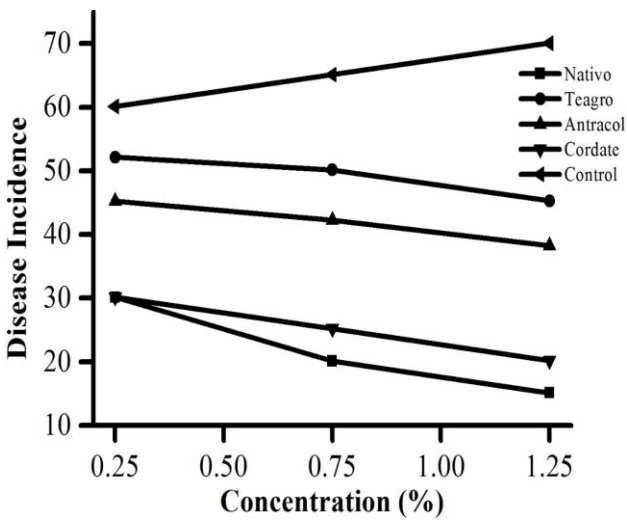

Fig. 6. Effect of different treatments at different concentration applied through soil drenching after 7 days on disease incidence of *Fol*on tomato under field conditions.

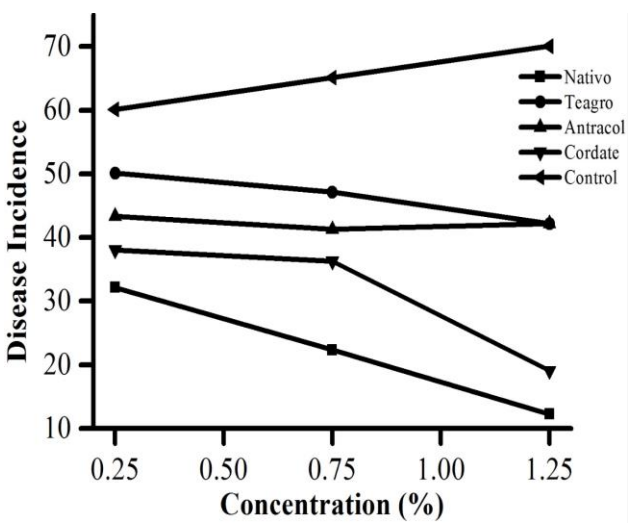

Fig. 7. Drenching-2.Effect of different treatments at different concentration applied through soil drenching after 14 days on disease incidence of *Fol* on tomato under field conditions.

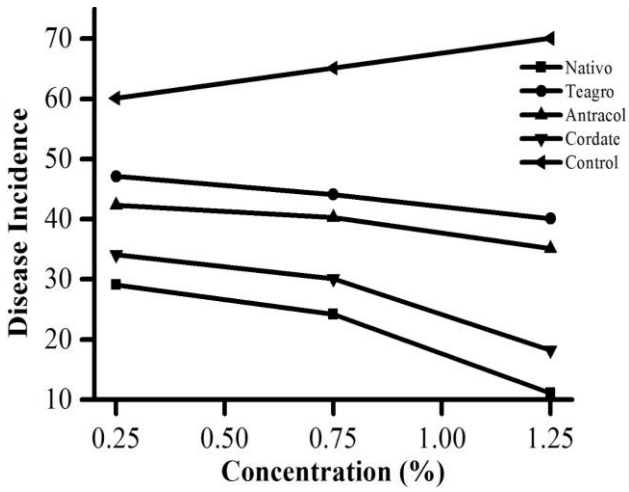

Fig. 8. Drenching-2.Effect of different treatments at different concentration applied through soil drenching after 21 days on disease incidence of *Fol* on tomato under field conditions.

Conclusion

It was concluded that inhibition in mycelial growth and spore germination of *F. oxysporium* was observed by the application of both fungicides and biopesticides. However fungicides used in this study prove to be better substitute to control the pathogenic *Fol* both *In vitro* and *In vivo* conditions and also for inhibit the spore germination. In conclusion.*Fusarium* wilt of tomato caused by *F. oxysporium* f.sp. *lycopersici* was effectively managed by Nativo and Teagro. Further field evaluation is required.

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