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Research Paper

In Vitro Efficacy of Systemic and Non-Systemic Chemicals on The Growth Inhibition of Alternaria Solani Causing Early Leaf Blight of Tomato

Agricultural Science

KEYWORDS : *Alternaria solani*, Chemical control, Propineb, Propiconazole

*** Somnath Koley**	Department of Plant Pathology, College of Agriculture, Orissa University of Agriculture & Technology, Bhubaneswar-751003 (Odisha), India * Corresponding author
Shyama Sundar Mahapatra	Department of Plant Pathology, College of Agriculture, Orissa University of Agriculture & Technology, Bhubaneswar-751003 (Odisha), India

ABSTRACT

In vitro testing of four non-systemic chemicals and six systemic chemicals against the fungus revealed that Propineb @ 0.1% among the non-systemic chemicals and Propiconazole @ 0.05% among the systemic chemicals were the best resulting in cent per cent and 96.91% growth inhibition of Alternaria solani, respectively. 0.3% concentration of non-systemic chemicals and 0.15% of systemic chemicals showed the highest growth inhibition (87.50% and 75.61%, respectively) of the fungus. As regards to the interaction effect between the tested chemicals and their concentrations, it was found that Mancozeb @ 0.3% was statistically at par with Copper oxychloride @ 0.2% and Difenoconazole @ 0.15% was statistically at par with Propiconazole @ 0.05%. These results will be helpful for the selection and rotation of the studied modern chemicals according to their potential lower concentration to reduce the environmental hazards and fungal resistance of the early blight fungus.

INTRODUCTION

Alternaria solani can cause diseases on leaf (leaf blight), stem (stem collar rot) as well as on fruit (fruit lesion) of tomato plant. Characteristic symptom of the *Alternaria* early blight is the concentric rings which looks like bull's eye (Akhtar *et al.*, 2004). Severe infection of this fungus leads to great reduction of the fruit yield in both quantitative and qualitative way.

Several previous reports suggested that Mancozeb, Carbendazim and Copper oxychloride are the best chemicals against *Alternaria* disease of tomato (Kumar *et al.* 2012; Sudarshana *et al.* 2012; Saha *et al.* 2013). Although spraying of these broad spectrum fungicides has been recommended for the control of early blight of tomato, while the numbers of applications of these chemicals are more, they are less persistent on foliage (Thind and Jyothi, 1982). Thus, the control achieved by these chemicals is inadequate. One of the reasons attributed to the low sensitivity of *Alternaria solani* to fungicides mentioned above is the production of dark brown to black pigment called melanin by the fungus, which enhanced its survival and competitive abilities, under certain environmental conditions (Bell and Wheeler, 1986). One another reason is the pathogen resistance. The best ways to prevent resistance are the use of new generation chemicals with new mode of action, use of multi-site active chemicals and minimize the concentration and number of application in a crop season (Staub, 1991; Brent and Hollomon, 2007).

In present day, triazoles are the largest class of fungicides for the reason of highly efficient broad spectrum products, resistance over time as a slow shift and decreasing pathogen sensitivity to their mode of action (Morton and Stub, 2008). The second largest class is the strobilurins which is the Quinone outside Inhibitors. Although dithiocarbamate group of fungicide is not the newer like previous two, but Propineb is the newer one within the group. In present days, the other commonly used group of fungicides are dithiocarbamate, benzimidazole, antifungal antibiotic, copper compound, phthalonitrile *etc.* for controlling severe diseases of different vegetable crops. Therefore, in the present study it is thought worthwhile to test the comparative efficacy of modern and more promising chemicals against the early blight fungus with the present day used chemicals. This fungicide research is also focused on the comparative efficacy of three different concentrations of the same systemic or non-systemic chemicals and their interaction effects.

MATERIALS AND METHODS

Preparation of medium: Potato dextrose agar (PDA) medium was selected for the culture of *A. solani*, as the fungus grows well in this solid semi-synthetic medium (Koley & Mahapatra, 2015). The medium contains 200 g peeled and sliced potato, 20 g dextrose and 20 g agar for 1litre preparation.

Collection of leaf samples, isolation, purification and multiplication of the pathogen: Tomato plants showing the distinct early leaf blight symptoms were collected from the research field of Central Agricultural Farm, O.U.A.T., Bhubaneswar. Ten such affected leaves were kept overnight in moist chamber for sporulation. Affected portions of the leaves were teased and examined under microscope which revealed the presence of characteristic mycelia, conidiophores and conidia of the fungus, *A. solani* (Ellis and Martin) Jones and Grout.

The part of leaves, showing typical symptoms of disease, were washed in running tap water. Affected portion of the leaf cut into small bits (2-3 mm) consisting of diseased as well as apparently healthy tissues of the leaf. These bits were surface sterilized with 1: 1000 (0.1%) mercuric chloride solution for 1 minute and then rinsed thoroughly with sterile water for 3-4 times to remove the last traces of mercuric chloride solution.

Finally with the help of a sterilized inoculating needle, the surface sterilized, diseased plant materials were transferred aseptically to the center of the sterilized PDA plates and were incubated at 27 ± 1°C for three days to obtain growth of the fungus. The small bit of fungal growth was transferred to PDA slants. The pure culture of the fungus was obtained by selecting "hyphal tip" from the periphery of actively growing colony for making the fresh culture under aseptic conditions. Sub-culturing of the pure fungus culture was necessary after every 14 days.

Table 1: Particulars of the fungicide used

Common Name	Trade Name	Chemical name
Non-Systemic Fungicides (Dose- 0.10%, 0.20% and 0.30%)		
Chlorothalonil	KAVACH 75% WP	Tetrachloro isophthalonitrile
Copper oxychloride	NAGCOPER 50% WP	Copper oxychloride
Mancozeb	KEM-45 75% WP	Manganese ethylene bisdithiocarbamate

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Propineb	ANTRACOL 70% WP	Polymeric zinc 1,2- propylenebis-dithiocarbamates
Systemic Fungicides (Dose- 0.05%, 0.10%, 0.15%)		
Carbendazim	BAVISTIN 50% WP	Methyl benzimidazol-2-ylcarbamate
Propiconazole	TILT 25% EC	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole
Thiophanate methyl	ROKO 70% WP	Dimethyl [(1,2-phenylene) bis-(iminocarbonothioyl) biscarbamate
Azoxystrobin	AMISTAR 23% EC	Methyl (2*E*)-2-(2-{[6-(2-cyanophenoxy) pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate
Difenoconazole	SCORE 25% EC	1-[2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole
Kasugamycin	Kasu-B 3% SL	Kasugamycin hydrochloride

Poisoned food technique: In order to determine the efficacy of different chemicals in inhibiting the mycelial growth of *Alternaria solani* causing early blight of tomato during the period of investigation, poisoned food technique was followed. The efficacy of chemicals on the growth of the test fungus was studied at three different doses. The doses for the systemic chemicals were 0.05%, 0.10% and 0.15% whereas the same for the non-systemic chemicals were 0.10%, 0.20% and 0.30%. The chemicals used in the present investigation for management of the test pathogen along with their formulation and doses are described in **Table 1**. During the study, required quantity of individual fungicide was added separately into molten and sparingly cooled potato dextrose agar so as to get the desired concentration of fungicides in the medium. Later 20 ml of the poisoned medium was poured into sterile Petri dishes under aseptic conditions inside an inoculation chamber. Medium without any fungicide served as control. Each Petri plate was inoculated at the centre with a mycelia disc of 5mm diameter taken from the periphery of five-day old colony of the test fungus with the help of sterilized cork borer and replicated thrice. The Petri plates were incubated at 27±1^0 C for 10 days.

Fungus growth inhibition measurement technique: The colony diameter was measured in each case, in two planes at right angles to each other on the 10th day of inoculation. Inhibition of mycelia growth was calculated by using Vincent formula (Vincent, 1947) as follows: Mean % inhibition= $\frac{C-T}{T}$× **100 (Where, C= Colony diameter in control, T= Colony diameter in treatment)**

Statistical analysis: The experiments are performed under controlled *in vitro* condition and the experimental data are statistically analyzed by the factorial completely randomized block design (factorial CRD).

RESULTS

Effect of non-systemic chemicals: Among the non-systemic chemicals tested (**Table 2**) against *A. solani*, Propineb was the best showing cent percent growth inhibition of the fungus, followed by Copper oxychloride, Chlorothalonil and Mancozeb (showing growth inhibition of 78.02%, 64.56% and 62.22%, respectively). As regards the concentrations of these non-systemic fungicides, it was found that 0.3% concentration resulted in significantly the highest growth inhibition (87.50%) of the test fungus. Among other treatments, it was found that Mancozeb @ 0.3% showing 77.03% growth inhibition of the test fungus, was statistically at par with that by Copper oxychloride @ 0.2% (80.73%). Similarly Mancozeb (0.2%), Copper oxychloride (0.1%) and Chlorothalonil (0.2%) were statistically at par with each other showing mycelial growth inhibition of 65.18%, 64.81% and 62.59%, respectively. Chlorothalonil (0.1%) and Mancozeb (0.1%) were also statistically at par with respective growth inhibition of 46.66% and 44.44%.

Effect of systemic chemicals: *In vitro* evaluation of some systemic chemicals (**Table 3**) against *A. solani* revealed that Propiconazole was the best showing highest growth reduction (96.91%), followed by Difenoconazole, Azoxystrobin and Thiophanate methyl (with growth inhibition of 81.73%, 69.25% and 50.75%, respectively). It was found that 0.15% concentration of all the chemicals achieved significantly the highest growth inhibition (75.61%) of the fungus. Among different chemicals, it was found that Difenoconazole @ 0.15% was statistically at par with Propiconazole @ 0.05%, both of them showing 90.74% growth inhibition. Similarly, Difenoconazole @ 0.1% was also statistically at par with Azoxystrobin @ 0.15%, respectively showing 82.59% and 78.51% inhibition. Difenoconazole @ 0.05%, Kasugamycin @ 0.15% and Azoxystrobin @ 0.1% were also observed to be statistically at par with 71.85%, 68.51% and 67.77% of growth inhibition, respectively. Thiophanate methyl @ 0.15%, Kasugamycin @ 0.10% and Azoxystrobin @ 0.05% were also observed to be statistically at par with each other, with respective growth inhibition of 63.33%, 61.85% and 61.48%. Similarly, Thiophanate methyl @ 0.10% and Carbendazim @ 0.15% with growth inhibition of 53.70% and 52.59%, respectively were also statistically at par with each other. Thiophanate methyl @ 0.05% and Carbendazim @ 0.05%, being statistically at par with each other resulted in growth inhibition of 35.18% and 31.48%, respectively.

Table 2: Efficacy of non-systemic chemicals on the growth inhibition of *A. solani (in vitro)*

Treatment	Mean per cent inhibition of growth			Mean (Chemicals)
	0.10% Conc.	0.20% Conc.	0.30% Conc.	
Chlorothalonil	46.66 (43.08)	62.59 (52.31)	84.44 (66.80)	64.56 (54.07)
Copper oxychloride	64.81(53.63)	80.73 (63.98)	88.51 (70.25)	78.02 (62.62)
Mancozeb	44.44 (41.81)	65.18 (53.84)	77.03 (61.40)	62.22 (52.35)
Propineb	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Mean (Conc.)	63.98 (57.13)	77.13 (65.03)	87.50 (72.12)	

	Chemical	Concentration	Chemical ×Conc.
S.E. (m) ±	0.52	0.45	0.90
CD (0.05)	1.52	1.32	2.64

***Figures in parentheses indicate corresponding angular values**

Table 3: Efficacy of systemic chemicals on the growth inhibition of *A. solani (in vitro)*

Treatment	Mean per cent inhibition			Mean (Chemicals)
	0.05% Conc.	0.10% Conc.	0.15% Conc.	
Carbendazim	31.48(34.12)	44.44(41.80)	52.59(46.49)	42.84(40.80)
Propiconazole	90.74(72.37)	100(90.00)	100(90.00)	96.91(84.12)
Thiophanate methyl	35.18(36.36)	53.70(47.12)	63.33(52.75)	50.75(45.41)
Azoxystrobin	61.48(51.64)	67.77(55.42)	78.51(62.39)	69.25(56.48)
Difenoconazole	71.85(57.97)	82.59(65.37)	90.74(72.32)	81.73(65.22)
Kasugamycin	11.85(19.72)	61.85(51.86)	68.51(55.88)	47.40(42.49)

Mean (Conc.)	50.43(45.36)	68.39(58.60)	75.61(63.31)	
	Chemical	Concentration	Chemical ×Conc.	
S.E. (m) ±	0.69	0.48	1.19	
CD (0.05)	1.97	1.39	3.40	

***Figures in parentheses indicate corresponding angular values**

DISCUSSION

The present finding revealed that Propineb and Propiconazole are most promising chemicals under *in vitro* condition against early blight fungus of tomato. All other tested chemicals caused also significant reduction in *Alternaria* growth and this inhibition is gradually increased with increasing concentration of the chemicals. From the study of interaction effect between chemicals and their concentrations, this research suggest that use of chemical with less concentration rather than chemical with higher concentration, which are statistically at par to each other, could reduce the chance of chemical environmental hazards as well as pathogen resistance.

Propineb is the best chemical option, irrespective of systemic & non-systemic classes, because it inhibits multi-sites in the metabolism of fungal cells. That's why Propineb can be more applicable in anti-resistance strategy of *A. solani*. In addition to pathogen control, Antracol (Propineb) has a beneficial physiological role by supplying zinc micronutrient in case of plant deficiency. Copper oxychloride acts as protectant fungicide which denatures proteins, resulting destroying enzymes, leading to stop cell function of the fungus (Zitter and Rosenberger, 2013). This present finding is in conformity with the reports of earlier workers like Abhinandan *et al.* (2004) and Singh and Singh (2006).

Propiconazole inhibits the growth of *Alternaria* by demethylation of C-14 during ergosterol biosynthesis which is critical for the fungal cell wall formation. Effect of Propiconazole against *A. solani* corroborates findings of Sali *et al.* (2010) and Sharma *et al.* (2011). Azoxystrobin inhibits the fungal respiration (MET-III, cytochrome bc_1), leading to stop spore germination (Bartlett *et al.*, 2002). This study confirms the finding of MacDonald *et al.* (2007) and Horsfield *et al.* (2010) who suggested that Azoxystrobin could suppress early blight of potato.

Koley *et al.* (2015) showed that the growth of *A. solani* is inhibited upto 52.77% and 57.03% by the use of bio-control agents and botanical extracts respectively. Although bio-control and botanical strategies are good for sustainable agriculture, but the chemical control is the best option for *Alternaria* disease (Mesta *et al.*, 2011) which is confirmed with the cent percent inhibition by all three concentration of Propineb and 0.10% and 0.15% concentration of Propiconazole in this finding. Present study could suggest that rotation of the best two chemicals Propineb and Propiconazole in an effective manner for the different crop season of tomato can prevent the fungal resistance, as these two fungicides have different mode of action. Further research into these chemicals will show the best integrated inhibition effect against tomato early blight fungus.

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