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Management of Anthracnose Disease of Mungbean Through New Fungicidal Formulations

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The mungbean crop suffers from several fungal diseases, among which anthracnose caused by *Colletotrichum gloeosporioides* is one of the serious disease observed regularly in mungbean growing areas of Gujarat and its incidence has increased alarmingly during the recent years in Gujarat. Among the nine fungicides at three concentrations were screened under *in vitro* condition against *C. gloeosporioides* by poisoned food technique revealed propiconazole, carbendazim (12%) + mancozeb (63%) and propineb completely inhibited the mycelial growth and proved to be most effective over rest of the treatments. The next best were azoxystrobin + difenconazole and trifloxystrobin + tebuconazole. Based on *in vitro* screening, promising fungicides were selected and re-evaluated under field condition against anthracnose of mungbean. Experiment under field condition revealed that anthracnose disease of mungbean can effectively managed by seed treatment with Thiram 75 SD, 3 g/kg seeds + two foliar sprays at 15 days interval starting from initiation of disease with trifloxystrobin + tebuconazole (75 WG), 0.075 per cent or with carbendazim + mancozeb (75 WP), 0.075 per cent. One of the key reasons for success of strobilurins that it gives a control of fungi from all four groups of plant pathogens *i.e. Ascomycota, Basidiomycota, Deuteromycota* and *Oomycota*.

Keywords: Mungbean, Fungicides, Anthracnose, *Colletotrichum gloeosporioides.*

The mungbean [*Vigna radiata* (L.) Wilczek, Syn.: *Phaseolus aureus* Roxb., *Phaseolus radiatus* L.] is one of the third most important pulse crop of India after chickpea and pigeon pea. The productivity of pulse crops became stagnant since last three decades because of less success in developing improved varieties and moreover, it is grown on marginal and sub-marginal lands. The crop suffers from many diseases caused by fungi, bacteria, viruses, nematodes and abiotic stresses. Among the fungal diseases, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (NFCCI 3151) is one of the economically important disease. The anthracnose disease of mungbean was first reported in India from Jorhat of Assam state in 1951 (Majid, 1953). The disease has been reported from all major mungbean growing states of India in mild to severe form and in tropical and subtropical areas causes considerable damage by reducing seed quality and yield (Sharma *et al*., 1971). The average seed yield loss of 40.18 per cent and stalk yield loss of 46.90 per cent was noticed due to anthracnose of mungbean (Kulkarni, 2009). Considering, the emerging and devastating nature of the disease and economic loss of the crop in this area, 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.

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MATERIALS AND METHODS

In vitro evaluation of fungicides against Colletotrichum gloeosporioides

Nine fungicides belonging to different chemical groups at three different concentrations were tested for their efficacy *in vitro* against *C. gloeosporioides* using poisoned food technique (Nene and Thapliyal, 1979). The concentrations of fungicides taken were those of active ingredients present in commercial formulation. 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 then, from this 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 actively growing pure culture of *C. gloeosporioides* grown on PDA.. Three repetitions were kept for each concentration of respective fungicide. The inoculated plates were incubated in B.O.D. at 27 ± 2^0C temperature. The observations on linear growth of fungus were recorded at 24 h interval till the entire plate in control was completely covered with mycelium. The percent 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= Percent growth inhibition

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

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

Management of anthracnose of mungbean through fungicides under field conditions

Considering the importance of disease and variation in the recommendations of different fungicides by various workers for the control of anthracnose disease, a field experiment was conducted with the fungicides, which were found effective under laboratory screening to test the relative field efficacy of different fungicides in controlling anthracnose disease of mungbean.

The field experiment was conducted at College Agronomy Farm, B. A. College of Agriculture, Anand Agricultural University, Anand during *kharif* 2014-15 in Randomized Block Design (RBD) with six treatments along with three replications using susceptible variety GM 4. All the recommended agronomical practices were followed during experimentation. The crop was sown with 45×15 cm spacing having gross plot size 3.0 x 1.5 mt. in the second week of July 2014. Seeds were treated with Thiram 75 SD, 3 g/kg seeds, prior to sowing. Two foliar sprays of fungicides were given, first at the initiation of the disease and second at 15 days after first spray. The intensity of anthracnose was recorded after seven days of each spray. Ten plants were selected randomly and labeled from each plot for scoring the disease intensity. The disease intensity was recorded by observing three trifoliate leaves, one each from basal, middle and upper portion of the plants. These selected plants were graded using 0-9 disease rating scale on the basis of percentage area of leaves infected by the pathogen (Mayee and Datar, 1986). The seed yield (kg/ha) was also recorded.

Disease Rating Scale for mungbean anthracnose

Scale	Description
0	No symptoms on leaves.
1	Small size lesions covering 1% or less of leaf area.
3	Small size lesions covering 1-10% of leaf area.
5	Lesions size big but not coalescing, covering 11-25% of the leaf area.
7	Lesions on leaves covering 26-50% of leaf area. Cankers on stem and pod infection.
9	Lesions on leaves covering 51% or more of leaf area. Defoliation of leaves, deep cankers on stem and pods, blighting of plant occurs.

The percent disease intensity (PDI) was calculated by using the formula given by Mayee and Datar (1986).

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{No. of leaves observed x Maximum ratings (9)}} x\,100$$

RESULTS AND DISCUSSION

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

In vitro evaluation of fungicides against Colletotrichum gloeosporioides

The screening of fungicides *in vitro* is very useful as it provides preliminary information about the bio-efficacy of fungicides against the pathogen. It saves time, labour, cost of testing costly fungicides in the field, and useful in planning of chemical control of the disease in field.

The observations on mycelial growth and percent growth inhibition (PGI) recorded after fifteen days of incubation are presented in Table 1. All the fungicides screened were found significantly superior in inhibiting the mycelial growth of *C. gloeosporioides* over control. Propiconazole (1000, 1500 ppm), carbendazim (12%) + mancozeb (63%) (1000, 2000, 2500 ppm) and propineb (2000 and 2500 ppm) completely inhibited the mycelial growth and thus appeared significantly superior over rest of the treatments. The next best treatments in order of merit in inhibiting mycelial growth was azoxystrobin (18.2%) + difenconazole (11.4%) at 500, 1000 and 1500 ppm concentration (84.90, 93.70 and 99.63%) which was at par with trifloxystrobin (25%) + tebuconazole (50%) at 500, 1000 and 1500 ppm concentration (87.08, 90.37 and 94.44%). The next best treatment was difenconazole at 500, 1000 and 1500 ppm concentration (81.38, 86.66 and 92.96%) followed by hexaconazole at 500, 1000 and 1500

Table 1. *In vitro* screening of fungicides against *Colletotrichum gloeosporioides*

S. No	Fungicides	Conc. (ppm)	Av. mycelial growth (mm)	Per cent Growth Inhibition(PGI)
1	Kresoxim methyl(45 SC)	500	65.00	27.77
		1000	61.66	31.48
		1500	56.66	37.03
2	Azoxystrobin (18.2%) +	500	13.58	84.90
	Difenconazole (11.4%) (29.6 SC)	1000	5.66	93.70
		1500	0.33	99.63
3	Hexaconazole(5 EC)	500	51.34	42.95
		1000	48.00	46.66
		1500	42.33	52.96
4	Propiconazole(25 EC)	500	1.33	98.51
		1000	0.00	100
		1500	0.00	100
5	Difenconazole(25 EC)	500	16.75	81.38
		1000	12.00	86.66
		1500	6.33	92.96
6	Trifloxystrobin (25%) +	500	11.62	87.08
	Tebuconazole (50%) (75 WG)	1000	8.66	90.37
		1500	5.00	94.44
7	Chlorothalonil(75 WP)	1000	64.00	28.88
		2000	60.00	33.33
		2500	53.66	40.37
8	Carbendazim (12%) +	1000	0.00	100
	Mancozeb (63%)(75 WP)	2000	0.00	100
		2500	0.00	100
9	Propineb(70 WP)	1000	1.00	98.88
		2000	0.00	100
		2500	0.00	100
10	Control	-	90.00	-
	S.Em.±	-	1.08	-
	C.D. at 5%	-	3.06	-

Table 2. Effect of fungicides on anthracnose disease intensity and yield of mungbean

Tr. No.	Fungicides	Fungicide formu-lation	Dose		Disease intensity (%)	Percent disease control	Seed yield (kg/ha)	Percent increase yield
			Concentration of a.i. (%)	Formulation (ml or g/ 10 liter of water)				
T_1	Azoxystrobin (18.2%) + Difenconazole (11.4%)	29.6 SC	0.03	10.0 ml	17.00	66.00	627	32.21
T_2	Propiconazole	25 EC	0.025	10.0 ml	21.16	57.66	522	18.58
T_3	Trifloxystrobin (25%) + Tebuconazole (50%)	75 WG	0.075	10.0 g	8.43	83.13	856	50.35
T_4	Carbendazim (12%) + Mancozeb (63%)	75 WP	0.075	10.0 g	11.83	76.33	779	45.44
T_5	Propineb	70 WP	0.10	15.0 g	22.66	54.66	515	17.47
T_6	Control (Untreated check)	-	-	-	55.00	-	425	-
	S.Em.±	-	-	-	1.23	-	62.87	-
	C.D. at 5%	-	-	-	3.86	-	140.00	-

Note: Seeds treated with Thiram 75 SD, 3 g/kg seeds, prior to sowing, except control treatment.

ppm concentration with 42.95, 46.66 and 52.96 per cent growth inhibition, respectively.

The next best were chlorothalonil at 1000, 2000 and 2500 ppm concentration with 28.88, 33.33 and 40.37 per cent and kresoxim methyl at 500, 1000 and 1500 concentration with 27.77, 31.48 and 37.03 per cent growth inhibition, respectively.

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 *C. gloeosporioides*.

Propiconazole is fungicide 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 slows or stops the growth of fungus and preventing further infection and/or invasion of host tissues.

The compound product *i.e.* carbendazim (12%) + mancozeb (63%) was also superior among all other treatments. Mancozeb acts by contact action. It is fungitoxic when exposed to air, converted into an isothiocyanate, which inactivates the sulphahydral groups of enzymes in fungi, causing disturbance in fungal enzyme functioning. Carbendazim having systemic activity acts by inhibiting fungal mitotic microtubule formation resulting in inhibiting the development of fungal germtube and growth of mycelium.

Shovan *et al*. (2008) evaluated five fungicides *viz*., propiconazole 25 EC, carboxin-200, iprodione 50 WP, mancozeb and cupravit at 100, 200 and 400 ppm concentration for their efficacy against *C. dematium* causing anthracnose of soybean and found complete inhibition with propiconazole 25 EC at all the tested concentration. Tasiwal *et al.* (2009) revealed that carbendazim at three different concentration (0.05, 0.1 and 0.15 %) found effective among all the tested chemicals in inhibiting mycelial growth of *C. gloeosporioides*.

Jagtap *et al*. (2013) screened nine fungicides under *in vitro* against *C. truncatum*, incitant of anthracnose/ pod blight of soybean and found least mean colony diameter (7.52 mm) and highest inhibition (91.63%) of mycelial growth with carbendazim over untreated control, followed by mancozeb, which recorded mean colony diameter of 10.38 mm and mycelial growth inhibition of 88.45

per cent. Thus, the results of earlier workers are also in line with the results obtained in the present investigations.

Management of anthracnose of mungbean through fungicides under field conditions

The data presented in Table 2 revealed that all the treatments significantly reduced the disease intensity as compared to control. Among them, trifloxystrobin (25%) + tebuconazole (50%), 0.075% was found significantly superior over the rest of treatments showing minimum (8.43%) disease intensity with maximum (83.13%) disease control (Plate 12).

The next best treatment was carbendazim (12%) + mancozeb (63%), 0.075% showed disease intensity of 11.83 per cent followed by azoxystrobin (18.2) + difenconazole (11.4%), 0.03% showed disease intensity of 17.00 per cent. Treatment of propiconazole, 0.025% and propineb, 0.1% were found mediocre with disease intensity of 21.16 and 22.66 per cent, respectively.

Regarding seed yield, the treatment effects were significant. Trifloxystrobin + tebuconazole, 0.075% was found significantly superior in obtaining highest yield (856 kg/ha). The next best treatment was carbendazim + mancozeb, 0.075% (779 kg/ha) followed by azoxystrobin + difenconazole, 0.03% (627 kg/ha). The rest of the treatment, propiconazole, 0.025% (522 kg/ha) which was at par with propineb, 0.1% (515 kg/ha) were moderately effective over control treatment (425 kg/ha).

In the present study, trifloxystrobin + tebuconazole, 0.075% was found significantly superior in reducing anthracnose disease and achieving the higher yield over rest of the treatments. The next effective treatment was carbendazim + mancozeb, 0.075% followed by azoxystrobin + difenconazole, 0.03%.

The more or less similar result was found by Ingle *et al*. (2014). They advocated two sprays of tebuconazole (0.1%) at 25 days interval for management of Colletotrichum leaf spot of soybean followed by propiconazole (0.1%), hexaconazole (0.05%) and azoxystrobin (0.1%). Ahiladevi and Prakasam (2013) studied the bioefficacy of azoxystrobin 25 SC at the rate of 100, 125, and 150 g a.i./ha against chilli anthracnose disease under field conditions and found maximum control of disease with 150 g a.i./ha.

Jagtap *et al*. (2013) conducted experiment to control *C. truncatum* causing anthracnose/ pod blight of soybean with fungicides and found carbandazim, 0.1% recorded minimum disease intensity (19.55%) and pod infection (9.63%), with highest seed yield (2605 kg/ha) followed by mancozeb, 0.1% which recorded the minimum disease intensity (21.50%) and pod infection (10.78%). Kumbhar and More (2013) studied the efficacy of five fungicides of triazole group *viz*., tebuconazole 25.9 EC, difenconazole 25 EC, hexaconazole 5 SC, tricyclazole 75 WP and propiconazole 25 EC against fruit rot disease of chilli. Fungicide, tebuconazole appeared the most effective among the fungicides tested, with reduction in fruit rot incidence and intensity to the tune of 69.96% and 73.56%, respectively over unsprayed control.

The new generation fungicides *i.e*. strobilurins (*i.e*. azoxystrobin and trifloxystrobin) are mostly contact fungicides as they absorbed into cuticle and not transported any further. The strobilurins act by inhibiting mitochondrial respiration of fungi. One of the key reasons for success of strobilurins that it gives a control of fungi from all four groups of plant pathogens *i.e*. *Ascomycota*, *Basidiomycota*, *Deuteromycota* and *Oomycota*.

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