

Original Research Article

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In vitro Efficacy of Non Systemic and Systemic Fungicides against *Macrophomina phaseolina* causing Charcoal Rot of Soybean

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Different non systemic and systemic fungicides are tested at four concentrations under *in vitro* over *Macrophomina phaseolina* causing charcoal rot of soybean. Among seven non systemic fungicides, mancozeb 75% WP and propineb 70% WP were found to be the most effective fungicides with cent per cent mean mycelial inhibition at all concentrations (1000, 1500, 2000 and 2500 ppm) tested. While, copper hydroxide 77% WP and copper oxychloride 50% WP were found as the least effective with 8.14 and 13.09 per cent mean mycelial inhibition, respectively. Among seven systemic fungicides, carbendazim 50% WP was significantly superior over rest of the treatments with mean mycelial growth inhibition of 82.98 per cent at 100, 250, 500 and 1000 ppm concentrations and fosetyl-Al 80% WP was found to be the least effective fungicide among all with the mean mycelial inhibition of 44.58 per cent. The sclerotial formation in all agrochemicals was found negatively correlated with the inhibition of growth.

Introduction

Soybean (*Glycine max* L.), a legume of commercial importance at both national and global market belongs to family Leguminaceae and sub-family Papilionoideae (Fabaceae). It can enrich the soil with nitrogen because of its N-fixing ability. Soybean is known as the ‘golden bean’ and ‘miracle crop’ because it contains about 43 per cent of good quality protein, 21 per cent carbohydrates, 20 per cent oil content, 5 per cent minerals, 8 per cent moisture, 20 per cent fat, 4 per cent fiber and reasonable amounts of

vitamins (Gautam *et al.*, 2018). USA, Brazil, Argentina, China, and India contribute about 89 per cent of the total soybean production in the world. In India, soybean oil is used for cooking and soya products are more famous due to the high protein content and also have high export value.

Soybean is subjected to many diseases caused by fungi, bacteria, viruses, mycoplasma and nematodes (Sweets, 2008). Among the different soil borne pathogen of soybean, *Macrophomina phaseolina* is an important fungus that causes charcoal rot having broad

host range like soybean, common bean, mung bean, sorghum, maize, cotton, peanut, sesame, cowpea, chickpea and cluster bean producing the symptoms of dry root rot, dry weather wilt, ashy stem blight and seedling blight (Su *et al.*, 2001). It has also been observed in Saurashtra region of Gujarat specifically during *kharif* season.

The pathogen causing charcoal rot may infect plants at any developmental stage. At seedling stage, hypocotyls have developed brown lesions (Wegulo *et al.*, 2000). Symptoms of infected plant shown with yellow coloured leaves prior to wilting, reddish brown discolouration of vascular tissues, dark brown to black colour at collar region followed by dried and death of plants. Due to similar symptom of pith tissue discoloration, charcoal rot may be confused with brown stem rot. However, pith tissue discoloration in plants with charcoal rot is limited to the lower part of the soybean stem, often no more than the fifth node, while the discoloration due to brown stem rot can reach higher portion of the stem. In severe cases, the upper one-third of the pods may have flat pods without seeds. Disease and symptom development is optimum under hot and dry weather which favours the disease development.

Impact on economic yield due to charcoal rot of soybean is nearly 35-40 thousand metric tonnes in India (Wrather *et al.*, 2010). To reduce the severity of yield loss and disease incidence, different systemic and non systemic fungicides were tested against *Macrophomina phaseolina*. Similar research was done by Salunke *et al.*, 2008, Suryawanshi *et al.*, 2008, Dinesh *et al.*, 2017.

Materials and Methods

Isolation and pathogenicity

Infected roots of soybean plant showing typical charcoal rot symptom were isolated

and followed by multiplication of pathogen by using tissue isolation method. The pathogenicity was proved with artificial inoculation of pathogen stated by Rayatpanah *et al.*, 2012 and Manoj, 2018.

In vitro evaluation of fungicides

Mycelial growth inhibition under different concentration of non systemic and systemic fungicides were tested against *M. phaseolina* under *in vitro* condition by employing poisoned food technique of Bagchi and Das (1968) using Potato Dextrose Agar (PDA) as a culture medium. The stock solution of each fungicide has been prepared with 100000 ppm concentration and the required concentration of each fungicide has been taken and mixed with measured quantity of autoclaved PDA medium before solidification in conical flasks respectively with the help of micropipette and then media was poured into sterilized Petri plates (90 mm dia.) in equal quantity (20 ml per Petri plate) to form a uniform layer.

After solidification the plates were inoculated with an actively growing fungal mycelial disc of 4 mm diameter from the multiplied pure culture plates was transferred under aseptic conditions over the solidified PDA medium. Fungal mycelial disc was inoculated in Petri plates containing PDA medium without fungicides were served as control. Then Petri plates were incubated at 28±2°C for 7 days and observations were recorded based on radial growth of mycelium in treated and control plates. Completely Randomized Block Design with factorial concept was used for analyzing the data. The per cent inhibition of the fungus in each treatment was calculated by using following formula (Vincent, 1947). The relative degree of sclerotial formation was recorded in Table 1.

$$I = \frac{C-T}{C} \times 100$$

Where,

$$\begin{aligned} I &= \text{Percent inhibition of mycelial growth} \\ C &= \text{Radial growth of fungus in control (mm)} \\ T &= \text{Radial growth of fungus in treatment (mm)} \end{aligned}$$

Sclerotial formations were counted in fungal culture suspension under microscope at low power (10x). The fungal culture suspension was prepared by vigorously shaking the 4 mm mycelial disc of the fungus in 10 ml sterilized distilled water after 15 days of incubation.

Results and Discussion

The mycelial growth inhibition and sclerotial formation of *M. phaseolina* causing charcoal rot of soybean was tested with different non systemic and systemic fungicides at various concentrations under *in vitro* condition were recorded in Table 2 and 3.

The efficacy of seven non-systemic fungicides were tested at various concentrations of 1000, 1500, 2000 and 2500 ppm (Table 2). Among them mancozeb 75% WP and propineb 70% WP gave 100 per cent mycelial growth inhibition at all concentrations which were found to superior over the rest of the non systemic fungicides (Fig. 1) followed by Zineb 75% WP with 79.67 per cent mycelial growth inhibition at 1000 ppm and 100 per cent mycelial growth inhibition at 1500, 2000 and 2500 ppm

concentrations respectively.

More than 70 per cent of mycelial inhibition was observed with thiram 75% WP at all concentrations tested. While copper hydroxide 77% WP was found the least effective with 8.14 per cent mean mycelial inhibition.

The production of sclerotia was found to be completely inhibited at all the concentrations tested with mancozeb 75 % WP and propineb 70% WP followed by zineb 75% WP and thiram 75% WP at 1500, 2000 and 2500 ppm concentrations respectively. The abundant sclerotial formation was observed with copper hydroxide 77% WP and copper oxychloride 50% WP at 1000, 1500 and 2000 ppm concentrations.

While chlorothalonil 75% WP at 1500 and 2000 ppm showed moderate sclerotial formation. Present investigation revealed that mancozeb and propineb which were inhibitory to mycelia could significantly halt the sclerotial formation.

The present results were also found in similar tune with Manoj (2018), Parmaret *et al.*, (2017), Suryawanshi *et al.*, (2008) and Salunke *et al.*, (2008). Dinesh *et al.* (2017) and Magar *et al.* (2011), where mancozeb was reported to inhibit charcoal rot pathogen at all the concentrations tested.

Table.1 Relative degree of sclerotial formation

No. of sclerotia per microscopic field (10X)	Grade	Sign
0	Absent	-
1-4	Scanty	+
5-8	Moderate	++
9-15	Good	+++
>15	Abundant	++++

Table 2 Effect of different systemic fungicides on mycelial growth inhibition and sclerotial formation of *M. phaseolina* under *in vitro* condition

Fungicides	Concentration (ppm) and Sclerotial formation				Mean fungicide inhibition (%)
	1000	1500	2000	2500	
Chlorothalonil 75% WP	43.30 (47.04)	45.11 (50.18)	47.90 (55.05)	50.83 (60.11)	46.79 (53.11)
	+++	++	++	+	
Copper hydroxide 77% WP	12.00 (4.32)	16.90 (8.45)	18.02 (9.57)	19.40 (11.03)	16.58 (8.14)
	++++	++++	++++	+++	
Copper oxychloride 50% WP	17.45 (8.99)	20.14 (11.85)	22.45 (14.58)	24.81 (17.60)	21.21 (13.09)
	++++	++++	++++	+++	
Mancozeb 75% WP	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)
	-	-	-	-	
Propineb 70% WP	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)
	-	-	-	-	
Thiram 75%WP	60.62 (75.93)	63.48 (80.06)	65.23 (82.44)	67.56 (85.43)	64.22 (81.09)
	+	-	-	-	
Zineb 75 %WP	63.20 (79.67)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	83.30 (98.64)
	+	-	-	-	
Concentration Mean (%)	53.80 (65.11)	59.37 (74.05)	60.52 (75.77)	61.80 (77.67)	
	Fungicide (F)	Conc. (C)	F x C		
S.Em. ±	0.65	0.49	1.29		
C. D. at 5%	1.84	1.39	3.68		
C.V. %			3.82		

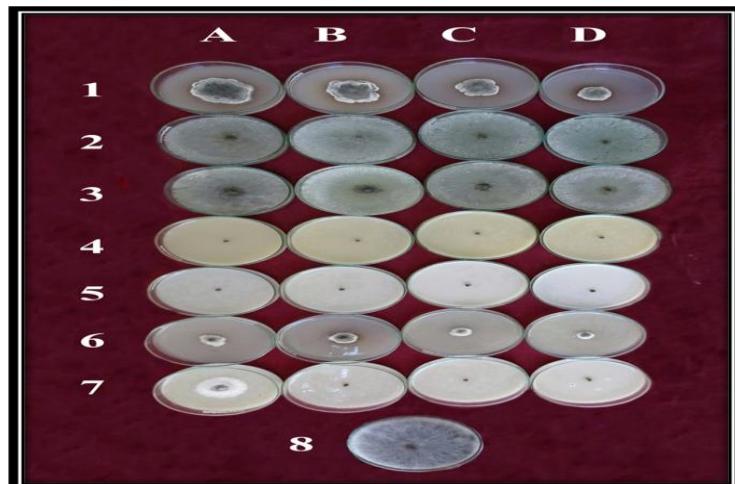
Data were arcsine transformed before analysis; Numerals in parentheses are re-transformed value

Table.3 Effect of different systemic fungicides on mycelial growth inhibition and sclerotial formation of *M. phaseolina* under *in vitro* condition

Fungicides	Concentration (ppm) and Sclerotial formation				Mean fungicide inhibition (%)
	100	250	500	1000	
Difenoconazole 25% EC	53.39 (64.43)	56.14 (68.95)	59.12 (73.66)	61.94 (77.88)	57.65 (71.36)
	++	+	-	-	
Carbendazim 50% WP	57.59 (71.27)	61.03 (76.54)	68.92 (87.06)	75.02 (93.31)	65.64 (82.98)
	-	-	-	-	
Fosetyl-Al 80% WP	32.47 (28.82)	37.47 (37.00)	45.21 (50.37)	52.41 (62.79)	41.89 (44.58)
	+++	+++	++	+	
Azoxystrobin 23% SC	27.98 (22.01)	44.68 (49.44)	51.54 (61.32)	56.22 (69.08)	45.11 (50.18)
	+++	++	+	-	
Picoxystrobin 25% EC	53.07 (63.89)	56.43 (69.42)	63.72 (80.40)	67.60 (85.48)	60.21 (75.31)
	++	+	-	-	
Tebuconazole 2% DS	45.21 (50.37)	52.94 (63.67)	61.88 (77.78)	66.66 (84.31)	56.67 (69.81)
	++	+	-	-	
Thiophanate methyl 70% WP	45.85 (51.48)	52.54 (63.01)	59.78 (74.66)	63.97 (80.74)	55.53 (67.97)
	++	+	-	-	
Concentration Mean (%)	45.08 (50.14)	51.60 (61.42)	58.60 (72.85)	63.40 (79.96)	
	Fungicide (F)	Conc. (C)	F x C		
S.Em. ±	0.60	0.46	1.21		
C. D. at 5%	1.71	1.29	3.42		
C.V. %	3.83				

Data were arcsine transformed before analysis; Numerals in parentheses are re-transformed value

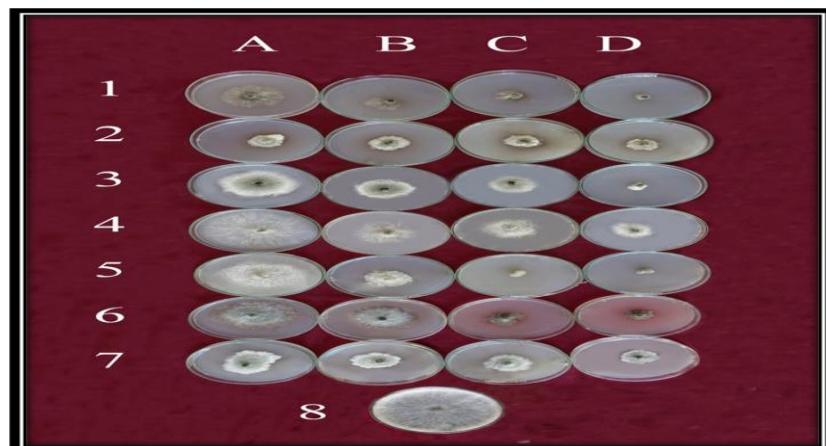
Fig.1 Non-systemic fungicides on mycelial growth inhibition of *M. phaseolina* under *in vitro*



Non-systemic fungicides Concentrations (ppm)

1. Chlorothalonil 75 % WP A. 1000
2. Copper hydroxide 77 % WP B. 1500
3. Copper oxychloride 50 % WP C. 2000
4. Mancozeb 75 % WP D. 2500
5. Propineb 70 % WP
6. Thiram 75 % WP
7. Zineb 75 % WP

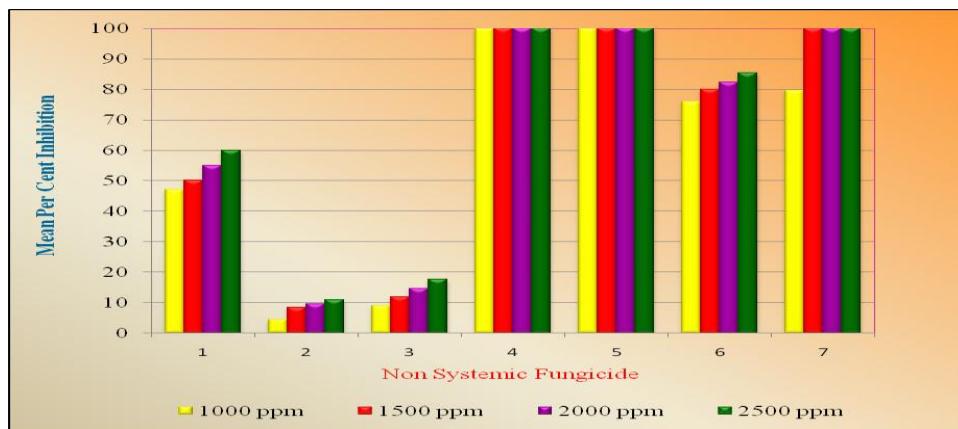
Fig.2 Systemic fungicides on mycelial growth inhibition of *M. phaseolina* under *in vitro*



Systemic fungicides Concentrations (ppm)

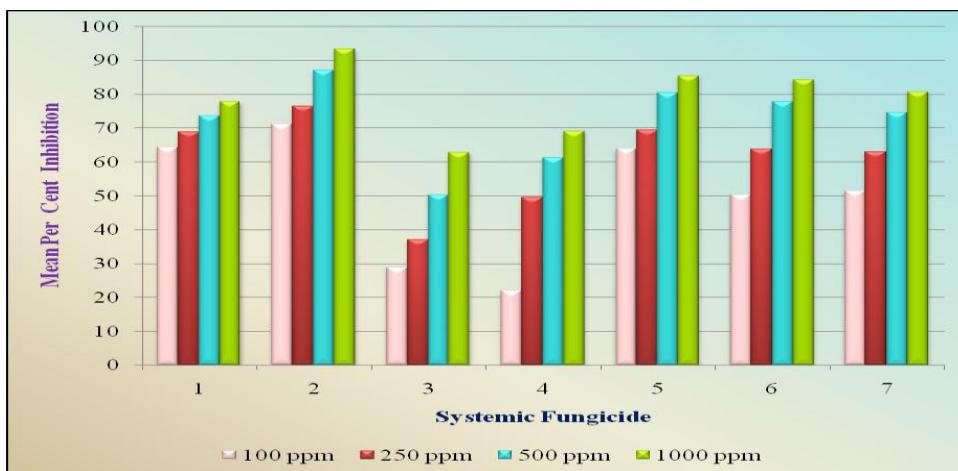
1. Difenoconazole 25 % EC A. 100
2. Carbendazim 50% WP B. 250
3. Fosetyl-Al 80% WP C. 500
4. Azoxystrobin 23 % SC D. 1000
5. Picoxystrobin 25% EC
6. Tebuconazole 2 % DS
7. Thiophanate methyl 70% WP

Graph.1 Effect of non-systemic fungicides on mycelial growth inhibition of *M. phaseolina* under *in vitro* condition



1. Chlorothalonil 75% WP
2. Copper hydroxide 77% WP
3. Copper oxychloride 50% WP
4. Mancozeb 75% WP
5. Propineb 70% WP
6. Thiram 75%WP
7. Zineb 75 %WP

Graph.2 Effect of systemic fungicides on mycelial growth inhibition of *M. phaseolina* under *in vitro* condition



1. Difenoconazole 25 % EC
2. Carbendazim 50% WP
3. Fosetyl-Al 80% WP
4. Azoxystrobin 23 % SC
5. Picoxystrobin 25% EC
6. Tebuconazole 2 % DS
7. Thiophanate methyl 70% WP

Similarly among seven systemic fungicides (Table 3), carbendazim 50% WP found to be best among all treatments and gave 71.27, 76.54, 87.06 and 93.31 per cent inhibition of growth of the fungus at all concentrations (100, 250, 500 and 1000 ppm) respectively as shown in Fig. 2. Picoxystrobin 25% EC (500 and 1000 ppm), tebuconazole 2% DS (1000 ppm) and thiophanate methyl 70% WP (1000 ppm) were shown more than 80 per cent mycelial growth inhibition. Fosetyl-Al 80% WP was found as least effective fungicide with 28.82, 37.00, 50.37 and 62.79 per cent at 100, 250, 500 and 1000 ppm concentrations respectively.

No sclerotial formation was observed at all the tested concentrations of carbendazim 50% WP and for all the treatments at 500 and 1000 ppm except with fosetyl-Al 80% WP and azoxystrobin 23% SC at 500 ppm. However, fosetyl-Al 80% WP at 100 and 250 ppm and azoxystrobin 23% SC at 100 ppm exhibited good sclerotial production.

The results obtained were in agreement with Iqbal and Mukhtar (2020), Ashish and Gohel (2018), Swamy et al., (2018), Dinesh et al., (2017), Arora et al., (2013), Suryawanshi et al., (2008) and Lambhate et al., (2002) who evaluated the efficacy of carbendazim on *M. phaseolina* of various crops.

In conclusion the present study revealed that mancozeb and propineb from non systemic as well as carbendazim from systemic fungicides were quite effective in controlling soybean charcoal rot pathogen.

Along with physical, cultural and biological practices, use of chemical measures with these effective fungicides in alternate application will give better result and will reduce the risk of resistance development in the pathogen.

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