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EVALUATION OF SOME FUNGICIDES FOR THE CONTROL OF EARLY BLIGHT DISEASE (*ALTERNARIA SOLANI*) OF TOMATO

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ABSTRACT

Five fungicides including Score, Cabritop, Carbofuran, Carbendazim and Aliette were evaluated for their efficiency against *Alternaria solani* under laboratory, greenhouse and field conditions. The fungicides Score, Cabritop, Carbendazim and Aliette decreased the growth of *A. solani* at the concentration of 0.5, 1, 3, 5 and 7µg. mL^{-1}. The fungicides Score and Aliette reduced the spore germination of *A. solani* at the concentrations of 0.5, 1 and 3 μgml^{-1}. Effect of Cabritop was not significant at low concentration ($0.5\mu g.mL^{-1}$) but it had significant effect at higher concentrations compared to positive controls. The fungicides Score, Cabritop, Aliette and Carbofuran at the concentration of $3\mu g.mL^{-1}$, significantly reduced disease severity by 50, 50, 40 and 60% (as per scale), respectively compared to the +ve controls. The fungicides Score and Aliette were further checked in greenhouse and field experiments and found adequately effective against *A. solani*. Both the fungicides Aliette and Score significantly increased the number of leaves, stem and root length, stem fresh weight and root fresh weights, stem dry weights and root dry weights over positive controls, while growth of negative control (water control) was, in general, substantially higher compared to fungicide treatments. Whereas, pigment contents (Chlorophyll a, b and carotene) were high in the order: -ve control > Score > Aliette while, pigment levels were substantially lower in +ve control.

Key-words: Early blight of tomato, *Alternaria solani*, chemical control, fungicides.

INTRODUCTION

Alternaria solani is known to cause early blight of tomato (Rotem, 1994) that is economically one of the most important diseases of tomato the world over, where it causes significant reductions in yield of 35 to 78 % (Jones, 1991). The disease is the most damaging to tomato due to complete defoliation (Peralta *et al*., 2005). The most important hosts of *A. solani* are eggplant, tomato, potato, chilli and black nightshade (Pscheidt, 1985). Other hosts include non-solanaceous species such as cucumber, zinnia, wild cabbage and horse nettle (Neergaard, 1945). Early blight is less frequent and less damaging on pepper and eggplants. The disease weakens plant tissues, either due to stress or wounding, and renders the plant even more susceptible to *Alternaria* infection than healthy tissues (Thomma, 2003). The important features of *Alternaria* spp. are the production of melanin in the spores and the production of host-specific toxins and nonspecific toxins such as alternaric acid, identified by Thomma (2003). This toxin, isolated from culture filtrate of *Alternaria* species causes chlorosis and necrosis when introduced in tomato plants and also damages non hosts of *Alternaria* pointing to its non-specificity (Langsdorf *et al.*, 1990). Tomato early blight can be controlled by cultural practices, fungicides, and biological control.

Fungicide application is a significant factor in early blight control in both the greenhouse and field experiments (Horsfield *et al*., 2010). Besides disease control, some fungicides exhibit beneficial physiological and/or growth-promoting effects on plants, including delaying of leaf senescence (Bertelsen *et al*., 2001), enhanced chlorophyll content (Butkute *et al*., 2008). Such physiological changes may contribute towards increased yield, even in the presence of the disease. Likewise, results from other field trials on target spot control (Shtienberg *et al*., 1996; Wick *et al*., 2005; Zitter and Drennan, 2005) showed that fungicides with systemic or translaminar activity were more effective than protective fungicides. The protective fungicides do not enter the leaf and a significant proportion of the products may be washed from the leaves and systemic/ translaminar fungicides are effective when applied both before and after infection. Researchers have suggested that the more effective fungicides should be applied late in the season when host susceptibility increases (Shtienberg *et al*., 1996). Miller and Miller (2005) indicated that using 'premium' fungicides around row closure may be too early for controlling early blight (depending on weather conditions).Fungicide treatments are generally the most effective control measures, but are not economically feasible in all areas of the world and may not be effective under weather conditions favorable for epidemics (Herriot *et al*.,1986). Among the many fungicides available, some products like chlorothalonil (Bravo) and mancozeb (Dithane M-45) are commonly used to control early blight (Bartlett *et al.*, 2002). Strobilurin compounds containing fungicides are very effective for a number of fungi including early blight (Bartlett *et al*., 2002; Pasche *et al.*, 2004).

The most effective way to control early blight to a non-damaging level is the fungicide treatment (Manohara, 1977). Fungicides are applied starting from two weeks after harvesting and in the wet season a fungicide treatment once or twice per week is necessary (Manohara, 1977). Such heavy use of chemicals imposes health concerns for growers, consumers and environmentalists and also they are not economically feasible for the generally resource-limited grower (Pasche *et al.*, 2004). Maximum fruit infection for 13 susceptible varieties was about 30% in the absence of fungicide and potential yield processing tomato and fruit size were reduced on an average by 30% and 10% respectively (Sherf and Macnab, 1986).

Sinha and Prasad (1991) observed that Dithane M-45 (Mancozeb) R 0.2% was the best and most effective treatment against *A. solani* in the field. Khade and Joi (1980) reported that Dithane M-45 (Mancozeb), blue Cu 50, Cuman L, Dithane Z-78 (Zineb) and Difolatan (Captafol) are able to reduce disease incidence and increase the yield. Choulwar and Datar (1989) stated that 0.2% Mancozeb applied at different times after transplanting, effectively reduced the intensity of early blight caused by *A. solani*. Vidhyasekaran (1983) reported that both Mancozeb and Captafol effectively controlled *A.solani*; they also reduce defoliation, increase fruit production more sugars and vitamins contents of fruit. Sobolewski and Robak (2004) examined the effectiveness of three fungicides (Unikat 75 WG (ethaboxam), Acrobat MZ 69 WP and MC 72.5 WP (mancozeb) against late and early blight disease and found that all three control the diseases. Smith and Littrell (1980) reported that after first appearance of symptoms spraying of fungicide decrease the disease severity. Mabbett and Phelps (1985) demonstrated that fungicides containing inorganic copper (cupric hydroxide) are more effective to check the growth of *A. solani* in low dosages. Comparing various fungicides, Maeso (1986) showed that captafol (Difolaton) was best for inhibiting the growth of *A. solani.* According to Maheshwari *et al*., (1991) copper oxychlorode most effective against *A. solani* up to 64.7% in a field trial among six fungal toxicants. Hawamdeh and Ahmed (2001) using seven fungicide (Antracol, Benlate, Copper oxychloride, Dithane M-45, Ridomil, Topas, and Topsin) against *Alternaria solani* found Dithane M-45 more effective than others. Miller and Miller (2005) indicated that using 'premium' fungicides around row closure may be too early for controlling early blight (depending on weather conditions).Many researchers found similar results that *A. solani* was best controlled by Iprodione containing fungicides (Bedlan, 1987; Hedges and Cole, 1988; Aponyine *et al*. 1988). Further, many investigators observed that Mancozeb was most active against *A. solani* (Sinha and Prasad, 1991; Devanthan and Ramanujam, 1995). Kumar and Kumar, (1996) tested seventeen fungicides and proved that Mancozeb, Captan, Achook, Ziram, Iprodione, and Ridomil ambly effectively inhibited the growth of *Alternaria* spp. Fugro *et al.*, (1994) reported that mycelium growth of *A. cucumerina* was inhibited by Dithane M-45. Further, Robak (1998) reported that propane containing fungicides were most effectively control *Alternaria* spp. Mondal *et al.*, (1989) demonstrated that *Alternaria* blight was best controlled by Captan in radish. Sarkar and Chaudhary (2004) reported that for the control of *A. solani* polyram was best rather than Captan for. Singh and Singh (2006) conduct experiment over different fungicides and observed that hexaconazole was very effective against *A. alternata.* Sidlauskiene *et al.*, (2003) found that *Alternaria* leaf spot in tomato was controlled by Amistar fungicide. Matharu *et al.* (2006) established that of *A. alternata* was best controlled by 2-chloro-benzal malono nitrite.

According to Chia and He (1999) and Swiech *et al*. (2001) low productivity of infected plants occurs due to low photosynthetic rate of necrotic and chlorotic leaves. In point of fact reduction in photosynthetic rate of the infected leaves is associated with growth of the symptoms (Platt, *et al*., 1979). Therefore, it is pertinent to examine the levels of pigments including Chlorophyll a and b and carotene.

The objective of the current investigation was to evaluate the effectiveness of five fungicides against *Alternaria solani* under laboratory, greenhouse and field conditions, the cause of early blight of tomato, potato, eggplant, etc.

MATERIALS AND METHODS

Chemical control using Fungicides

Five fungicides, including Score, Cabritop, Carbofuran, Carbendazim and Aliette were evaluated for their efficiency against *Alternaria solani in vitro* and *in vivo* (Table 1).

Evaluation of fungicides under laboratory condition

Effect of fungicides on mycelial growth of *Alternaria solani* under laboratory condition

This experiment was conducted to observe the effect of different concentrations of the fungicides on the rate of mycelial growth of *A. solani*. Five different concentrations of fungicides (0.5, 1, 3, 5 and 7 μg mL^{-1}) were prepared for this experiment. Each concentration was added to 100 ml PDA medium in an Erlenmeyer flask; thereafter mixture was poured into Petri plates. 5mm mycelial disk of 10-days-old-culture of the fungus *A. solani* were

inoculated in all Petri plates. Mycelial growth rate of *A. solani* was measured after 5 days. Plates were incubated at 30±1°C. The experimental design was completely randomized with five replicates for each concentration and each fungicide. Mycelium growth rate and inhibition (MGR) was calculated using the formula adduced by Elad *et al*. (1981).

Growth inhibition (%) = [((Dc-Dt) /Dc)*100]

Where Dc= diameter of colony in the control (mm), Dt= Diameter of colony in the treatment (mm).

Table 1. Fungicides Manufacturer and Composition.

Fungicides	Manufacturer	Composition	Active ingredients
Aliette ®	Bayer	Ethyl hydrogen Phosphonate	Aluminum tris (O-ethyl phosphonate) 800g/kg
Score ® 250EC	Syngenta	Difenoconazol	250g/litre of Difenoconazole
Cabritop	Basf S.A.	Metrian+ piraclostrobin	Matiram complex 55 %, Pyraclostrobin 5 %
Carbofuran	FMC corporation	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate	98 % min
Carbendazim®	Noor industries	Methyl *1H*-benzimidazol-2-ylcarbamate	Carbendazim 500g/L

Effect of fungicides on spore germination of *Alternaria solani* under laboratory condition

To evaluate the effect of the fungicides, Score, Cabritop and Aliette on spore germination of *A. solani* at the concentrations of (0.5, 1 and 3μg mL^{-1}) were chosen. Each concentration was added to 100 ml PDA medium in flask. The mixture was poured in Petri plates, All Petri plates were inoculated with conidial suspension of *A. solani* with spore concentration of 120 spores/ml. Plates were incubated at 30±1°C for 5 days before measuring spore germination. The experiment was conducted using completely randomized design with three replications.

Evaluation of fungicides under greenhouse condition

The greenhouse experiment was carried out with four treatments and three replicates in Federal Urdu University (Gulshan-e-Iqbal campus). Four-week-old tomato plants were transferred in pots. After 30 days of transplantation, tomato plant were sprayed with *A. solani* spore suspension (142 spore/ mL) while control plants remained untreated with *A. solani* for negative control. Positive controls were sprayed with *A. solani* spore suspension. Fungicides Score and Aliette at the concentration of 0.5 μg ml^{-1}were tested in this experiment. After 30 days of inoculation of *A. solani* when symptoms of early blight were observable in the pots, then each plant in the treatment pots was sprayed with 50 ml of fungicides. Positive controls remained untreated with fungicides. After one month of fungicides spray three plants were randomly selected stem length, root length, fresh and dry weights of root and stem was recorded.

Determination of pigment content under greenhouse condition

Chlorophyll a, b and carotene were measured in greenhouse experiment and the leaves were collected from control and treated three month old plants. 1g of fresh leaves from each plant separately in 100 mL of 80% acetone. The leaves were grounded using pestle and mortar after that Homogenized for five minutes at 1000 rpm by a homogenizer, and the extract centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the absorbance at 470, 645, 663 nm recorded on (UV-1201.Shimadzu, Japan) spectrophotometer.

Evaluation of fungicides under field conditions

Effect of fungicides on disease severity of *Alternaria solani* under field conditions

Eight-weeks-old tomato plants were sprayed with *A. solani* spore suspension ($3x10^5$ spores/ mL) under greenhouse condition. After 30 days, inoculated plants were sprayed with the following fungicides (Score, Cabritop, Carbofuran and Aliette) at the concentrations of (1 and 3 μg mL^{-1}a.i.). Each plant was sprayed with 40 ml of the fungicide suspension. The control plants were not treated with fungicide but sprayed with *A. solani*. Each treatment was replicated three times. Disease severity was evaluated after 30 days of incubation as disease (%) in accordance with the scale (Table 2) developed by Abed (2007).

Effect of fungicides on growth of *Alternaria solani* under field condition

The field experiment was performed in accordance with a randomized complete block design (RCBD) with four treatments and three replicates in Karachi University Campus (experimental field of the Institute of Environmental Studies) three-week old tomato plants grown in a nursery were transplanted into the field in a plots measuring 1 x 1 m. There were 3 rows with 4 plants. Fungicides Score and Aliette at the concentration of 0.5 µg. mL^{-1}were tested in this experiment. After 10 days of transplantation, tomato plant were sprayed with *A. solani* spore suspension (100 spores/ mL) while control plants remained untreated with *A. solani* for negative control. Positive controls were sprayed with *A. solani* spore suspension. After 10 days of inoculation of *A. solani* when symptoms of early blight were obvious in the field, then each plant in the treatment plots was sprayed with 40 mL of the fungicide suspension using a hand sprayer. Positive controls remained untreated with fungicide. Four plants were randomly selected and harvested after fifteen days number of leaves, stem and root length; fresh and dry weights of root and stem were recorded.

Table 2. Disease severity scale.

Disease (%)	Scale
0-20	1-10 spots on leaves
20-40	10-20 spots on leaves and spots start to appear on stems
40-60	20-30 spots on leaves and 10 spots on stems
60-80	30-40 spots on leaves and 20 spots on stems
80-100	Completely blighted plant

RESULTS

Results of laboratory methods

Effect of fungicides on mycelial growth of *Alternaria* under laboratory condition

Table 3 shows the fungicides Score, Cabritop, Carbofuran, Carbendazim and Aliette effectively suppressed the mycelium growth rate of *A. solani* at the concentration of (0.5, 1, 3, 5 and 7µg mL^{-1}). The fungicide Carbendazim gave the lowest growth rates of *A. solani* mycelium at all concentrations. Score, Cabritop and Aliette arrested mycelial growth completely. Carbofuran inhibited mycelial growth at high concentration. The five fungicides, Score, Cabritop, Carbofuran, Carbendazim and Aliette significantly ($p<0.01$) reduced the mycelial growth of *A. solani* at the concentrations of (0.5, 1, 3, 5 and 7µg mL^{-1}). The growth reduction recorded for different fungicides varied in the order Score > Cabritop > Aliette. Carbofuran suppressed the mycelia growth at concentrations > 3µg ml^{-1}.

Table 3. Effect of fungicide on mycelial growth of *A. solani* in Petri plates.

	Mycelial Growth Inhibition %				
Concentration	Score	Cabritop	Aliette	Carbofuran	Carbendazim
0	10	10	10	10	10
0.5ppm	100	95	90	60	84
1ppm	100	95	90	70	82
3ppm	100	100	100	75	81
5ppm	100	100	100	100	72
7ppm	100	100	100	100	67

Effect of fungicides on spore germination of *Alternaria solani* under laboratory condition

The results are given in Fig. 1. The fungicides Score and Aliette reduced the spore germination of *A. solani* at the concentrations of (0.5, 1 and 3 µg mL^{-1}). Cabritop was slightly (though significantly $p<0.05$) less effective than the other two fungicides tested at 0.5µg mL^{-1}) but it's effect at high concentrations was comparable to Score and Aliette.

Results of greenhouse method

Effect of two fungicides on disease severity of *Alternaria solani* under greenhouse condition

The results of greenhouse experiment were also subjected to single factor ANOVA. Most fungicides showed significant effect on number of leaves, fresh and dry weight of root and stem ($p<0.01$) but stem length and root length were found non-significant. The results of greenhouse experiment were also analyzed by two multivariate

methods including cluster analysis and PCA (principal component analysis) ordination. The dendrogram derived from Ward's method of cluster analysis (Fig. 2) shows considerable grouping of fungicide treatments (Aliette and Score) and the negative and positive controls. On the left side are the three replicates of positive control and the negative controls tend to be separated on the right side while the two fungicide treatment remains in between negative and positive controls. The two-dimensional PCA ordination (Fig.3) basically repeats the pattern depicted by the dendrogram. A remarkable separation of +ve and -ve controls can be seen in the ordination configuration. Both group 1 and group 2 obtained in cluster analysis clearly separated out.

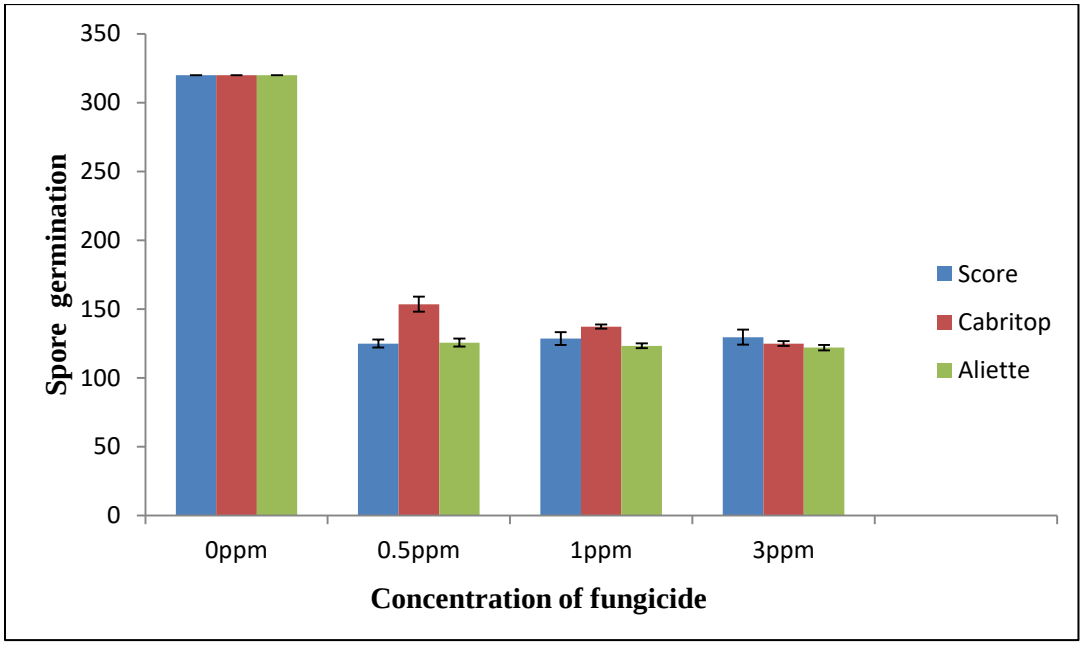


Fig. 1. Effect of fungicide on spore germination of *A. solani*.

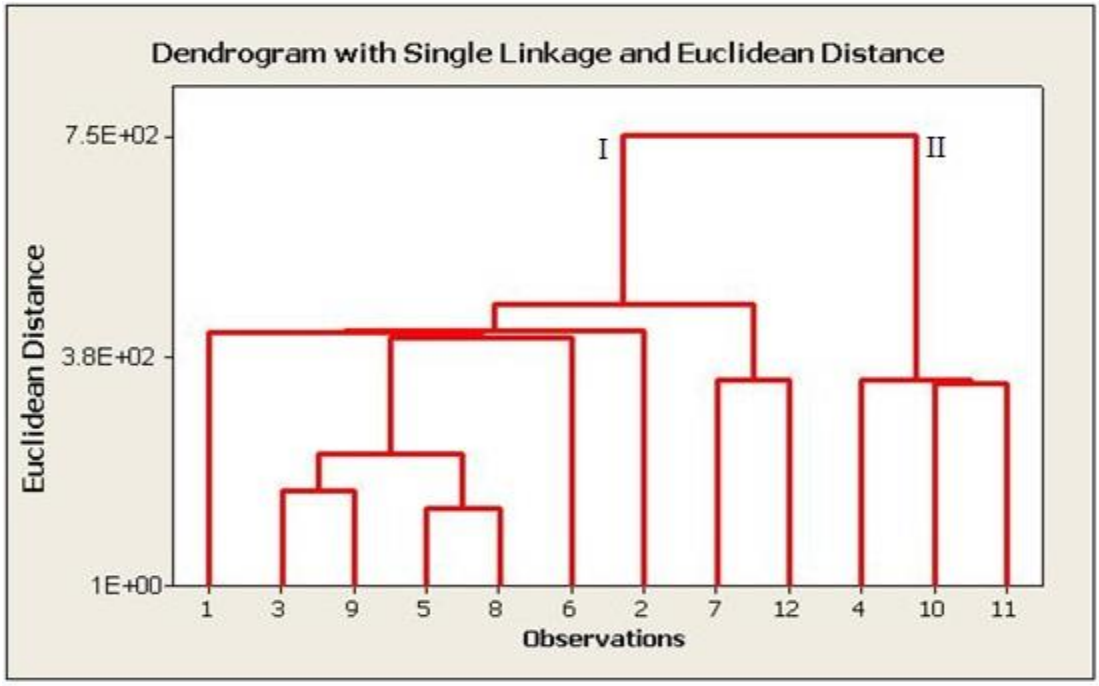


Fig. 2. Dendrogram derived from the data of the effect of fungicides on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under greenhouse conditions. (1,2,3) = positive control, (4,5,6) = Aliette, (7,8,9) = Score and (10,11,12) = negative control.

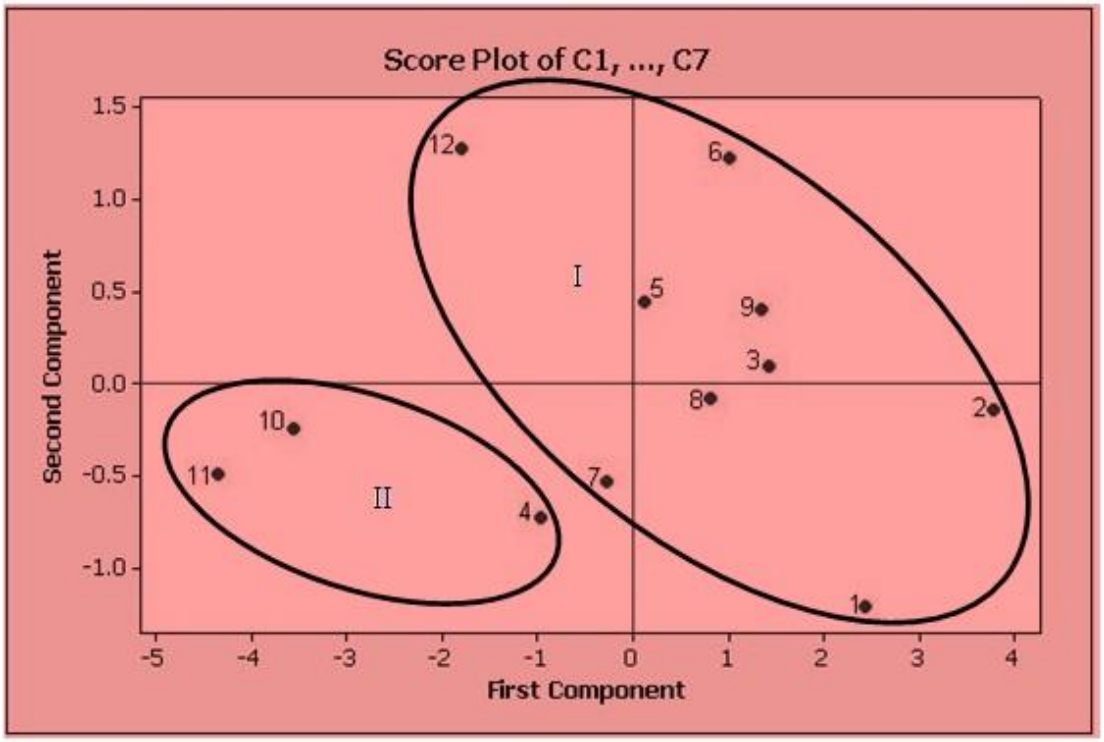
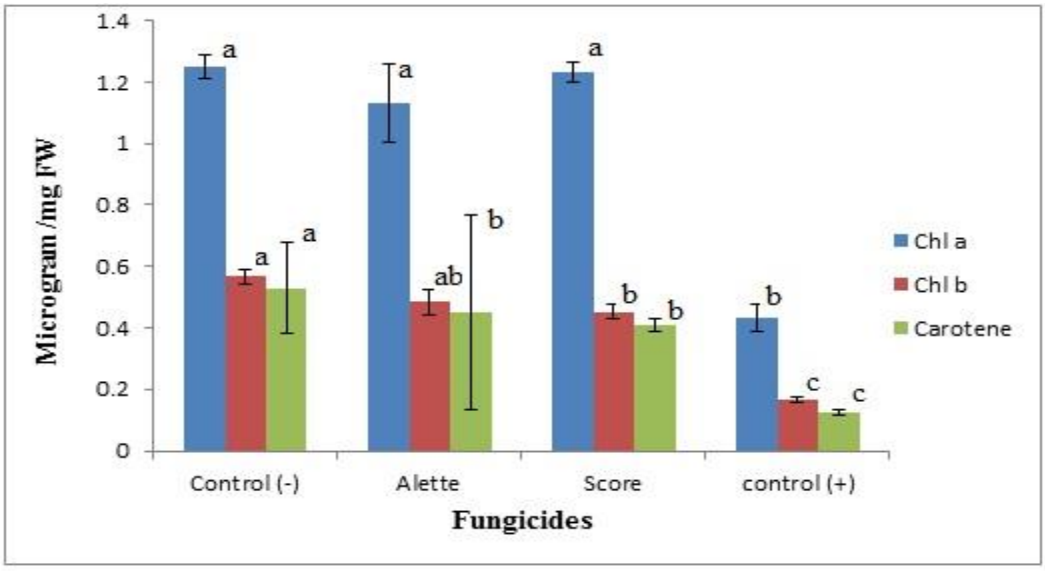


Fig. 3. Two-dimensional PCA ordination of the data of the effect of fungicides on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under greenhouse condition. (1, 2, 3) = positive control, (4, 5, 6) = Aliette, (7, 8, 9) = Score and (10, 11, 12) = negative control.



LSD $_{0.05}$= Chl a (0.23) Chl b(0.0894) Carotene (0.0663)

Fig. 4. Pigment content measured from the data of the effect of Fungicides on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under greenhouse condition.

Pigment content

Pigment content was measured following infection with *Alternaria solani* and treatment with fungicides under greenhouse condition. Chlorophyll a, b and carotene were measured (Fig. 4). Pigment contents were high in the order – ve control > Score > Aleitte while pigment levels were lower in +ve control. One factor ANOVA was

performed on the data of pigment content under field condition. All Fungicides significantly increased chlorophyll a, b and carotene content ($p<0.001$) over the +ve control ($p<0.001$) but that of -ve control was greater than that of +ve control (Fig. 4).

Results of field method

Effect of three fungicides on disease severity of *Alternaria solani* under field condition

The fungicides Score, Cabritop, Aliette and Carbofuran at the concentrations (3µg mL^{-1}), significantly reduced disease severity by 50, 50, 40 and 60%, respectively compared to the controls (Fig. 5).

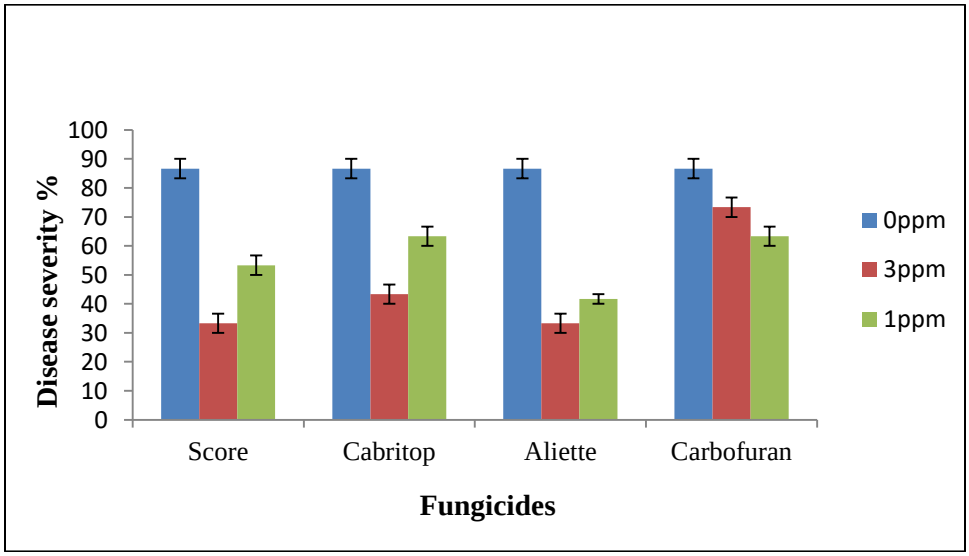


Fig 5. Effect of fungicide on disease severity of *A. solani* under field condition.

Effect of fungicides on growth of *Alternaria solani* under field condition

Table 5 shows the growth characteristics of tomato plants at 56 days of growth in negative and positive controls and fungicide treated plants. All variable showed significant results for control and treatments (P at the most 0.01). Both the fungicides Aleitte and Score significantly increased the number of leaves, stem length, stem fresh weight, root fresh weight, stem dry weight and root dry weights over positive controls, negative control (water control) were, in general, substantially higher compared to fungicide (plants sprayed with *A. solani*) (P at the most 0.01). However, root length was reduced compared to positive control ($P<0.05$). The magnitude of growth parameters for treatments (P at the most 0.05).

The results were also analyzed by two multivariate methods including cluster analysis and PCA (principal component analysis) ordination. The dendrogram derived from Ward's method of cluster analysis (Fig.6) shows considerable separation of fungicide treatments (Aleitte and Score) and the negative and positive controls. On the left side are the three replicates of negative control (water control) which form a separate group because of the greater magnitude of the growth parameters. The negative controls tend to be separated on the right side while the two fungicide treatments remain in between negative and positive controls. The two-dimensional PCA ordination also shows a remarkable separation of treatments and –ve and +ve controls (Fig.7). Appendix 1b shows the results of PCA with regard to explained variance by the first three components and the cumulative variance. The first components explained 82.1 % while the first three components cumulatively explained 95.9% of the total variance inherent in the data set. The multivariate test statistics (for MANOVA) including, Wilk's lambda, Lawley-Hotelling, Pillai and Roy's were all found to be significant (P at the most 0.05).

Table 4. Effect of fungicides on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* at 120 days of growth under greenhouse condition. Control (-) = Water control, no spray of fungicide and *A. solani*. Control (+) = Plants sprayed with *A. solani*, no spray of fungicide. Means are followed by ± Standard error (SE).

LSD $_{.05}$=	4.54	8.848	3.646	0.597	0.0891	0.0920	0.0119

Treatment	No. of Leaves	Stem Length(cm)	Root Length(cm)	Stem Fresh Wt.(g)	Root Fresh Wt.(g)	Stem Dry Wt.(g)	Root Dry Wt.(g)
Control (+)	17.33 ± 1.45a	28 ± 1.73a	10.33 ± 0.88a	4.966 ± 0.240a	0.196 ± 0.02a	1.05 ± 0.04a	0.054 ± 0.002a
Aleitte	24.66 ± 1.45b	32 ± 1.15ab	10 ± 1.15ab	5.63 ± 0.233b	0.38 ± 0.011ab	1.153 ± 0.01b8	0.063 ± 0.003b
Score	23.33 ± 1.45b	32 ± 1.73ab	10.6 ± 1.20ab	5.76 ± 0.120b	0.313 ± 0.029b	1.11 ± 0.0145bc	0.058 ± 0.005bc
Control (-)	30.66 ± 1.201c	39.33 ± 4.70b	13.33 ± 1.20b	6.733 ± 0.088c	0.416 ± 0.03c	1.37 ± 0.011c	0.097 ± 0.004c

Table 5. Effect of fungicides on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* at 56 days of growth under field condition. Control (-) = Water control, no spray of fungicide and *A. solani*. Control (+) = Plants sprayed with *A. solani*, no spray of fungicide. Means are followed by ± Standard error (SE).

Treatment	No of Leaves	Stem Length(cm)	Root Length(cm)	Stem Fresh Wt.(g)	Root Fresh Wt.(g)	Stem Dry Wt.(g)	Root Dry Wt.(g)
control (-)	8.33 ± 0.33	4.4 ± 0.15	6.7 ± 0.17	3.32 ± 0.05	2.12 ± 0.06	0.66 ± 0.02	0.41 ± 0.01
Aleitte	5.66 ± 0.33	26.8 ± 2.71	3.8 ± 0.40	2.53 ± 2.57	1.5 ± 0.1	0.52 ± 0.07	0.30 ± 0.01
Score	6 ± 0.57	28.4 ± 3.33	5.43 ± 0.28	2.66 ± 0.33	1.66 ± 0.08	0.54 ± 0.06	0.33 ± 0.01
control (+)	4.33 ± 0.33	18.33 ± 0.44	4.86 ± 0.08	1.43 ± 0.12	0.96 ± 0.12	0.28 ± 0.02	0.19 ± 0.02

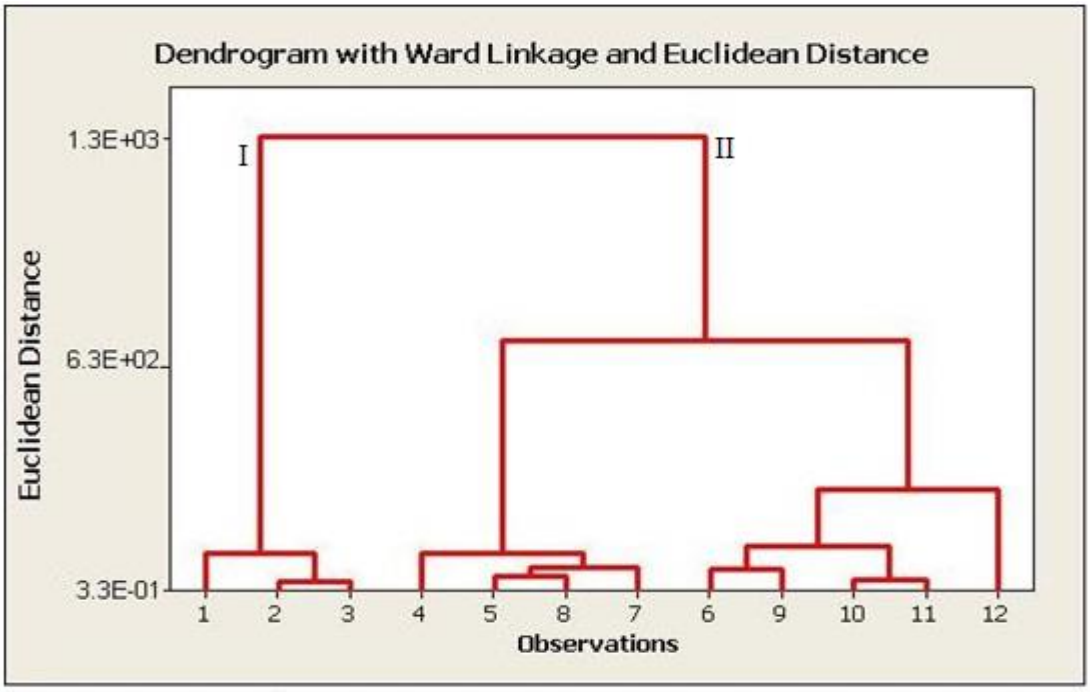


Fig. 6. Dendrogram derived from the data of the effect of fungicides on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under field conditions. (1,2,3) = negative control, (4,5,6) = Aliette, (7,8,9) = Score and (10,11,12) = positive control.

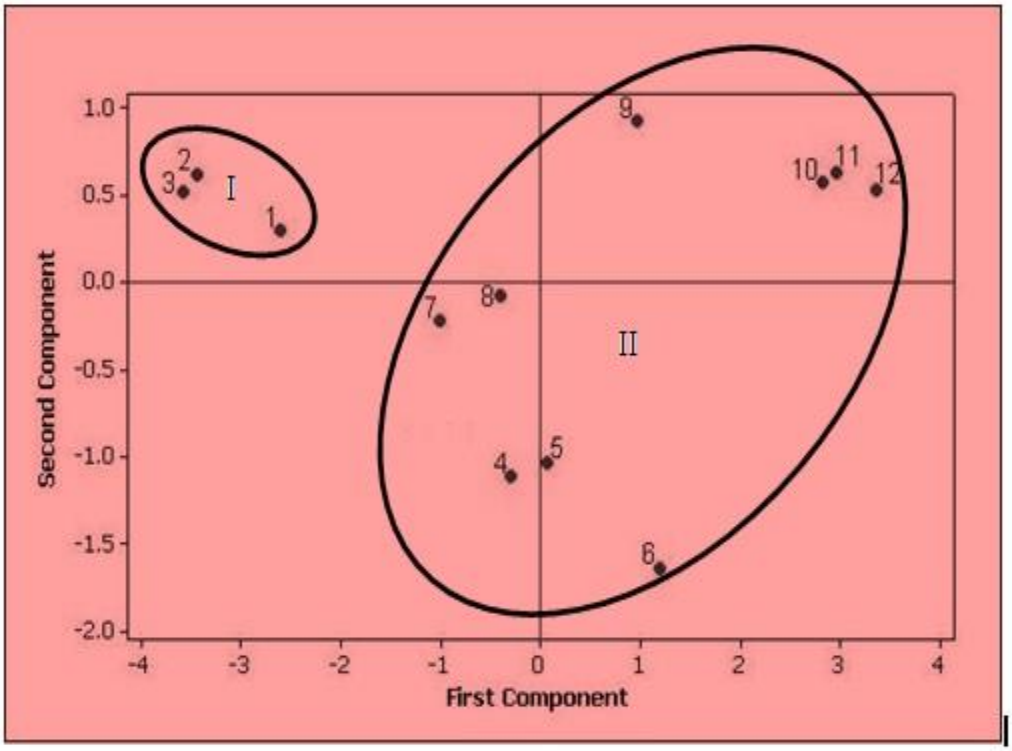


Fig. 7. Two-dimensional PCA ordination of the data of the effect of fungicides on the growth characteristics of tomato (*Solanum lycopersicum* L.) infected with *Alternaria solani* under field condition. (1, 2, 3) =negative control, (4, 5, 6) = Aliette, (7, 8, 9) = Score and (10, 11, 12) = positive control.

DISCUSSION

Fungicides are the most effective and conventional method for fungal disease control. The results of this experiment disclosed that Score, Cabritop, Carbendazim and Aliette strongly inhibited the growth of *A. solani*. Similar results were found by Datar (1996) that Carbendazim was most effective for fruit rot caused by *A. alternata*. Further, Thippeswamy *et al*., (2006) demonstrated that carbendazim and captaf checked the growth of *A. solani* since Carbendazim (methylbenzim-idazole carbamate) group of fungicides are recognized to inhibit cell division and mitosis in fungi (Seiler, 1975 and McCarroll *et al.*, 2002). Previous research revealed the inhibitory property of these fungicides due to polymerization of β-tubulin in microtubules reducing their proliferation and dynamic instability (Gupta, *et al*., 2004; Davidse 1986; Koo *et al*., 2009). Further, methylbenzim-idazole carbamate fungicides suppress the meeting of spindle microtubules owing to disturbed chromosomal alignment and microtubule-kinetochore interactions at the metaphase plate causing chromosome loss and chromatid loss, in pathogenic fungi (Rathinasamy and Panda 2006). They also manipulate the supportive arbuscular mycorrhizal fungi (Carr and Hinkley, 1985) and mammalian cells (Clement *et al*., 2008; Bi *et al*., 2009).

Horsfield *et al*., (2010) showed that score suppresses the cause early blight. Researchers have suggested that the more effective fungicides should be applied late in the season when host susceptibility increases (Shtienberg *et al*., 1996). Difenoconazol belonging to trizole family exhibits high activity against a large number of plant pathogens. Including ascomycetes, basidiomycetes and deutromycetes (Jellis *et al*., 1989; Ruess *et al*., 1989) it belongs to SI fungicides (inhibait ergosterol biosynthesis) SI fungicides differ in their spectra and level of disease control activity in their systematic properties within the plant system (Gisi, *et al*., 1986; Gupta and Kumar, 1985; Kelley and Jones, 1981).

Similarly, Lucas (1995) established that Aliette (phosphonate fungicide) combined with another fungicide, mancozeb, enhanced turf quality and controlled what has been referred to as “summer decline of bentgrass”. The mode of action of phosphonate fungicides is still in argument and obscurity because some scientists consider that these fungicides have direct effect on the fungal pathogen; while others believe on that together a direct effect of pathogenic fungus and a natural host defenses stimulation combine to prevent disease but it was assumed that phosphonate fungicide itself was not directly involved in killing the fungus, rather it was involved in stimulation of the plant’s natural physical and chemical defenses against the disease (McDonald *et al*., 2001). Cabritop, a

pyraclostrobin based fungicides, inhibits spore germination because germinating fungal spores respire actively and highly demanding energy in fungal developmental (Allen, 1965). This fungicides affects the leaf surface through inhibiting pathogen‘s spore germination as a result reduce the initial establishment of the disease in early stages (Reuveni, 2000).

The photosynthetic rate is an important sign of physiological status of the plant that is connected to the chlorophyll content. According to Howlett (2006) reduction in chlorophyll a, b and carotenoids in infected tomato leaves as a consequence of *Alternaria* infection may be due to the discharge of transported toxins, and release of reactive oxygen species that result in programmed cell death.

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