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In vitro evaluation of fungicides and organic extracts against *Macrophomina phaseolina* (Tassi) Goid. isolated from pigeonpea [*Cajanus cajan* (L.) Millsp.]

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ABSTRACT

Ten fungicides at three different concentrations were screened *in vitro* by poisoned food technique for evaluating their efficacy against *M. phaseolina*, in which mancozeb (dithane M-45, 75% WP), carbendanzim (bavistin, 50% WP), carbendanzim + mancozeb (sixer, 75%WP) and metalexyl 18% + mancozeb 64% (ridomil, 75% WP) were proved to be highly toxic to the growth of the *M. phaseolina*. Eight different organic extracts were tested against *M. phaseolina* by poisoned food technique with different concentration *in vitro*. All the extracts were inhibitory to *M. phaseolina* significantly lower mycelium growth was recorded in *Neem* cake followed by coconut. Next best were FYM, mustard cake, sesamum cake and vermicompost. While lowest inhibition of mycelial growth of *M. phaseolina* was observed in groundnut cake and castor cake.

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INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is known as red gram, tur, arhar, tuvarica, congobean, thogari or gandul, is economically and nutritionally an important legume and is a major source of protein for the poor communities of many tropical and subtropical regions of the world. Globally, it is cultivated on (4.79 M ha) in 22 countries (FAO, 2008) but which only a few major producer in the world. In Asia, India has largest acreage under pigeonpea (3.90 M ha) with a total production and productivity of 2.89 mt and 741 kg/ha, respectively (DAC, 2011). Pigeonpea is attacked by more than 100 pathogens (Nene *et al*., 1989). This includes *viz*., fungi, bacteria, viruses, nematodes and phytoplasmas. Only a few of them cause economic losses and the distribution of the most important diseases is geographically restricted (Kannaiyan *et al*., 1984). Various economic importance diseases are associated with pigeonpea *viz*., Fusarial wilt (*Fusarium udum* B.), Sterility Mosaic (*PPSMV* S.), Phytophthora Blight (*Phytophthora drechesleri* T.),

Macrophomina stem canker (*Macrophomina phaseolina*), Alternaria blight (*Alternaria alternate* K.) and pearly cyst nematode (*Globodera rostochiensis* S.) in the Indian sub-continent. In Gujarat, among these diseases, stem canker is one of the major constraints. This is the first report of the occurrence of the disease on pigeonpea from Nepal (Maubl.). The stem canker is caused by *Macrophomina phaseolina* is one of most serious and oldest known disease (Nene *et al*., 1984).

MATERIAL AND METHODS

This work was conducted in Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University. Navsari during 2014 to determine the antifungal activity of *Bougainvillea spectabilis, Zingiber officinali, Datura stramonium, Curcuma longa, Ocimum sanctum, Hibiscus bombycideron* and *Tegetes erecta* against fungal *Macrophomina phasiolina* by using food poisoning technique (Naz *et al*., 2006).

Isolation of pathogens:

Pigeonpea plants (GT-1) showing the typical stem canker symptoms were collected from N.A.R.P. Research Station, NAU, Bharuch as well as from the farmers' field and brought to the laboratory and subjected to tissue isolation. After 48 hrs of incubation the isolated fungus initially started to grow as dirty white mycelial growth, then turn to fluffy, blackish mycelial growth on sterilized potato dextrose agar (PDA) medium (potato starch: 20 g, dextrose: 20 g, agar: 20 g and distilled water to make the volume 1 liter. After eight days, minute black sclerotial bodies formed on PDA. The culture was further purified by single hyphal tip method and the purified culture was maintained on PDA slants for further studies. The periodical sub-culturing and multiplication were made on PDA plates to keep the culture fresh and to use throughout the investigations. After purification of the pathogen as described cultural and morphological characters of the fungus on PDA, were studied for identification and compared with those described in the literature. The pure culture was also sent to Indian Type Culture Collection (I.T.C.C), Division of Plant Pathology, I.A.R.I., New Delhi-110 012 and was identified as *Macrophomina phaseolina* (Tassi.) Goid (I.T.C.C. No. 9572.14). The studies on the cultural and morphological characters of isolated *Macrophomina* sp. showed its close identity with *Macrophomina phaseolina* (Tassi.) Goid as described by Nakarni (1991) and Agrawal (1993) were also similar with our present finding. Thus, the *M. phaseolina* causing pigeonpea stem canker.

Preparation of culture media:

For fungicides evaluation:

The different fungicides was taken and weighed by micro weight machine as per requirement to prepare their concentration in ppm. After that all fungicides were individually added into previously sterilized PDA plates @ 10 per cent and mixed thoroughly at the time of pouring in the previously sterilized Petri plates. The Petri plates were inoculated aseptically after solidification by placing 5 mm diameter mycelial disc at the center, cut aseptically with cork borer from 10 days old pure culture of *M. phaseolina*. Three replications of each treatment were maintained. The plate without plant extracts served as control. The Petri plates were incubated at 27 ± 2^0 C temperature till the complete coverage in control plate.

For organic manures/cakes extracts:

Different organic manures/cakes are minced with the help of a grinder by adding 50 ml water. The extracts were filtered through double layered muslin cloth in 150 ml conical flasks and plugged with non-absorbent cotton. These extracts were individually added into PDA plates @ 5 per cent and mixed thoroughly at the time of pouring in the Petri plates. The Petri plates were inoculated aseptically after solidification by placing 5 mm diameter mycelial disc at the center, cut aseptically with cork borer from 10 days old pure culture of *M. phaseolina*. Three replications of each treatment were maintained. The plate without plant extracts served as control. The Petri plates were incubated at 27 ± 2^0 C.

Observations on colony diameter were recorded up to the complete coverage of control plates, which were inoculated with only pathogen. Radial growth of the pathogen was recorded and per cent growth inhibition was calculated by following formula (Vincent, 1947):

$$\text{Growth inhibition (\%)} = \frac{C - T}{C} \times 100$$

where,

C = Growth of pathogen in control after incubation

T = Growth of pathogen in treatment after incubation

RESULTS AND DISCUSSION

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

Fungicides evaluation:

The results presented in Table 1, and depicted graphically in Fig. 1 indicated that all fungicides evaluated significantly reduced the growth of *M. phaseolina* as compare to control but all the fungicides and their concentrations significantly differ within themselves. Among all concentration, the higher concentration of each fungicide produced maximum growth inhibition of the pathogen. From fungicides, mancozeb 75% WP, carbendazim 50% WP, metalaxyl 8% + mancozeb 64%, 75% WP and carbendazim 12% + mancozeb 63% at all the three concentration completely inhibited growth of the pathogen. The next best in order of merit were propiconazole at 250ppm (90.48%), 500ppm (91.67%) and 1000ppm (92.86%) followed by trifloxystrobin 25% + tebuconazole 50% at 500ppm (80.95%), 1000 (83.33%)

Table 1 : Evaluation of different fungicides against *Macrophomina phaseolina in vitro*

Sr. No.	Fungicides	Concentration (ppm)	Average Colony diameter (mm)	Growth inhibition over control (%)
1.	Mancozeb 75% WP	1500	0.71* (0)	100
		2000	0.71 (0)	100
		2500	0.71 (0)	100
2.	Copper oxychloride 50 % WP	1500	5.34 (28)	66.67
		2000	5.15 (26)	69.05
		2500	5.10 (25.5)	69.64
3.	Trifloxystrobin 25% + Tebuconazole	500	4.06 (16)	80.95
	50%	1000	3.81 (14)	83.33
		1500	3.54 (12)	85.71
4.	Chlorothalonil 75% WP	1500	4.74 (22)	73.81
		2000	4.64 (21)	75.00
		2500	4.53 (20)	76.19
5.	Carbendazim 12%+ Mancozeb 75%	1500	0.71 (0)	100
	WP	2000	0.71 (0)	100
		2500	0.71 (0)	100
6.	Kresoxim methyl 50 SC	250	5.96 (35)	58.33
		500	5.52 (30)	64.29
		1000	5.34 (28)	66.67
7.	Benomyl 50% WP	250	4.64 (21)	75
		500	4.18 (17)	79.76
		1000	4.06 (16)	80.95
8.	Carbendazim 50% WP	250	0.71 (0)	100
		500	0.71 (0)	100
		1000	0.71 (0)	100
9.	Propiconazole 25% EC	250	2.92 (8)	90.48
		500	2.74 (7)	91.67
		1000	2.55 (6)	92.86
10.	Matalexyl 18% + Mancozeb 64%,	1000	0.71 (0)	100
	75% WP	1500	0.71 (0)	100
		2000	0.71 (0)	100
11.	Control		9.19 (84)	--
	S.E.±		0.50	
	C.D. (P=0.05)		1.47	
	C.V. %		8.67	

Mean of three repetitions
*Figures are SQR + 0.5 transformed values
Figures in parenthesis are original values

Table 2 : Efficacy of different organic manure/cakes extracts (10%) against *Macrophomina phaseolina in vitro*

Sr. No.	Name of extract	Average Colony diameter of pathogen (mm)	Growth inhibition over control (%)
1.	FYM	59	29.76
2.	Coconut cake	48	42.86
3.	Sesamum cake	63	25.00
4.	*Neem* cake	41	51.19
5.	Castor cake	73	13.10
6.	Groundnut cake	70	16.67
7.	Mustard cake	61	27.38
8.	Vermicompost	64	23.81
9.	Control	84	
	S.E.±	1.30	
	C.D. (P=0.05)	3.86	
	C.V. %	6.57	

and 1500ppm (85.71%), benomyl 50% WP at 250ppm (75.00%), 500ppm (79.76%) and 1000ppm (80.95%), chlorothalonil at 1500ppm (73.81%), 2000ppm (75.00%) and 2500ppm (76.19%), copper oxychloride at 1500ppm (66.67%), 2000ppm (69.05%) and 2500ppm (69.64%), kresoxinmethyle 50% SC at 250ppm (58.33%), 500ppm (64.29%) and 1000ppm (66.67%) inhibiting the growth of *M. phaseolina*.

It is evident from the results that the growth inhibition of *M. phaseolina* increased as increase in the concentration of the chemicals. Mancozeb 75% WP, carbendazim 50% WP, carbendazim 12% + mancozeb 63% and metalaxyl 8% + mancozeb 64%, 75% WP were proved most effective.

Lambhate *et al*. (2002) tested the efficacy of fungicides against *M. phaseolina*, root rot pathogen of cotton in vitro and reported that bavistin, ridomil M Z-72 and topsin-M at 0.1, 0.2 and 0.3 per cent showed cent per cent inhibition of mycelial growth of the fungus. Jaiman *et al.* (2009) reported that carbendazim 50% WP @ 2g/kg, were superior in reducing pre and post emergence seedling rot and root rot disease (*M. phaseolina*) of cluster bean.

Organic extracts/cakes extracts:

The aqueous extracts of different organics were evaluated for their inhibitory effect on *M. phaseolina*. The results presented in Table 2 indicated that organic extracts produced significant inhibitory effect on the fungal growth. Among all the organic extracts, minimum growth was recorded in the extract of *Neem* cake (41mm) followed by coconut cake (48mm), FYM (59mm), mustard cake (61mm), sesamum cake (63mm), vermincompost (64mm). Whereas, groundnut cake (70mm) and castor cake (73mm) were poor in inhibiting growth of the pathogen.

Maximum per cent growth inhibition of *M. phaseolina* was recorded in *Neem* cake (51.19%) followed by coconut cake (42.86%), FYM (29.76%), mustard cake (27.38%), sesamum cake (25.00%), vermicompost (23.81%). Whereas, groundnut cake (16.67%) and castor cake (13.10%) were least effective in inhibiting the growth of the *M. phaseolina.*

From this study, it is clear that *Neem* cake and coconut cake were found effective in reducing the growth of *M. phaseolina* causing stem canker in pigeonpea. The present investigation is more or less similar to the work done by earlier workers Chavan (2006) reported that extracts of groundnut and *Neem* cake inhibited the growth of *M. phaseolina* by 70.00 and 46.88 per cent, respectively in laboratory condition. Effect of *Neem* cake @ 10% recorded maximum (53.69%) growth inhibition against *M. phaseolina* (Dhingani, 2011).

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