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Studies on Fungicidal Management of Foliar Fungal Pathogens of Cowpea In vitro

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Abstract

Foliar diseases of cowpea include Myrothecium leaf spot (*Myrothecium roridum*), Anthracnose (*Colletotrichum lindemuthianum* and *C. dematium*), stem and leaf blight (*Macrophomina phaseolina*) and web blight (*Rhizoctonia solani*). *Macrophomina phaseolina, Colletotrichum dematium* and *Dreschlera tetramera* were isolated from infected leaves of cowpea collected from Akola, Amravati and Washim districts of Vidarbha region of Maharashtra. Among these fungi, *Macrophomina phaseolina, Myrothecium roridum* and *Colletotrichum dematium* were found pathogenic to cowpea. To know the possible sources of diseases infection, these fungi were used for further studies by leaf, soil and seed inoculation methods. Under leaf inoculation method, symptoms were produced on leaves after 12 to 15 days from the date of inoculation. Maximum infection (80%) was observed in *Colletotrichum dematium.* Seed and soil inoculation method were observed to be more or less similar in both cases. Seed rot was observed in the range of 24 to 43 per cent, pre and post-emergence damping off 17.33 to 34.21 per cent and seedling blight 14.29 to 29.07 per cent. Hence, it is concluded that, seed and soil borne inoculum of these fungi are responsible for causing the diseases in cowpea. Among fungicides, Curzate M 8, mancozeb, penconazole and combination of carbendazim + mancozeb showing 100 per cent mycelia growth inhibition were found effective in reducing the growth of the fungi namely *Macrophomina phaseolina, Myrothecium roridum* and *Colletotrichum dematium.*

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Cowpea, disease, infection, fungal, foliar, pathogen.

Introduction

Cowpea (*Vigna unguiculata* L. Walp) a dicotyledonous plant belonging to the family Fabaceae, genus *Vigna* is of major importance to the livelihood of millions of people in the tropics. Cowpea is one of the important *Kharif* legumes grown in India. It is a warm season crop, well adapted to many areas of the humid tropics and subtropical zones. Cowpea is tolerant to heat and dry conditions, but is intolerant to frost (Davis *et al*., 2000) and it also has the useful ability to fix atmospheric nitrogen through its root nodules. In India, cowpea is grown on about 0.5 million ha with an average productivity of 600 to 750 kg grains ha^{-1} (Ahlawat and Shivakumar, 2005). It is grown throughout India for its long, green vegetable pods, seeds, and foliage for fodder (Mandal *et al*, 2009). Now-a-days foliar diseases of cowpea have become a major constraint for vegetable growers. Sometime the disease(s) cause huge crop loss. During last few years, the weather has undergone a

significant change world over. This had a direct effect on bearing cowpea disease outbreak and consequently the crop losses. In India, the weather had a pronounced effect on development of new virulence of different foliar fungal pathogens. Hence, a suitable disease incidence warning system is very much essential for the vegetable growers in order to reduce the crop losses during period of severe outbreaks. Keeping these views in mind, the present investigation were undertaken with the objectives to study the management strategy through fungicides against fungal foliar diseases of cowpea.

Materials and Methods

An experiment was conducted at Department of Plant Pathology, Post Graduate Institute, Dr. PDKV, Akola to know the sources of disease infection of foliar fungal pathogens of cowpea and management strategy against them through fungicide. Isolation of the fungi was made from diseased material collected from Akola, Amravati and Washim districts of Vidarbha region of Maharashtra. PDA as a basal medium was used for fungal studies.

The usual tissue isolation method was followed for the isolation of fungi causing leaf spot in cowpea. Fungal cultures were observed under microscope for identification and identity of each of the isolates was confirmed by comparing the published literature. Isolates obtained above were purified by following hyphal tip transferred method. Seven days old cultures were used in further studies. Pathogenicity of all fungal isolates obtained earlier was tested on cowpea seedling grown in cage house.

Three weeks old test plant were used for inoculation. After appearance of symptoms on artificially inoculated leaves, re-isolation of the pathogen from infected tissue was done to confirm the identity of the pathogens, so as to prove the Koch's postulates. As regards the pathogenicity test of *Macrophomina phaseolina,* seven days old sclerotial culture of *Rhizoctonia bataticola* was used and pearl millet leaf water agar medium (Chidambaram and Mathur, 1975) was used to induce pycnidial formation to obtain the pycnidiospores of *Macrophomina phaseolina.* Leaf, soil and seed inoculation method was followed to study the sources of disease infection of fungal pathogens of cowpea. Evaluation of fungicides was done by employing poisoned food technique. After incubation period, inhibition of mycelial growth was recorded in mm and per cent mycelia inhibition was calculated by following formula,

$$\text{Per cent inhibition} = \frac{\text{Growth in control plate} - \text{Growth in treatment plate}}{\text{Growth in control plate}} \times 100$$

Results and Discussion

Diseased leaves of cowpea showing small circular to irregular, reddish to brown, blighted and coalesced spots were collected from Akola, Amravati and Washim districts of Vidarbha region of Maharashtra. Fungi were to be associated with these samples and the majority of isolation, yielded the fungi namely *Macrophomina phaseolina, Myrothecium roridum, Colletotrichum dematium* and *Dreschlera tetramera.* It is seen form the Table 1 that, the majority of diseased bits yielded the fungi viz., *Macrophomina phaseolina* (27.28 to 36.36%), *Myrothecium roridum* (23.00 to 30.90%) and *Colletotrichum dematium* (18.20 to 30.30%). However, frequency of *Dreschlera tetramera* was observed in the range of 14.54 to 19.08 per cent. Hence, these isolates were tested in further studies including pathogenicity. The association of these fungi with leaf spot diseases of cowpea and other leguminous host like mungbean, urdbean, gaur, mothbean, chickpea and cucurbits have ben reported by Mishra *et al.,* (1975); Sinha and Khare (1977); Laksman and Menon (1979); Jamaluddin (1979); Das and Kaushik (1981); Zote *et al.,* (1983); Byadgi and Hegde (1985); Demony and Burke (1990); Prameela Devi and Singh (1998); Lenne (1992) and Seebold *et al.,* (2005).

Pathogenicity

Pathogenicity of all the fungal isolates obtained earlier was tested on three weeks old plants of cowpea. After appearance of symptoms, the infected leaves were collected and resorted for re-isolation. Re-isolation of infected leaves yielded the same pathogens and their identity was confirmed and evident that, *Macrophomina phaseolina, Myrothecium roridum* and *Colletotrichum dematium* were pathogenic to cowpea causing leaf spot diseases (Table 2). Hooda and Grover (1982) observed pathogenic behaviour of *Macrophomina phaseolina (Rhizoctonia bataticola)* on the foliage of mungbean and reported 26.70 to 57.50 per cent leaf blight index. Jamaluddin (1979) tested the pathogenicity of *Myrothecium roridum* on cowpea pods by spraying the conidial suspension on uninjured pods which later on covered the whole plants (Sharma and Gupta, 1982;

UditNarain *et al.,* 1982 and Seebold *et al.,* 2005). Similar type of symptoms was recorded under pathogenicity test in which scattered to minute, round to irregular brownish spots were noticed on leaves of inoculated plants. *Colletotrichum dematium* had produced symptoms on cowpea in the form of small circular, oval reddish to brownish spots. Similar symptoms and pathogenicity of *Colletotrichum capsici* were reported on mungbean leaves by Nath *et al.,* (1970) and Mathur and Tyagi (1982) on moth bean.

Source of infection as leaf inoculation method

By leaf inoculation method (Table 3), *Colletotrichum dematium* caused maximum per cent of leaf infection (80%) and also expressed the symptoms earlier than *Macrophomina phaseolina* and *Myrothecium roridum*. Infected leaves due to *Myrothecium roridum* and *Macrophomina phaseolina* were 76 and 72 per cent, respectively. Thus, leaf inoculation of these test fungi plays an important role in causing leaf spot diseases of cowpea. These findings were similar with Laksman and Menon (1979); Jamaluddin (1979); Dass and Kaushik (1981); Sharma and Gupta (1982); Zote *et al.,* (1983) and Seebold *et al.,* (2005).

Source of infection as soil inoculation method

Soil inoculation was done by sick soil method. Three fungal pathogens were inoculated separately in earthen pots containing sterilized soil. Highest seed rot of 40 per cent was noticed by *Colletotrichum dematium.* Maximum pre (34.21%) and post-emergence (23.37%) damping of was observed in *Macrophomina phaseolina* followed by *Myrothecium roridum* (27.10 and 21.38%, respectively). Minimum pre and post emergence damping off i.e., 24.45 and 19.05 per cent, respectively was observed in soil inoculated with *Colletotrichum dematium.* Maximum seedling blight (19.52%) was recorded in *Myrothecium roridum* inoculated soil followed by *Macrophomina phaseolina* (18.42%) and *Colletotrichum dematium* (16.50%) (Table 4). Efficiency of soil inoculation method was observed to be more or less similar also recorded by Baross *et al.,* (1985); Bhardwaj and Singh (1986); Mittal (1994) and Smith *et al.,* (1998).

Source of infection as seed to plant transmission

Seeds of cowpea were inoculated with the cultures of test fungi and sown in earthen pots containing sterilized soil. Observations were recorded on seed rot, pre and post-emergence damping off and seedling blight (Table 5) and also observed for the symptoms developed on seedlings (seedling emergence and per cent seedling showing symptoms) were recorded 10 days after sowing of seeds. From the table 5 it is noticed that, the highest seed rot was observed in *Myrothecium roridum* (43%) followed by *Macrophomina phaseolina* (40%).

Maximum pre and post emergence damping off (24.76 and 23.17%) and seedling blight (21.07%) was observed in seed inoculated with *Colletotrichum dematium*. Per cent emergence was maximum in *Macrophomina phaseolina* inoculated seeds (74%) and minimum in *Myrothecium roridum* (69%). Per cent seedling showing symptoms on cotyledons/ true leaves was maximum in *Macrophomina phaseolina* (28.98%) and minimum in *Colletotrichum dematium* (23.55%).

However, all the three fungal pathogens produced symptoms on cotyledons/true leaves in the range of 23.55 to 28.98 per cent (Table 6). From the infected cotyledons/ true leaves tissue isolation was done on PDA to know the responsible fungi associated with the diseased cotyledons/ true leaves.

Under isolation, *Macrophomina phaseolina (Rhizoctonia bataticola), Myrothecium roridum* and *Colletotrichum dematium* were obtained from the diseased tissue of the seedlings inoculated with the cultures of respective fungi. Thus, the studies indicated that, all three fungi causing leaf spot diseases in cowpea were transmissible from seed to plant. Present findings are in line with Baross *et al.,* (1985); Demony and Burke (1990) and Sharma and Singh (2000).

Evaluation of fungicides by poisoned food technique (Table 7)

Macrophomina phaseolina (Rhizoctonia bataticola)

Curzate M-8, penconazole and combination of carbendazim + mancozeb have shown 100 per cent inhibition of mycelium while more than 90 per cent inhibition of growth of *Macrophomina phaseolina* was recorded with the fungicides *viz*., mancozeb, chlorothalonil, metalaxyl, thiopahanate methyl and propiconazole.

However, 88 per cent inhibition was observed in carbendazim and copper oxychloride. All the fungicides were significantly superior over control in inhibiting the mycelial growth of the fungi.

Table.1 Frequency of the fungal pathogens causing foliar disease in cowpea

S. N.	Locations of the diseased sample collected	No. of bits used for isolation	No. of bits yielded fungi	Per cent occurrence of fungi			
				M. phaseolina	*M. roridum*	*C. dematium*	*D. tetramera*
1	Akola	45	33	27.28	27.27	30.30	15.15
2	Amravati	68	55	36.36	30.90	18.20	14.54
3	Washim	61	52	29.92	23.00	28.00	19.08

Table.2 Infected plants of cowpea in pathogenicity test

S. N.	Isolate	Spore load ml^{-1} for testing	No. of plants tested	No. of plants infected	Days to initiation of symptoms
1	*Macrophomina phaseolina*	5×10^4	10	7	12
2	*Myrothecium roridum*	2×10^6	10	8	15
3	*Colletotrichum dematium*	5×10^4	10	8	10
4	*Dreschlera tetramera*	2×10^6	10	-	-

Table.3 Effect of leaf inoculation of fungal pathogens on cowpea

S. N.	Fungi	No. of leaves inoculated	No. of leaves infected	Per cent leaves infected	Days to initiation of symptoms
1	*Macrophomina phaseolina*	25	18	72.00	13
2	*Myrothecium roridum*	25	19	76.00	15
3	*Colletotrichum dematium*	25	20	80.00	12

Table.4 Pre and post-emergence mortality in fungus inoculated soil

S. N.	Fungi	Number of seeds		Per cent disease observed			
		Sown	Germinated	Seed rot	Pre-emergence damping off	Post-emergence damping off	Seedling blight
1	*Macrophomina phaseolina*	100	76	24.00	34.21	23.37	18.42
2	*Myrothecium roridum*	100	68	32.00	27.10	21.38	19.52
3	*Colletotrichum dematium*	100	80	40.00	24.45	19.05	16.50

Table.5 Effect of seed inoculation of fungal pathogens on cowpea

S. N.	Fungi	Number of seeds		Per cent disease observed			
		Sown	Germinated	Seed rot	Pre-emergence damping off	Post-emergence damping off	Seedling blight
1	*Macrophomina phaseolina*	100	60	40.00	23.34	17.33	19.33
2	*Myrothecium roridum*	100	57	43.00	21.38	21.33	14.29
3	*Colletotrichum dematium*	100	69	31.00	24.76	23.17	21.07

Table.6 Seed to plant transmission of foliar fungal pathogens of cowpea

S. N.	Fungi	No. of seed tested	Per cent emergence	Per cent seedling showing symptoms on cotyledons, true leaves
1	*Macrophomina phaseolina*	100	74.00	28.98
2	*Myrothecium roridum*	100	69.00	25.78
3	*Colletotrichum dematium*	100	72.00	23.55

Table.7 Efficacy of fungicides against foliar fungal pathogens of cowpea by poisoned food technique (*in vitro*)

S.N.	Treatments	Conc. (%)	*Macrophomina phaseolina*		*Myrothecium roridum*		*Colletotrichum dematium*	
			Avg. Colony dia. (mm)	Per cent growth inhibition	Avg. Colony dia. (mm)	Per cent growth inhibition	Avg. Colony dia. (mm)	Per cent growth inhibition
1.	Curzate M-8	0.2	0.00	100.00	0.00	100.00	0.00	100.00
2.	Mancozeb	0.3	1.72	98.04	0.00	100.00	0.00	100.00
3.	Chlorothalonil	0.2	4.11	95.33	3.64	90.80	3.11	96.10
4.	Carbendazim	0.1	10.43	88.15	8.20	79.24	7.33	90.83
5.	Metalaxyl MZ	0.2	1.59	98.19	1.63	95.87	1.54	98.07
6.	Thiophanate methyl	0.2	7.82	91.13	6.77	82.84	6.69	91.62
7.	Copper oxychloride	0.3	10.53	88.04	7.56	80.87	10.30	87.12
8.	Propiconazole	0.1	3.32	96.23	2.87	92.90	2.27	96.52
9.	Penconazole	0.1	0.00	100.00	0.00	100.00	0.00	100.00
10.	Carbendazim + mancozeb	0.2	0.00	100.00	0.00	100.00	0.00	100.00
11.	Control	-	88.17	-	39.55	-	80.00	-
'F' Test			Sig.		Sig.		Sig.	
SE (m)±			0.35		0.23		0.14	
CD (P=0.01)			1.42		0.90		0.50	

Myrothecium roridum

Curzate M-8, mancozeb, penconazole and combination of carbendazim + mancozeb caused 100 per cent inhibition of mycelium whereas, other fungicides *viz*., chlorothalonil, carbendazim, metalaxyl MZ, thiophanate methyl, copper oxychloride and propiconazole showed inhibition of mycelium in the range of 79.24 to 95.87 per cent. All fungicides significantly superior over control.

Colletotrichum dematium

Curzate M-8, mancozeb, penconazole and combination of carbendazim + mancozeb have shown 100 per cent inhibition of mycelium followed by chlorothalonil, carbendazim, metalaxyl MZ, thiophanate methyl, copper oxychloride and propiconazole. All fungicides significantly superior over control.

Efficacy of carbendazim + mancozeb in present study confirms the results of Sudhakar *et al.,* (2002) who have reported 100 per cent inhibition of mycelial growth of *Macrophomina phaseolina.* Efficacy of mancozeb, chlorothalonil, carbendazim, thiophanate methyl, copper oxychloride and combination of carbendazim + mancozeb against *Macrophomina phaseolina, Myrothecium roridum and Colletotrichum dematium* of the present studies are in agreement to those reported by Chauhan and Suryanarayana (1972); Thakur and Singh (1973); Sakhuja and Parshotam (1974); Abdul Hasan and Khan (1979); Jamaluddin (1979); Gupta and Bhardwaj (1980); Das and Kaushik (1981); Farnando *et al.,* (1986); Sharma and Gupta (1986); Thakur and Khare (1990); Bhardwaj and Thakur (1991); Mahanta and Dhal (2000); Dod and Deshmukh (2003) and Giri *et al.,* (2005) who have reported either the suppression of growth or effectiveness of these fungicides in controlling the

disease under field conditions. Perusal of these above results leads to conclude that, the fungicides viz., Curzate M-8 (0.2%), mancozeb (0.3%), combination of carbendazim + mancozeb (0.2%) and penconazole (0.1%) are efficient in restricting the growth of these fungi. *Macrophomina phaseolina, Myrothecium roridum and Colletotrichum dematium* are commonly occurring in Vidarbha region of Maharashtra during growing season of the crop and for management of these fungi, causing diseases, any of the fungicide as above can very well be used under field conditions.

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