

Research Article

Potential of few fungicides and plant extracts for managing charcoal rot of soybean caused by *Macrophomina phaseolina* (Tassi) Gold. in Madhya Pradesh, India

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Article Info

<https://doi.org/10.31018/jans.v12i3.2335>

Received: July 26, 2020

Revised: August 21, 2020

Accepted: August 24, 2020

How to Cite

Sanjay *et al.* (2020). Potential of few fungicides and plant extracts for managing charcoal rot of soybean caused by *Macrophomina phaseolina* (Tassi) Gold. in Madhya Pradesh, India. *Journal of Applied and Natural Science*, 12(3): 388 - 393. <https://doi.org/10.31018/jans.v12i3.2335>

Abstract

Charcoal rot disease of soybean caused by *Macrophomina phaseolina* is a serious problem in most of the soybean growing area of Madhya Pradesh. In this study, seven plant extracts viz., leaves of *Azadirachta indica*, *Citrus limon*, *Polyalthia longifolia*, *Parthenium hysterophorus* and *Ricinus communis*, bulb of *Allium sativum* and *Allium cepa* and eight fungicides viz., Captan (0.25%), Mancozeb (0.25%), Carbendazim + Mancozeb (25%), Thiophanate Methyl (0.1%), Pyraclostrobin (0.2%), Carbendazim (0.1%) and Blue copper (0.3%) were evaluated for an effective management of charcoal rot of soybean caused by *M. phaseolina* (Tassi) Goid under *in vitro* and *in vivo* condition. Among plant extracts, garlic clove extract was found most effective showing 77.3% growth inhibition and poor microsclerotia formation of *M. phaseolina* by 77.3% followed by parthenium leaf extract (75.2% inhibition) at 15% concentration. Two soil drenching of garlic clove extracts @ 15.0% concentration also found most effective for the management of disease under field condition recorded minimum disease incidence (13.5%) and highest yield (14.6q/ha). Among fungicides, Carbendazim (0.1%) and Thiophanate Methyl (0.1%) showed 100% inhibition of radial growth and microsclerotia production of *M. phaseolina* under *in vitro* condition. Two soil drenching of Carbendazim @ 0.1% found to be most effective for the management of charcoal rot of soybean under field condition showing minimum disease incidence (5.36%) and producing highest yield (16.0 q/ha) followed by Thiophanate Methyl. These results suggested that the toxic effect of Carbendazim and Thiophanate Methyl and *A. sativum* inhibited maximum mycelium growth *in vitro* and provide management of charcoal rot disease under field conditions.

Keywords: Charcoal rot, Fungicide, *M. phaseolina*, Plant extract, Soybean

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] plants are susceptible to root and stem base rots caused by soil pathogens at all growth stages. One of these diseases is charcoal rot of soybean, caused by the polyphagous fungus *Macrophomina phaseolina*. This pathogen infects a wide host range of nearly 500 species in more than 100 families around the world (Reznikov *et al.* 2016). The disease is also a serious problem in most of the soybean growing area of Madhya Pradesh,

India (Amrate *et al.*, 2020). The infection is seen in seedlings and proves to be fatal in most cases. The disease incidence was noticed in all the locations surveyed with a range from 16.91 to 26.76%. Although considerable research related to the biology, ecology and management of pathogen has been conducted, it continues to cause economic losses in soybean. No single management is effective under farmer's field (Reznikov *et al.*, 2016). Keeping in view, the above-mentioned facts an investigation was undertaken to

assess the efficiency of fungicides, and plant extracts for management of charcoal rot of soybean.

MATERIALS AND METHODS

Isolation and identification of pathogen: The causal organism was isolated from infected portion of the plants collected from Soybean experimental field, Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya- Jabalpur and was purified by single culture method, and pathogenicity of the pathogen was confirmed. The pathogen was identified by comparing characters with the description of the standard reference (Bernett, 1980) and its identity was confirmed as *M. phaseolina* and maintained from time to time by transfer to potato dextrose agar slants.

Evaluation of plant extract: In order to find out the efficacy of different plant extract against *M. phaseolina*, seven plant extracts viz., leaves of *Azadirachta indica*, *Citrus limon*, *Polyalthia longifolia*, *Parthenium hysterophorus* and *Ricinus communis*, bulb of *Allium sativum* and *Allium cepa* were used. Fresh leaves/ bulbs were collected and washed carefully in clean water. 100 g of each washed plant material was ground in Pestle and Mortar by adding equal amount (100ml) of sterilized water (1:1V/W) and heated at 80°C for 10 minutes in hot water bath. The materials were filtered through double-layered muslin cloth followed by filtering through sterilized What man No. 1 filter paper and the filtrate so obtained formed the standard plant extract solution (100%). The stock solutions 5, 10 and 15 % concentration were made by adding 95, 90 and 85 ml of sterilized potato dextrose agar media to obtain 5, 10 and 15 % concentration of plant extract. The inhibitory effect of plant extract on radial growth of *M. phaseolina* was studied at 5, 10 and 15 % concentrations were used by applying poison food techniques under *in vitro* condition. Eight treatments having three replications were maintained. Five mm discs of 7 days old culture of *M. phaseolina* were cut with a sterilized cork borer and placed in the centre of plant extract amended petri plates. The control petriplates having PDA alone were inoculated in the same manner. These petriplates were incubated at 25±2°C. The observations were recorded on radial growth at 96 hrs of incubation.

The seven plants extracts were further evaluated at 15 % concentration as soil drenching on charcoal rot of soybean under field condition. The experiment was conducted in soybean breeding Farm JNKVV Jabalpur (M.P.) during 2016-17 in Randomized Block Design with three replications using variety JS 335 with a row spacing of 45 x 15 cm and plot size of 4 x 1.5 m². Standard plant extracts were prepared in cold water as per the method described above. The extract thus obtained was diluted to 15 % by water and drenched

around the root zone in the soil after disease initiation. Plot drenched with water only served as check. Two consecutive drenched at an interval of 15 days were given. Observations on disease incidence and % disease control were recorded 120 days after sowing. The yields were recorded after harvest of the crop. Data were analyzed statistically.

Evaluation of fungicides: Eight fungicides viz., Captan (0.25%), Mancozeb (0.25%), Carbendazim + Mancozeb (25%), Thiophanate Methyl (0.1%), Pyraclostrobin (0.2%), Carbendazim (0.1%) and Blue copper (0.3%) and control having PDA alone were evaluated against *M. phaseolina* by "Poison Food Techniques" as described by Morton and Straube (1955). The required quantity of each fungicide was thoroughly mixed with 100 ml of sterilized PDA medium contained in 200 ml flakes. It was then mixed thoroughly and was poured in petriplates and allowed to solidify. Each treatment replicated thrice. The control petriplates having PDA alone were inoculated in the same manner. Five mm diameter of pathogen colony from seven days old culture of *M. phaseolina* was cut with the help of cork borer and inoculated at the center in each Petridish. The inoculated Petri-dishes were incubated at 25±2°C. Observations were recorded on radial growth at 96 hrs of incubation.

Fungicides were further evaluated as soil drenching under field condition on charcoal rot of soybean under field conditions. The experiment was conducted in soybean breeding Farm JNKVV Jabalpur (M.P.) during 2016-17 in Randomized Block Design (RBD) with three replications using variety JS 335 with row spacing of 45 x 15 cm and plot size of 4 x 1.5 m². The date of sowing was 23 June 2016. Standard fungicides solutions were prepared and drenched around the root zone of the crop after disease initiation. Plot drenched with water only served as check. Two consecutive drenched at an interval of 15 days were given. Observations on disease incidence and % disease control were observed 120 days after sowing. The yields were recorded after harvest of the crop. Data were analyzed statistically using R-Statistics program.

RESULTS AND DISCUSSION

Effect of plant extracts: Out of the seven plants extracts viz., *A. indica*, *C. limon*, *P. longifolia*, *P. hysterophorus* and *R. communis*, *A. sativum* and *A. cepa* evaluated, *A. sativum* clove extract was most effective as they inhibited the growth and poor sclerotia formation of *M. phaseolina* by 77.3 % followed by *P. hysterophorus* leaf extract (75.2%) at 15 % concentration (Table-1). They were also found effective at 10 % concentration also as they produced growth inhibition ranging respectively, between 63.6 to 71.3%. *A. indica* leaf, *P. longifolia* leaf and *R. communis* leaf were also

Table 1. Effect of plant extracts on radial growth and microsclerotia formation of *M. phaseolina*.

S.No.	Name of plant extracts	Local name	Parts used	Radial growth (mm) of target pathogen (4 DAI)*			Percent growth inhibition			No. of microsclerotia formed after 21 days
				5%	10%	15%	5%	10%	15%	
1	<i>Allium sativum</i>	Garlic	Clove	40.7	25.8	20.4	54.7	71.3	77.3	Poor
2	<i>Parthenium hysterophorus</i>	Parthenium	Leaf	54.2	32.7	22.3	39.7	63.6	75.2	Poor
3	<i>Polyalthia longifolia</i>	Ashok	Leaf	87.7	79.1	34.8	2.5	12.1	61.3	Fair
4	<i>Citrus limon</i>	Citrus	Leaf	61.4	52.5	48.5	31.8	41.6	46.1	Fair
5	<i>Azadirachta indica</i>	Neem	Leaf	53.3	40.7	31.1	40.7	54.7	65.4	Poor
6	<i>Ricinus communis</i>	Castor	Leaf	55.3	51.7	37.7	38.5	42.5	58.1	Fair
7	<i>Allium cepa</i>	Onion	Bulb	76.7	60.6	52.8	14.7	32.6	41.3	Fair
8	Control (only PDA)	-	-	90.0	90.0	90.0	00.0	00.0	00.0	Excellent
	SE (m)			0.48	0.56	0.54				
	CD (0.05)			1.20	1.30	1.27				

Average of 3 replications

Table 2. Effect of plant extracts on disease incidence and yield of soybean.

S.No.	Plant extracts	Doses (%)	Disease incidence (%)	Percent disease control over check (%)	Yield (q/ha)	% increase in yield over check
1	<i>Allium sativum</i>	15	13.5	65.2	14.6	56.4
2	<i>Azadirachta indica</i>	15	16.4	57.2	12.6	35.0
3	<i>Polyalthia longifolia</i>	15	21.6	43.6	10.9	17.1
4	<i>Parthenium hysterophorus</i>	15	17.4	55.6	11.9	27.5
5	<i>Ricinus communis</i>	15	33.7	12.0	10.3	10.3
6	<i>Citrus limon</i>	15	35.0	8.6	9.6	3.5
7	<i>Allium cepa</i>	15	23.5	38.6	11.0	17.8
8	Control (only water)	--	38.3	--	9.3	--
	SE (m)		0.79		0.52	
	CD (0.05)		1.90		1.26	

Average of 3 replications

found very promising as they produced only 65.4, 61.3 and 58.1 % inhibition, respectively, at 15 % concentration. At lower concentrations, i.e. 5 and 10 % growth inhibition was due to *A. indica* leaf, *P. longifolia* leaf and *R. communis* leaf extract, *A. cepa* bulb and *C. limon* leaf extract were less than 55%. No plant extracts were found effective at 5 % concentration. The poor microsclerotia formation was observed in *Allium sativum* and *Parthenium hysterophorus* while fair to abundant microsclerotia formation was observed in other treatment. The result of the present study are in accordance with the findings of Savaliya et al., (2015), Hussain et al., (2014) and Chaudhary et al. (2019). Hussain et al. (2014) reported that *A. sativum* inhibited

maximum mycelium growth followed by *P. hysterophorus* at all concentrations against *M. phaseolina in vitro*. The inhibition of fungal growth may be due to the presence of antifungal compounds such as glycoside, steroids, saponins, medicagenic acid, tannins, terpenoids and phobol esters (Anusha, 2003).

All the plant extracts at 15 % concentration tested under field condition significantly ($p=0.05$) reduced the disease incidence when compared with the check (Table 2). Two soil drenching of *Allium sativum* extracts @ 15 % showed minimum disease incidence (13.5%) and maximum yield (14.6 q/ha) followed by *Azadirachta indica* extract (16.4%; 12.6 q/ha) as compared to 38.3% disease incidence and 9.3 q/ha yield

Table 3. Effect of fungicides on radial growth and microsclerotia formation of *M. phaseolina*.

S. No	Fungicides	Doses (gm/liter)	Radial growth (mm) after 72 hrs*	Growth inhibition over control (%)	Radial growth (mm) after 96 hrs	Growth inhibition over control (%)	No. of microsclerotia formed after 21 days
1	Pyraclostrobin	0.2	30.9	63.6	39.6	55.9	Fair
2	Mancozeb	2.5	44.4	47.7	65.5	27.2	Fair
3	Captan	2.5	10.0	88.2	26.0	71.1	Poor
4	Copper oxychloride	3.0	76.4	10.0	85.6	4.8	Excellent
5	Carbendazim	1.0	0.0	100.0	0.0	100.0	Nil
6	Thiophanate Methyl	1.0	0.0	100.0	0.0	100.0	Nil
7	Carbendazim + Mancozeb	2.5	77.7	8.4	88.0	2.2	Excellent
8	Control (Only PDA)	--	85.0	-	90.0	-	Excellent
	SE (m)		0.48		0.40		
	CD (0.05)		1.20		1.29		

Average of 3 replications

Table 4. Effect of soil drenching of fungicides on disease incidence and yield of soybean.

S. No	Fungicides	Doses (gm/ lit)	Disease incidence	Percent inhibition over control	Yield (q/ha)	% increase in yield over check
1	Pyraclostrobin	0.2	15.6	63.6	13.0	59.9
2	Mancozeb	2.5	17.8	58.4	11.2	38.3
3	Captan	2.5	10.0	76.6	14.0	72.2
4	Copper oxychloride	3.0	29.0	31.7	9.9	22.0
5	Carbendazim	1.0	5.3	87.5	16.0	96.8
6	Thiophanate methyl	1.0	6.3	85.2	15.0	85.1
7	Carbendazim + Mancozeb	2.5	23.4	45.4	10.8	32.8
8	Control (Plain water)	--	42.96	--	8.1	--
	SE (m)		0.68		0.10	
	CD (0.05)		1.31		1.44	-

Average of 3 replications

in control (Table 2). Datar (1999) reported that clove extract of *A. sativum* and bulb of *A. cepa* was found most effective in inhibiting mycelial growth of *M. Phaseolina*. Kane et al., (2002) reported that crude extract of a clove of *A. sativum* effective in inhibiting the mycelial growth of the *M. Phaseolina* to the extent of a cent %. Dubey and Dwivedi (1991) found fungi toxic properties of clove extracts of *A. sativum* against vegetative growth and sclerotial viability of *M. phaseolina*. Muzammil et al., (2014) reported that leaf extract of *A. indica* extract was effective to inhibit the

growth of the fungus at 15 % concentration. These results support the present result in which *A. sativum* clove extract and leaf extract of *A. indica* was found most effective in managing the disease under field conditions.

Effect of fungicides: *In vitro* evaluation of fungicides provides useful preliminary information regarding its efficacy against a pathogen within the shortest period of time and therefore serves as a guide for further field testing. In the present study among the fungicides viz., Captan, Mancozeb, Carbendazim + Mancozeb, Thi-

ophanate Methyl, Pyraclostrobin, Carbendazim and Blue copper evaluated, Carbendazim and Thiophanate methyl were found best fungicide which completely inhibited the radial growth and sclerotia formation of *M. phaseolina* (Table 3). Captan, Pyraclostrobin and Mancozeb were second next in order of toxicity resulting, respectively 71.11, 55.93 and 27.22 % inhibition of radial growth. Copper oxychloride, Carbendazim + Mancozeb were not found effective in inhibiting the growth and microsclerotia production of *M. phaseolina*. Similar findings have been observed by Konde et al. (2008), Ray & Kumar (2008), Shovan et al. (2008), Mallesh and Narendrappa (2008), Ramesh (2009), Muzammil et al. (2014), and Kumar et al. (2019) against *Macrophomina* spp. Most of them reported that Carbendazim was effective in inhibiting mycelia growth of *M. phaseolina*. It was concluded from the Table-3 that Carbendazim and Thiophanate methyl significantly ($p=0.05$) inhibited the mycelia growth of *M. phaseolina*. The mechanism of fungicide is binding the β -tubulin polymer of pathogens which are responsible for nuclear division and directly stop the microtubulin activity. Carbendazim directly stops tubulin activity which is important for the growth of pathogens. All the fungicides tested reduced the disease incidence when compared with a check under field condition. Two soils drenching of Carbendazim @ 0.1% showed minimum disease incidence of 5.36 % and highest yield (16.0 q/ha) followed by Thiophanate Methyl (6.34%, 15.05 q/ha) (Table 4). Soil drenched with Captan, Pyraclostrobin, Mancozeb and Carbendazim + Mancozeb were second next in order of toxicity resulting, respectively 10.03, 15.62, 17.87 and 23.43 % incidence. Copper oxychloride was least effective in controlling charcoal rot of soybean. The disease incidence recorded in the check plot was 42.96%. The result of the present study are in accordance to the findings of Konde et al. (2008), Muzammil et al. (2014) and Andrabi et al. (2011) who reported that Carbendazim 50 % WP was more effective @ 2g/kg seed treatment showing least disease intensity (22.6%) of *Rhizoctonia* root rot of soybean as against 49.3 % in control. Kumar et al. (2019) reported that seed treatment with Carbendazim was the most effective against *Rhizoctonia* root rot of soybean.

Conclusion

It was concluded that the charcoal rot disease of soybean is prevalent in all major soybean growing areas like Jabalpur, Rewa, Satna and Indore districts of Madhya Pradesh. Among the plant extracts viz., leaves of *A. indica*, *C. limon*, *P. longifolia*, *P. hysterophorus* and *R. communis*, bulb of *A. sativum* and *A. cepa* and eight fungicides viz., Captan Mancozeb, Carbendazim + Mancozeb, Thiophanate Methyl, Pyra-

clostrobin, Carbendazim and Blue copper evaluated for managing charcoal rot disease of soybean, *Allium sativum* clove extract, Carbendazim and Thiophanate methyl significantly ($p=0.05$) inhibited mycelium growth *in vitro*. The study would be helpful in the management of charcoal rot disease under field conditions.

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