INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 8 | ISSUE 1 | APRIL, 2015 | 169-173
e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/8.1/169-173

Evaluation of newer fungicides against *Alternaria alternata* (Fr.) Keissler causing fruit rot disease of chilli

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ARITCLE INFO

Received : 12.12.2014
Revised : 06.03.2015
Accepted : 20.03.2015

KEY WORDS :
Fungicides, *Alternaria alternata*, Fruit rot, Chilli, *Capsicum annum, In vitro, In vivo*

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ABSTRACT

Among the ten newer fungicides evaluated under *in vitro* condition by poisoned food technique against *Alternaria alternata* revealed tebuconazole, hexaconazole and azoxystrobin (18.2%) + difenconazole (11.4%) at all the three concentrations (500, 1000 and 1500 ppm) completely inhibited the mycelial growth of the pathogen and proved to be most effective. Based on *in vitro* screening, five promising fungicides were selected and reevaluated under field condition against fruit rot of chilli and the result revealed that two foliar application of tebuconazole (50%) + trifloxystrobin (25%) (75 WP) @ 0.05 per cent at an interval of 15 days, commencing from the initiation of disease was most effective in reducing fruit rot intensity and increasing fruit yield over control. Tebuconazole which was found effective under *in vitro* but could not reduce fruit rot intensity effectively under field conditions.

How to view point the article : Ginoya, C.M. and Gohel, N.M. (2015). Evaluation of newer fungicides against *Alternaria alternata* (Fr.) Keissler causing fruit rot disease of chilli. *Internat. J. Plant Protec.*, **8**(1) : 169-173.

INTRODUCTION

Chilli (*Capsicum annum* L.) crop is vulnerable to many diseases and pests due to its extreme delicacy and succulence. Diseases caused by fungi, bacteria and viruses are major constrains to chilli production. In India, the first report of *Alternaria* sp. was made from Delhi by Dutt in 1937. Mathur and Agnihotri (1961) reported fruit rot of chilli caused by *Alternaria tenuis* Nees from Rajasthan. Sreekantiah *et al*. (1973) reported a virulent strain of *A. alternata* causing leaf spot and fruit rot of chilli from Mysore, while from Maharashtra, Khodke and Gahukar (1993) also reported *Alternaria* sp. associated with chilli. Bhatt *et al*. (2000) recorded *A. alternata* causing fruit rot on chilli from Kumaon hillis of Uttar Pradesh, India. Narain *et al*. (2000) reported *Alternaria alternata* causing fruit rot of chilli on fruits, as initially small blackish brown, circular to elongated water soaked depressed lesions are formed on the pericarp of fruits, which leads to rotting of fruits in later stage. The characteristic lesions observed at semi ripe stage of chilli fruits. Fruit rot is a major constraint in chilli causing several losses in terms of quality and quantity (Sreekantiah *et al*., 1973). Mathur and Agnihotri (1961) and Singh (1987) reported 5-85 per cent yield losses due to this disease. An attempt was made to evaluate the newer fungicides both *in vitro* and *in vivo* for the control of this important disease in middle Gujarat condition.

MATERIAL AND METHODS

***In vitro* screening of fungicides against *A. alternata* :**

Ten fungicides belonging to different chemical groups at three different concentrations were tested for their efficacy *in vitro* against *A. alternata* using poisoned food technique (Nene and Thapliyal, 1993).

The required quantities of each test fungicides were incorporated in a conical flask containing 100 ml melted PDA medium so as to get required concentration in parts per million (ppm). The flask containing poisoned medium was well shaken to facilitate uniform mixture of fungicides and 20 ml was poured in each sterilized Petriplates. On solidification of the medium, the plates were inoculated in the centre by placing 5 mm diameter mycelial culture block cut aseptically with the help of cork borer from 10 days old pure culture of *A. alternata.* Three repetitions were kept for each concentration of respective fungicide. The inoculated plates were incubated at 27± 1ºC temperature. The observations on linear growth of fungus was recorded at 24 h interval till the entire plate in control was completely covered with mycelium. The per cent growth inhibition (PGI) of the pathogen over control was worked out by using formula given by Arora and Dwivedi (1979).

$$PGI = \frac{100\ (DC - DT)}{DC}$$

where,

PGI= Per cent growth inhibition

DC=Average diameter (mm) of mycelial colony in control treatment

DT=Average diameter (mm) of mycelial colony in treated set.

The concentrations of fungicides taken were those of active ingredient presenting in commercial formulation. Each fungicide was tested at three different concentrations.

In vivo evaluation of fungicides against fruit rot :

Considering the importance of disease and variation in the recommendations of different fungicides by various workers for the control of fruit rot disease, a field experiment was carried out with the fungicides, which were found effective under laboratory screening to test relative field efficacy of different fungicides in controlling the Alternaria fruit rot disease of chilli.

A field trial was conducted at Main Vegetable Research Station, Anand Agricultural University, Anand during *Kharif* 2013-14 in Randomized Block Design (RBD) with six treatments along with four replications. All the recommended agronomical practices were followed during experimentation. Thirty-five days old seedlings of chilli cv. GVC-101 were transplanted with 60×60 cm spacing in the second week of August 2013. Two foliar sprays of fungicides were given commencing from the appearance of fruit rot disease and a subsequent spray was given after 15 days of first spray. The intensity of Alternaria fruit rot was recorded after seven days of each spray. Ten plants were selected randomly and labeled from each plot for scoring the disease intensity. From each plant, three fruits from upper, middle and lower portions were randomly observed. These selected plants were graded using 0-9 disease rating scale on the basis of percentage area of fruits infected by the pathogen.

Disease rating scale for scoring the intensity of chilli fruit rot : 0 = No infection; 1 = 1-10 per cent; 3 = 11-25 per cent; 5 = 26-50 per cent; 7 = 51-75 per cent and 9 = >75 per cent infection on fruits.

The Per cent Disease Intensity (PDI) was calculated by adopting the following equation as given by Mayee and Datar (1986):

$$PDI = \frac{\text{Summation of all numerical ratings}}{\text{Number of fruits observed} \times \text{Maximum grade value (9)}} \times 100$$

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads :

***In vitro* screening of fungicides against *A. alternata* :**

The observations on mycelial growth and per cent growth inhibition (PGI) recorded after fifteen days of incubation (Table 1). Tebuconazole, hexaconazole and azoxystrobin (18.2%) + difenconazole (11.4%) completely inhibited the mycelial growth at all the three concentrations tested and thus appeared significantly superior over rest of the treatments. The next best treatments in order of merit in inhibiting mycelial growth was fusilazole (12.5%) + carbendazim (25%) at 500, 1000 and 1500 ppm concentration (84.22, 87.47 and 90.71%) which was at par with tebuconazole (50%) + trifloxystrobin (25%) at 1000, 1500 and 2000 ppm concentration (82.83, 86.07 and 87.23%). The next best treatment was fenamidone (10%) + mancozeb (50%) at 1000, 1500 and 2000 ppm concentration (64.04, 67.04 and 69.14%) followed by isoprothiolane at 1000, 1500 and 2000 ppm concentration with 47.09, 64.49 and 69.37 per cent growth inhibition, respectively.

The results of Mane *et al*. (2011) who reported cent per cent inhibition of radial growth with propiconazole, current M-45 and copper hydroxide, while difenconazole and hexaconazole were best among triazole group fungicides in inhibiting the radial growth of *A. alternata*. Patel (2008) reported hexaconazole, penconazole, propiconazole and compound fungicide *i.e.* hexaconazole (5%) + captan (70%) were highly fungitoxic against *A. alternata* and cent per cent growth inhibition of *A. alternata* was noted at all three concentration over control.

Singh and Singh (2006) screened seven fungicides against *A. alternata* found that hexaconazole (5 EC) at all the tested concentrations (50, 100, 200, 500 and 1000 ppm) gave cent per cent inhibition of the mycelial growth.

Akbari and Parakhia (2007) found that the propiconazole,

Table 1 : *In vitro* screening of newer fungicides against *Alternaria alternata*

Sr. No.	Treatments	Concentration (ppm)	Average mycelial growth (mm)	Per cent growth inhibition (PGI)
1.	Tebuconazole (25 EC)	500	0	100
		1000	0	100
		1500	0	100
2.	Hexaconazole (5 EC)	500	0	100
		1000	0	100
		1500	0	100
3.	Kresoxim methyl (45 SC)	500	59.67	16.92
		1000	52.83	26.45
		1500	44.33	38.28
4.	Mancozeb (75 WP)	2000	50.00	30.39
		2500	48.17	32.93
		3000	47.33	34.10
5.	Carbendazim (12%) + Mancozeb (63%) (75 WP)	1000	46.67	35.02
		2000	36.33	49.42
		3000	30.00	58.23
6.	Azoxystrobin (18.2%) + Difenconazole (11.4%) (29.6 SC)	500	0	100
		1000	0	100
		1500	0	100
7.	Isoprothiolane (40 EC)	1000	38.00	47.09
		1500	25.50	64.49
		2000	22.00	69.37
8.	Fusilazole (12.5%) + Carbendazim (25%) (37.5 SC)	500	11.33	84.22
		1000	9.00	87.47
		1500	6.67	90.71
9.	Fenamidone (10%) + Mancozeb (50%) (60 WG)	1000	25.83	64.04
		1500	23.67	67.04
		2000	22.16	69.14
10.	Tebuconazole (50%) + Trifloxystrobin (25%) (75 WP)	1000	12.33	82.83
		1500	10.00	86.07
		2000	9.17	87.23
11.	Control (only test pathogen)	---	71.83	---
S.E. ±			0.74	
C.D. (P = 0.05)			2.12	

Table 2 : Effect of fungicides on fruit rot intensity and yield of chilli

Sr. No.	Treatments	Concentration (%)	Fruit rot intensity (%)	Percent disease control	Fruit yield (kg./ha)
1.	Tebuconazole (25 EC)	0.05	34.03	50.68	750
2.	Hexaconazole (5 EC)	0.05	19.13	72.27	834
3.	Azoxystrobin (18.2%) + Difenconazole (11.4%) (29.6 SC)	0.05	24.69	64.22	812
4.	Fusilazole (12.5%) + Carbendazim (25%) (37.5 SC)	0.15	39.88	42.21	598
5.	Tebuconazole (50%) + Trifloxystrobin (25%) (75 WP)	0.20	11.23	83.72	992
6.	Control	-	69.01	-	440
	S.E. ±		1.76		47.87
	C.D. (P = 0.05)		5.55		150.84

tridemorph, difenconazole and hexaconazole were completely inhibitory to *A. alternata* causing blight of sesame even at minimum concentration of 50 ppm. Non-systemic fungicides, both thiram and mancozeb gave cent per cent inhibition of *A. alternata* at minimum concentration of 500 ppm. Singh and Chowdhary (2008) screened different six fungicides against *A. solani* incitant of fruit rot of chilli and found that mycelial growth was least in carbendazim 12 per cent + mancozeb 63 per cent (75 WP) with highest per cent inhibition of 83.93 per cent, 94.27 per cent and 97.64 per cent at 500, 750 and 1000 ppm concentration, respectively.

Kumar *et al.* (2013) tested twelve fungicides among them, carbendazim, mancozeb, chlorothalonil, vitavax and thiram were proved most effective and inhibit the pathogen completely. Thus, the results of earlier workers are also in line with the results obtained in the present investigations.

Effectiveness of the fungicides found promising in present *in vitro* study can be attributed to their mode of action leading to adverse effect on growth and development of *A. alternata.* Tebuconazole and hexaconazole are fungicides of triazole group. They cause demethylation of C-14 during ergosterol biosynthesis leading to accumulation of C-14 methyl sterols. The biosynthesis of these ergosterols is critical to the formation of cell walls of fungi. Lack of normal sterol production slower down or stops the growth of the fungus preventing further infection and/or invasion of host tissues.

The compound product *i.e.* azoxystrobin (18.2%) + difenconazole (11.4%) was also superior among all other treatments. Among them, difenconazole and azoxystrobin belongs to triazole and strobilurin group, respectively. Strobilurin fungicides are quinone outside inhibitors (QoI), which interfere with energy production in fungal cell. They block the electron transfer at the site of quinol oxidation in the cytochrome bc1 complex, thus preventing ATP production.

In vivo evaluation of fungicides against fruit rot :

All the treatments significantly reduced the per cent fruit rot intensity as compared to control (Table 2). Among them, tebuconazole (50%) + trifloxystrobin (25%) was found significantly superior over the rest of treatments showing minimum (11.23 %) fruit rot intensity.

The next best treatment was hexaconazole (19.13%) followed by azoxystrobin (18.2%) + difenconazole (11.4%) showed disease intensity of 24.69%. Treatment of tebuconazole (34.03%) and fusilazole (12.5%) + carbendazim (25%) with disease intensity of 39.88 per cent were found mediocre.

The treatment effects were significant in relation to fruit yield (Table 2). Tebuconazole (50%) + trifloxystrobin (25%) was found significantly superior in giving highest fruit yield (992 kg ha^{-1}). The next best treatment was hexaconazole (834 kg ha^{-1}) which was at par with azoxystrobin (18.2%) + difenconazole (11.4%) (812 kg ha^{-1}). The rest of the treatment, tebuconazole (750 kg ha^{-1}) followed by fusilazole (12.5%) + carbendazim (25%) (598 kg ha^{-1}) were moderately effective over control (440 kg ha^{-1}).

In the present study, tebuconazole (50%) + trifloxystrobin (25%) (0.05%) was found significantly superior in reducing fruit rot and achieving the higher yield over rest of the treatments. The next effective treatment was hexaconazole, which was at par with azoxystrobin (18.2%) + difenconazole (11.4%). Surprisingly, tebuconazole showed promising fungicides under *in vitro* test, but found ineffective in reducing disease under field condition.

The more or less similar result was found by Akbari and Parakhia (2007). They proved that the field performance of propiconazole (0.05%) was remarkable, gave effective control of sesame blight caused by *A. alternata* in leaves (80%), stem (78%) and capsule (80%) and higher seed yield (886 kg ha^{-1}). Carbendazim (0.05%) and hexaconazole (0.005%) were also found effective and remained at par with propiconazole. Mane *et al.* (2011) proved that among different seven fungicides, hexaconazole (0.05%) was most effective followed by mancozeb (0.3%) and carbendazim (0.2%) with significant higher yield when applied four times at 15 days interval starting from 30 days after transplanting.

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