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In vitro evaluation of systemic and non systemic fungicides against Early blight (*Alternaria solani* Ellis and Martin) Jones and Grout of tomato under temperate conditions of Kashmir

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Abstract
In vitro studies were conducted to evaluate the comparative efficacy often systemic and non-systemic fungitoxicants against *Alternaria solani* (Ellis and Martin). Both systemic and non systemic fungicides were evaluated for their efficacy against the mycelial growth of *A. solani* by using poisoned food technique. On an overall mean basis, Mancozeb, proved the most effective exhibiting mean mycelial growth inhibition of 83.10 per cent followed by Dodine and Polyram causing 77.00 and 57.10 per cent mean inhibition, respectively, while Propineb proved the least effective among test non systemic fungitoxicants showing only 51.88 per cent mean inhibition. The maximum inhibition of 77.11 per cent was achieved at 500µg a.i ml^{-1} which decreased as the fungitoxicant concentration decreased. The minimum inhibition of 53.65 per cent was recorded at a concentration of 50 µg a.i ml^{-1} concentration. Amongst the systemic fungicides Difenoconazole, proved the most effective exhibiting mean mycelial growth inhibition of 81.08 per cent followed by Flusilazole and Hexaconazole causing 74.47 and 70.82 per cent mean inhibition, respectively, while Myclobutanil proved the least effective among test systemic fungitoxicants showing only 41.83 per cent mean inhibition. The maximum inhibition of 72.63 per cent was achieved at 50 µg a.i ml^{-1}which decreased as the fungi toxicant concentration decreased. The minimum inhibition of 51.85 per cent was recorded at a concentration of 10 µga.i ml^{-1}concentration.Mancozeb and dodine caused 91.66 and 90.00 per cent mycelial growth inhibition at 500 µga.i ml^{-1} Difenoconazole and Flusilazole caused 96.25 and 89.74 per cent mycelial growth inhibition at 50 µga.i ml^{-1}.

Keywords: *Alternaria solani*, fungitoxicants, mycelial growth

Introduction
Tomato (*Solanum lycopersicum* Mill.) is one of the most remunerable and widely grown vegetables in the world. It belongs to family *solanaceae*, commonly known as nightshade family include tomato, potato, chilli, pepper and eggplant [1]. Tomato (*Lycopersicon esculentum* L.) Karst is one of the most important dominant vegetable crop after potato by virtue of its high nutritive value and is grown throughout the world. Tomato crop is attacked by several diseases of biotic and abiotic nature leading to great losses to cultivators. Out of 15 vegetables listed by the FAO, tomato is placed sixth in terms of total annual world production. Tomato is a heavy feeder of nutrients, especially potash as compared to cereals [2]. On an average, a tomato crop producing 30 t ha-1 would require approximately 280 kg N, 55 kg P2O5 and 540 kg K2O ha-1 [2, 3]. Globally tomato is cultivated in 140 countries of the world with an annual production of 16.82 metric million tonnes (Anonymous, 2012) [4]. In India, major tomato growing states are Maharashtra, Bihar, Uttar Pradesh, Karnataka and west Bengal. The total area under crop in India is about 1204 thousand hectares with annual production of about 19402 metric tonnes which accounts for 11.5% of the total vegetable production [5]. The production of tomato in Jammu and Kashmir during the year 2014 was 0.008 metric million tonnes which accounts for 28.50 per cent of the total vegetable production of the State [6]. Inspite of quite favorable edaphic and environmental conditions for tomato cultivation in the Kashmir valley, the yield have not been encouraging. The wide gap between the yield potential of cultivars and the yields realized is chiefly attributed to a number of biotic and abiotic stresses [7-10].

Kodemelwar *et al*. (1973) [11] reported that copper, based fungicides gave the best control of *A. solani in vitro* while Lodha and Prasad (1975) [12] reported that Dithane Z-78 effectively checked the growth of *A. solani in vitro*. Choulwar *et al*. (1989) [13] reported that Mancozeb (0.2%) was most effective for inhibiting the mycelial growth of *Alternaria solani*. The effectiveness of Mancozeb in controlling early blight of tomato was confirmed by Singh *et al*. (2001) [14]. Ferial and Zovaqui (2010) [15] reported that Difenoconazole had a better effectiveness than chlorothalonil in inhibition of mycelial growth and condia germination of *A. alternata*.

Insects attack in tomato from the time of planting until the fruit is harvested. Insects can cause death of the tomato plant and damage to fruits in the form of tissue destruction and aberration in shape or colour. Insects can also introduce decay organisms in to fruit or can act as vector for many viruses and several mycoplasms that cause growth disorders or death of the plant. The major insect pests which plays most important role in the economic losses of tomato crop are leaf miner, aphid, jassid, whitefly and fruit borer. Dhamdhere (1990) [16] mentioned *Bemisia tabaci* and *Helicoverpa armigera* as regular pests. Salas (1992) [17] recorded *Liriomyza* spp. as major pest in tomato. Srinivasan (1993) [18] reported white fly and fruit borers as major pests of tomato. Nair (1995) [19] mentioned *Helicoverpa armigera* as more destructive pest. Gravena (1999) [20] reported that *Bemisia tabaci*, *Helicoverpa armigera* and *Liriomyza trifolii* as major pests of tomato crop. Chaudhuri *et al*. (2001) [21] recorded that the aphid (*Aphis gossypii*), whitefly (*Bemisia tabaci*), leaf miner (*Liriomyza trifolii*), tingid bug (*Urentius hystricellus*) and fruit borer (*Helicoverpa armigera*) attack tomato crop.

Methods and Materials

The present investigations were conducted in the Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, Srinagar. Ten systemic and non-systemic fungitoxicants, both were evaluated *in vitro* for their efficacy against the mycelial growth of *A. solani* by using poisoned food technique (Nene and Thapliyal, 1993) [22]. Each test fungi toxicant was evaluated at five different concentrations including check as under:

Table 1: Type of chemical name

S. No.	Common name	Nomenclature chemical name
1.	Myclobutanil 10WP	2-(4-chlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)hexanenitrile
2.	Thiophenate methyl 70WP	Dimethyl4,4–(o-phenylene)bis(3-thioallophanate)
3.	Flusilazole 40EC	1-((bis(4-flourophenyl)methylsilyl)methyl)-1-H-1 triazole
4.	Difenoconazole 25EC	Cis,trans-3-chloro-4-[4-methyl-2(1-H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether
5.	Pyraclostrobin 20WG	Methyl-*N*-[2-[[[1-(4-chlorophenyl)pyrazol-3-yloxymethyl]]-*N*-methoxycarbanilite
6.	Hexaconazole 5EC	2-(2,4-dichlorophenyl)-1-(1 h-1,2,4 triyl) hexan-2-ol
7.	Dodine 65WP	*N*-dodecyl guanadineoxitate
8.	Mancozeb 75WP	Manganese ethylene bis-di thiocarbamate plus zinc
9.	Polyram 70 WG	(1-methyl-1,2-ehanediyl)bis-carbamodithioc acid
10.	Propineb 70 WP	Zinc ethylene bis di thiocarbamate

Poisoned food technique

The non-systemic and systemic chemical fungitoxicants were assayed *in vitro* against *Alternaria solani*. On the basis of active ingredient, the non-systemic fungitoxicants were evaluated at concentration of 50, 100, 150, 250 and 500 µg a.iml^{-1} and the systemic fungitoxicants at concentrations of 10, 20, 30, 40 and 50 µg a.iml^{-1}. Fifteen ml double strength PDA, sterilized at 1.05 kg cm and 121ºC for 20 minutes, were aseptically poured into sterilized petri plates, containing equal amount of double strength test fungi toxicant prepared in distilled sterlized water. Each treatment was replicated four times. Suitable control without fungi toxicant was maintained simultaneously. Each petri plate containing solidified medium was inoculated with 5 mm mycelial disc taken from the periphery of an actively growing 7 days old culture of the fungus already grown on potato dextrose agar medium. The inoculated Petri plates were incubated at 24 ± 2ºC and observation on mycelial growth of fungus recorded after seven days of incubation. The per cent inhibition mycelial growth due to various fungi toxicant treatment at different concentrations was calculated by the following formula by Vincent (1947) [23].

$$\text{Per cent growth inhibition} = \frac{C\text{-}T}{C} \times 100$$

Where,

C = Mycelial growth in check

T = Mycelial growth in treatment

Results and Discussion

In vitro evaluation of non-systemic fungitoxicants

Mycelial growth

The data (Table 2) revealed that all the test fungi toxicants significantly inhibited the mycelial growth at all the test concentrations. On an overall mean basis, Mancozeb, proved the most effective exhibiting mean mycelial growth inhibition of 83.10 per cent followed by Dodine and Polyram causing 77.00 and 57.10 per cent mean inhibition, respectively, while Propineb proved the least effective among test non systemic fungitoxicants showing only 51.88 per cent mean inhibition. In general, the efficacy varied significantly with change in fungicidal concentration. The maximum inhibition of 77.11 per cent was achieved at 500µg a.i ml^{-1} which decreased as the fungitoxicant concentration decreased and recording a minimum of 53.65 per cent at a concentration of 50 µg a.i ml^{-1}

Table 2: Per cent mycelial growth inhibition of *Alternaria solani* at different concentrations of various non-systemic fungitoxicants *in vitro*

Fungitoxicant	Fungitoxicant concentration (µg a.i.ml^{-1})					Mean
	40	100	150	250	500	
Mancozeb 75 WP	70.41 (8.45)	78.91 (8.88)	84.60 (9.19)	89.92 (9.48)	91.66 (9.57)	83.10 (9.11)
Propineb 70 WP	30.67 (5.53)	52.84 (7.33)	55.85 (7.54)	57.91 (7.67)	62.14 (7.88)	51.88 (7.20)
Polyram 76 WG	50.22 (7.15)	53.89 (7.40)	56.83 (7.60)	59.89 (7.80)	64.67 (8.10)	57.10 (7.55)
Dodine 65 WP	63.33 (7.95)	70.00 (8.36)	76.67 (8.75)	85.00 (9.21)	90.00 (9.48)	77.00 (8.77)
Mean	53.65 (7.32)	63.91 (7.99)	68.48 (8.27)	73.18 (8.55)	77.11 (8.78)	67.27 (8.20)

C.D (P≤0.05)
Fungitoxicant: 0.07
Concentration: 0.08
Fungitoxicant × concentration: 0.01
Figures in parenthesis are square root transformation.

Concentration. There is also a significant interaction between fungitoxicants and their concentrations. Mancozeb and dodine caused 91.66 and 90.00 per cent mycelial growth inhibition at 500 µga.i ml^{-1} whereas Propineb provided by 62.14 per cent inhibition even at the highest concentration of 500 µga.i ml^{-1}. All the fungitoxicants caused more than 50 per cent growth inhibition at the lowest test concentration of 50 µga.i ml^{-1} except Propineb which showed 30.67 per cent growth at same concentration.

In vitro evaluation of systemic fungitoxicants

Mycelial growth

The data (Table 3) revealed that all the test fungitoxicants significantly inhibited the mycelial growth at all the test concentrations. On an overall mean basis, Difenoconazole, proved the most effective exhibiting mean mycelial growth inhibition of 81.08 per cent followed by Flusilazole and Hexaconazole causing 74.47 and 70.82 per cent mean inhibition, respectively, while Myclobutanil proved the least effective among test systemic fungitoxicants showing only 41.83 per cent mean inhibition. In general, the efficacy varied significantly with change in fungicidal concentration. The maximum inhibition of 72.63 per cent was achieved at 50 µga.i ml^{-1}which decreased as the fungi toxicant concentration was recording a minimum of 51.85 per cent at a concentration of 10 µga.i ml^{-1}concentration.There is also a significant interaction between fungitoxicants and their concentrations. Difenoconazole and Flusilazole caused 96.25 and 89.74 per cent mycelial growth inhibition at 50 µga.i ml^{-1}, whereas Myclobutanil provided by 47.85 per cent inhibition even at the highest concentration of 50 µga.i ml^{-1}. All the fungitoxicants caused more than 50 per cent growth inhibition at the lowest test concentration of 10 µga.i ml^{-1} except Myclobutanil and Thiophanate methyl which showed 36.73 and 37.48 per cent growth, respectively.

Table 3: Per cent mycelial growth inhibition of *Alternaria solani* at different concentrations of various systemic fungitoxicants *in vitro*

Fungitoxicant	Fungitoxicant concentration (µg a.i.ml^{-1})					Mean
	10	20	30	40	50	
Difenoconazole 25 EC	67.50 (55.24)	75.00 (60.00)	78.33 (62.26)	88.33 (70.02)	96.25 (78.84)	81.08 (64.19)
Flusilazole 40 EC	60.00 (50.76)	63.75 (52.97)	74.17 (59.45)	84.70 (9.25)	89.74 (9.52)	74.47 (59.62)
Hexaconzole 25 EC	59.15 (45.14)	60.00 (50.76)	71.67 (8.52)	79.77 (8.98)	83.53 (9.19)	70.82 (59.62)
Pyroclostrobin 20 WG	50.25 (45.14)	61.77 (7.92)	64.22 (8.07)	66.92 (8.24)	69.48 (8.39)	62.52 (52.23)
Myclobutanil 10 WP	36.73 (6.14)	38.95 (6.32)	41.25 (6.50)	44.41 (6.73)	47.85 (6.98)	41.83 (52.23)
Thiophenatemethyl 70 WP	37.48 (6.20)	39.83 (6.39)	42.16 (6.57)	45.43 (6.81)	48.94 (7.06)	42.76 (40.81)
Mean	51.85 (46.04)	56.55 (48.74)	61.96 (51.89)	68.26 (55.68)	72.63 (58.43)	62.25 (52.07)

C.D (P≤0.05)
Fungitoxicants : 0.0049
Concentrations : 0.0045
Fungitoxicants × concentrations: 0.011
Figures in parenthesis are square root transformation

Studies on *in vitro* evaluation of four non systemic and six systemic fungitoxicants at different concentrations against the mycelial growth and conidial germination of *Alternaria solani* were conducted to get a preliminary idea about the fungitoxicants to be used under natural field conditions against the disease. Among the four nonsystemic fungitoxicants evaluated against *Alternaria solani*, Mancozeb was the most effective in checking the mycelial growth of *Alternaria solani* causing mycelial inhibition of 83.10 per cent followed by Dodine with 77.00 per cent mycelial growth inhibition. Among the six systemic fungitoxicants evaluated *in vitro* Difenoconazole and Flusilazole were most effective causing mycelia growth inhibition of 81.08 and 74.47 per cent, respectively. The efficacy of Difenoconazole and Flusilazole against *Alternaria solani* has been confirmed by many workers. Choulwar *et al.* (1989) [13] reported that Mancozeb (0.2%) was most effective for inhibiting the mycelial growth of *Alternaria solani.* The effectiveness of Mancozeb in controlling early blight of tomato was confirmed by Singh *et al.* (2001) [14]. Ferial and Zovaqui (2010) [15] reported that Difenoconazole had a better effectiveness than chlorothalonil in inhibition of mycelial growth and condia germination of *A. alternata.*

Conclusion

In vitro evaluation of fungitoxicants against *Alternaria solani* indicated that all the fungitoxicants significantly inhibited the mycelial growth of the test pathogen. Among the non-systemic fungitoxicants, Mancozeb proved the most effective exhibiting mean mycelial growth inhibition of 83.10 per cent, Similarly, Difenoconazole, proved the most effective exhibiting mean mycelial growth inhibition of 81.08 per cent. Hopefully present study will be beneficial for management control agencies.

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