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Evaluation of Fungicides and Bio-Agents under *in vitro* Condition against *Macrophomina phaseolina* Causing Stem Canker of Pigeonpea

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A B S T R A C T

In recent years *Macrophomina phaseolina* causing stem canker of pigeonpea is more problematic in pigeonpea growing parts of Karnataka. Present investigation was taken on evaluation of non-systemic, systemic fungicides and bio-agents against *M. phaseolina* under laboratory condition. Five non systemic, four non systemic and three combi products were evaluated. Similarly two isolates of *Trichoderma* spp. four *Pseudomonas fluorescens* isolates, one *Pseudomonas putida* and one *Bacillus subtilis* were evaluated against *M. phaseolina* by poison food technique and dual culture method respectively. Among fungicides mancozeb, copper hydroxide, carbendazim, benomyl, thiophanate methyl, carbendazim 25 % + mancozeb 50 %, zineb 68 % + hexaconazole14 % and tricyclazole 18 % + mancozeb 62 %, carbendazim 12 % + mancozeb 63 %, recorded maximum inhibition of (100 %) mycelial growth. Among the bio-agents tested *Trichoderma harzianum* (Th-R) was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (41.86 %) of *M. phaseolina.*

Introduction

Pigeonpea [*Cajanuscajan* (L.) Mill spaugh] is one of the major legume crops grown in the tropics and sub tropics which accounts for about five per cent of world legume production. India is largest producer of pigeonpea. Similarly Gulbarga is a very potential district in the country for extensive cultivation of pigeonpea. It is also grown in Bidar, Bijapur, Dharwad, Bellary and Belgaum districts of Northern Karnataka. Pigeonpea is affected by biotic stresses (fungal pathogens, insects and storage pests) that limit the realization of true potential of yield. The most widespread and destructive of which is *Fusarium* wilt (*Fusarium udum* Butler), sterility mosaic and *Phytophthora* blight (*Phytophthora drechsleri* f.sp. *cajani*) which are important in. Stem canker incited by *Macrophomina phaseolina* (Tassi) Goid has emerged as one of the very important diseases of pigeonpea.

Macrophomina Stem Canker of pigeonpea caused by *Macrophomina phaseolina* is primarily soil borne that incites disease by producing microsclerotia/pycnidia. It infects a

wide host range of approximately 500 species from 75 plant families. It causes stem canker, seedling blight, charcoal rot, stem rot and root rot diseases in various crops. Hot (>30 ^{0}C) and dry weather encourage disease development which is more prevalent on vertisols than alfisols. Rain after a prolonged dry spell pre-disposes plants to the disease. The pathogen poses a great problem in pigeonpea cultivation and causes considerable loss. The disease incidence varied from 2 to 70 % in most fields and its severity ranged from 17 to 55 % in the aforesaid regions of Eastern Uttar Pradesh (Kaur *et al.,* 2009).

In Northern Karnataka, there is no earlier report on Macrophomina Stem Canker of pigeonpea but recent year's disease was more sever in pigeonpea growing parts of Karnataka (Chikkanna Swamy *et al.,* 2014 and 2017). Fungicides, which have been reported to be effective against *M. phaseolina,* are Captan, Thiram and Agallol (Grewal and Vir, 1958; Clinton, 1960; Bhargava, 1965; Sahai, 1969 and Masih *et al.,* 1970). The efficacy of various fungicides and observed that Carbendazim, Quintozene and Mancozeb reduced *M. phaseolina* population under laboratory conditions (Ilyas *et al.,* 1975).

The role of antagonists in suppressing the growth of soil-borne pathogens was well documented (Garret, 1980). Biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for the future. Use of antagonistic organisms against *Macrophomina* root rot was well documented in several crops (Mukhopadhyay, 1987). In Karnataka lack of information is available on evaluation fungicides and bio-agents against Macrophomina Stem Canker of pigeonpea. Hence present investigation was undertaken on evaluation of fungicides and bio-agents were against stem canker diseases to find out the efficient fungicides and bio-agents for the better management of stem canker disease.

Materials and Methods

Collection, isolation and proving pathogenicity of *M. phaseolina*

The symptomatic parts of *Macrophomina* Stem Canker diseased plants collected from Raichur and pathogen was isolated by standard tissue isolation method described by Kaur *et al.,* (2013). Pathogen was transferred to PDA slants and stored at 4±1 °C for further studies. Isolated pathogen was proved the pathogenicity by pigeonpea plants of TS-3R variety was selected during flowering stage.

Stems were superficially wounded by peeling 1 mm deep and 0.5 cm long to the epidermis with razor blade. The wounded area was inserted with *M. phaseolina* culture using a sterilized needle and smeared by 2 % sugar solution and covered with cellophane tape (Chikkanna *et al.,* 2014). This method was replicated for thrice under glass house condition. Water stress was given after 15 days of inoculation to create favourable condition. Similarly pathogenicity was also proved under laboratory by cellophane tape technique.

In vitro evaluation of systemic and non-systemic fungicides

The experiment was carried out in (CRD). The details of treatments for *in vitro* evaluation of fungicides are listed in Table 1. Twenty ml of PDA medium initially mixed with chemicals listed below were poured in to 90 mm diameter Petri dishes. Control was maintained without addition of fungicides.

After solidification, 5 mm discs of *M. phaseolina* were placed at the centre of the plate. Each set of experiment was replicated thrice and plates were incubated at 30 ±1 ^{o}C for control when reached the periphery of plates. Observations were taken on parameters

such as colony diameter and per cent inhibition of growth which was calculated using the formula (Vincent, 1927).

In-vitro evaluation of bio-agents against *M. phaseolina*

Two isolates of *Trichoderma* spp. four *Pseudomonas fluorescens* isolates, one *Pseudomonas putida* and one *Bacillus subtilis* were evaluated for their efficacy through dual culture technique. The source of bio-agents is presented in Table 2. The fungal bio-agent and the test fungus were inoculated side by side on a single Petri plate containing solidified PDA medium. Whereas, the bacterial bio-agents were streaked one day earlier to test the pathogen. Three replications were maintained for each isolate with one control by maintaining only pathogen and bio-agent. They were incubated for control reaches periphery of plates. The diameter of the colony of both bio-agent and the fungus was measured in both directions and average was recorded and the per cent inhibition on growth of the test pathogen was calculated by using the formula given below by (Vincent, 1927).

Results and Discussion

Collection, isolation and proving pathogenicity of *M. phaseolina*

Artificial inoculation of the fungus was carried out to prove the pathogenicity using cellophane tape method under glass house condition. Mycelium was inoculated in to 90 days old plant of pigeonpea variety TS-3R. After 21 days of inoculation, symptoms were expressed by formation of spindle shaped lesion producing numerous pycnidia on stem. *In vitro* detached stem technique was also carried out to prove pathogenicity, after six days of inoculation good number of pycnidia were noticed on stem incubated in moist chamber at 30 ±1 °C. The fungus was re isolated and was found to resemble the original culture of *M. phaseolina*.

In vitro evaluation of systemic and non-systemic fungicides

In vitro evaluation of fungicides provides useful preliminary information regarding its efficacy against a pathogen with in a shortest period of time and therefore serve as guide for further field testing. Efficacy of five contact fungicides was tested against *M. phaseolina* by poisoned food technique. Among contact fungicides mancozeb, copper hydroxide and a combiproducts of carbendazim 12 % + mancozeb 63 % recorded maximum inhibition of (100 %) mycelial growth at all concentrations (0.10 %, 0.20 % and 0.30 %) and captan showed 92.20 % inhibition at 0.10 per cent concentration, 100 per cent inhibition at 0.2 and 0.3 per cent concentrations. Least inhibition of 86.00, 88.88 and 89.33 per cent was observed in case of ziram at 0.10, 0.20 and 0.30 per cent respectively (Table 3 and Fig. 1). Bavistin was most effective fungicide in reducing the mycelial growth of *M. phaseolina* and gave complete inhibition of mycelia growth and action of these chemicals inhibit the germination, growth and multiplication of the fungus or are directly toxic (Kumari *et al.*, 2012). Ramadoss and Sivapraskasam (1994) reported that sclerotial production of *M. phaseolina* was completely inhibited by carbendazim.

Efficacy of seven systemic fungicides was tested against *M. phaseolina* by poisoned food technique. Among systemic fungicides carbendazim, benomyl, thiophanate methyl, carbendazim 25 % + mancozeb 50 %, zineb 68 % + hexaconazole14 % and tricyclazole 18 % + mancozeb 62 % showed 100 % inhibition at all concentrations (0.05, 0.10 and 0.20 %). Least inhibition was found in hexoconazole with 88.88, 90.44 and 91.88 per cent at 0.05, 0.10 and 0.20 per cent concentrations

respectively with significant difference (Table 4 and Fig. 1). The efficacy of the triazoles fungicides in a combiform such as hexaconazole and tricyclazole may be attributed to their interference with the biosynthesis of fungal sterols and inhibition of ergosterol biosynthesis. In many fungi, ergosterol is essential to the structure of cell wall and its absence causes irreparable damage to cell wall leading to death of fungal cell. A similar study was reported for the effectiveness of triazoles, which inhibit the biosynthesis pathway in fungi (Nene and Thapliyal, 1973). Complete 100 per cent inhibition of carbendazim, thiophanate methyl, carbendazim 25 % + mancozeb 50 % and tricyclazole 18 % + mancozeb 62 % were reported earlier.

Table.1 List of systemic and non-systemic fungicides used for *in-vitro* evaluation against *M. phaseolina*

Sl. No.	Common name	Chemical name	Trade name
Non-systemic fungicides			
1.	Captan	N-Trichloromethyl-1-thio-4-cyclohexane-1, 2 dicorboximide	MERIMAIN 50% WP
2.	Mancozeb	Manganese ethylene bis dithiocarbamate	Dithane M-45 75% WP
3.	Ziram	Zinc dimethyl dithiocorbomate	Deviziram 27 %SC
4.	Copper hydroxide	Copper hydroxide	Kocide 77% WP
5.	Carbendazim 12 % + Mancozeb 63 %	Methyl 1-1-2 benzimidazole carbonate + Manganese ethylene bis dithiocarbamate	SAAF 75% WP
Systemic fungicides			
1.	Carbendazim	2-methoxy-carbamoyl-benzimidazole	Bavistin 50 WP
2.	Benomyl	Methyl-N-(1-butyl carbamyl) 2-benzimidazole carbomate	Benofit 50 % WP
3.	Hexaconazole	2-2,4-dichlor phenyl 1-(1H,1,2,4-triazol-1y1) hexan-2-01	Hexalife 5% EC
4.	Thiophanate methyl	1,2, bis (3-methoxy caboryl-2-thioureido benzene)	Roko70%WP
5.	Carbendazim 25 % + Mancozeb 50 %	Methyl 1-1-2 benzimidazole carbonate + Manganese ethylene bis dithiocarbamate	SPRINT 75% WP
6.	Hexaconazole 4% + Zineb 68%	2-2,4-dichlor phenyl 1-(1H,1,2,4-triazol-1y1) hexan-2-01 + Zinc ethylene bis dithiocarbamate	Avtar 72% WP
7.	Tricyclazole 18 % + Mancozeb 62 %	5-methyl 1,2,4 triazole (3, 4, 6) beza thiozole +Manganese ethylene bis dithiocarbamate	MERGER 80%WP

Table.2 *In-vitro* evaluation of contact fungicides on the growth of *M. phaseolina*

Sl. No.	Fungicides	Per cent inhibition at different concentration*			
		0.10%	**0.20%**	**0.30%**	**Mean**
1	Capton	92.20* (84.60)	100 (90.00)	100 (90.00)	97.40 (80.72)
2	Ziram	86.00 (68.02)	88.88 (70.52)	89.33 (70.93)	88.07 (69.82)
3	Mancozeb	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
4	Copper hydroxide	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5	Carbendazim+ Mancozeb	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6	Control	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)
	Mean	79.70 (63.22)	81.48 (64.51)	81.55 (64.56)	80.91 (64.09)
		S. Em±	**CD @1 %**		
Fungicides (F)		0.36	1.40		
Concentration (C)		0.25	0.99		
F x C		0.63	2.44		

Table.3 *In-vitro* evaluation of systemic fungicides on the growth of *M. phaseolina*

Sl. No.	Fungicides	Per cent inhibition at different concentration			
		0.05%	**0.10%**	**0.20%**	**Mean**
1	Carbendazim	100* (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
2	Benomyl	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3	Hexaconazole	88.88 (70.52)	90.44 (71.98)	91.88 (73.44)	90.40 (71.98)
4	Thiophanate methyl	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5	Carbendazim 25% + Mancozeb 50%	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6	Zineb 68 % + Hexaconazole l4 %	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
7	Tricyclazole 18% + Mancozeb 62%	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
8	Control	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)
	Mean	86.11 (68.11)	86.30 (68.27)	86.48 (68.42)	86.30 (68.27)
		S. Em±	**CD @1 %**		
Fungicides (F)		0.10	0.39		
Concentration (C)		0.06	0.24		
F x C		0.17	0.68		

*Mean average of three replications
Figures in parentheses are arc sine values

Table.4 *In vitro* evaluation of bio-agents against *M. phaseolina* in dual culture

Sl. No.	Bio-agents	Per cent of inhibition
1	*Trichoderma viride* (Tv-R)	39.07* (38.66)
2	*T. harzianum* (Th-R)	41.86 (40.31)
3	*Pseudomonas fluorescens* (Pf-46)	18.33 (25.34)
4	*P. fluorescens* (Pf-31)	18.77 (25.67)
5	*P. fluorescens* (Pf-16)	23.10 (28.72)
6	*P. fluorescens* (Pf-41)	12.66 (20.84)
7	*P. putida* (56)	27.22 (31.44)
8	*Bacillus subtilis*	14.85 (22.66)
9	Control	0.00 (0.00)
	S. Em±	0.55
	CD @ 1%	2.26

*Mean average of three replications
Figures in parentheses are arc sine values

Fig.1 *In vitro* evaluation of non-systemic and systemic fungicides on inhibition of mycelial growth of *M. phaseolina*

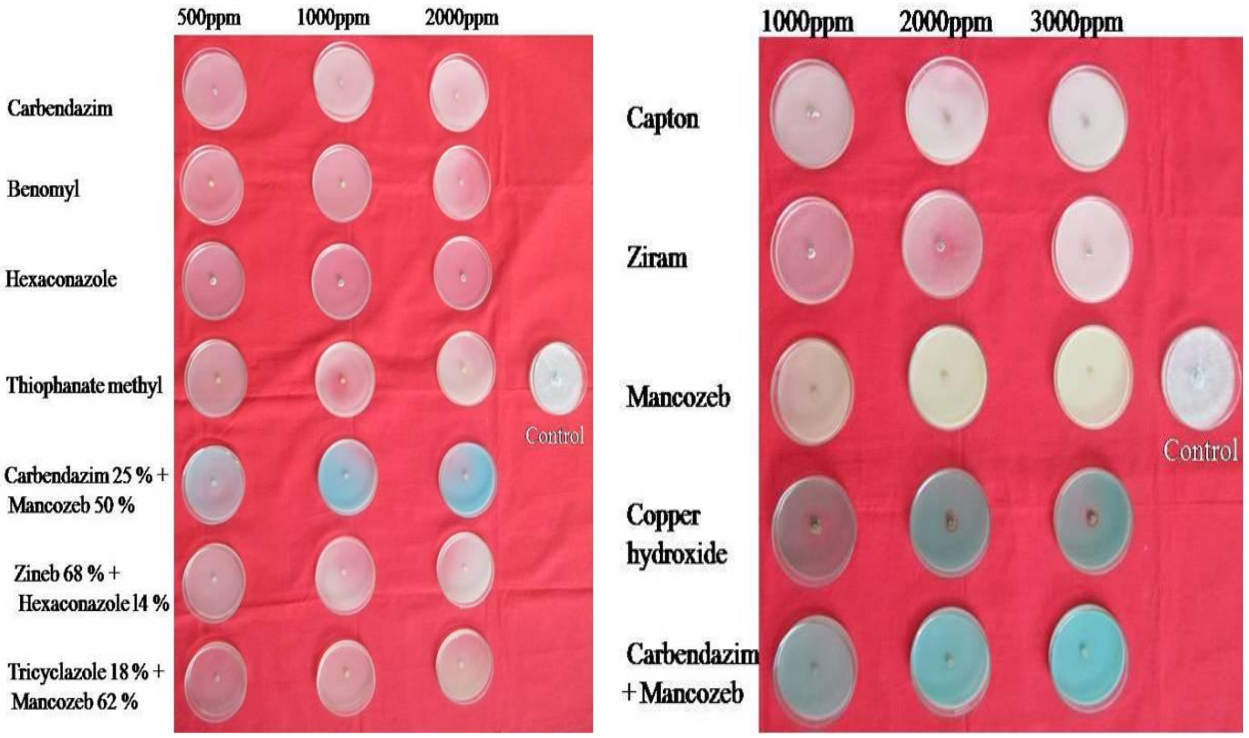
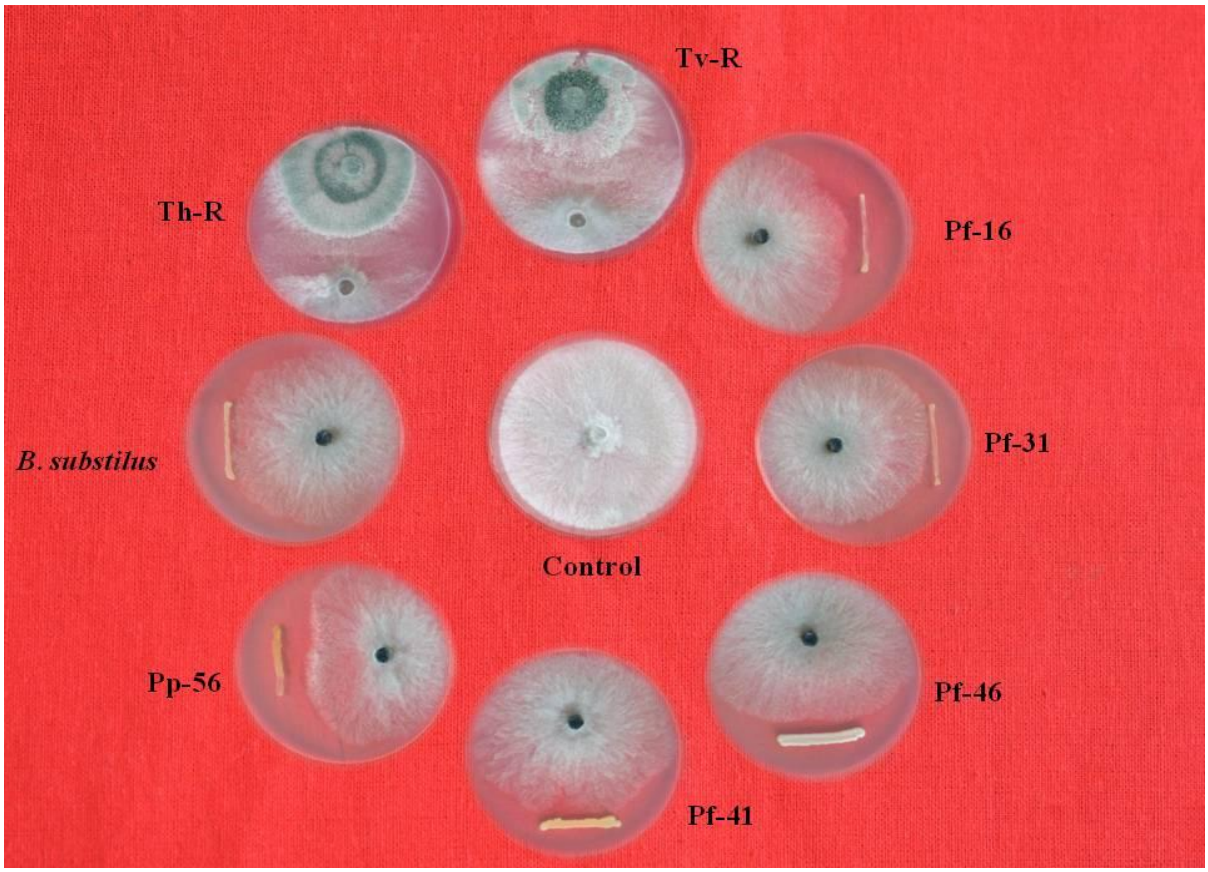


Fig.2 Efficacy of bio-agents in inhibition of mycelial growth of *M. phaseolina*



In-vitro evaluation of bio-agents against *M. phaseolina*

Biological control is a potential non-chemical and eco-friendly means for plant disease control by reducing harmful effects of a pathogen through the use of other living entities. It is now widely recognized that biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for future and can be successfully utilized especially within the frame work of integrated disease management system (Muthamilan and Jeyarajan, 1996). In the present study, investigations were carried out on the *in vitro* evaluation of bio-agents on inhibition of growth of *M. phaseolina.*

Efficacy of bio agents was studied under *in vitro* and the results on inhibition of mycelial growth of *M. phaseolina* were recorded. Among the bio-agents tested (Table 5 and Fig. 2), *T. harzianum* (Th-R) was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (41.86 %) of *M. phaseolina* followed by *T. viride* (39.07 %). *P. putida* (RP- 56) was inhibited to the extent of 27.22 % compared to other strains of *Pseudomonas* spp. Among *P. fluorescens* strains, RP-41 showed least inhibition, RP-46 and RP-31 were on par with each other, RP-16 showed 23.10 per cent inhibition and *Bacillus subtilis* recorded 14.85 per cent of inhibition (Plate 14). Mechanisms for bio-control of plant pathogens by *Trichoderma* are antibiosis, lysis competition and mycoparasitism. *T. harzianum* and *T. viride* both suppressed the growth of *M. phaseolina* and this may be due to coiling and disintegration of hyphae of the test fungus

resulting in loss of competitive saprophytic ability (Naik and Sen, 1995 and Naik *et al.*, 2009). Manczinger *et al.,* (2002) reported that *T. harzianum*, *T. viride* and *T. polysporum* have a strong antagonistic against soil borne pathogens.

In vitro screening of fungicides and antagonists provides preliminary information regarding their efficacy against. *M. phaseolina* and with a hope to utilize the promising bio-agents and fungicides for management of stem canker of pigeonpea under field conditions by integrating the methods to apply bio-agents, fungicides and with other components of integrated disease management. In present study mancozeb, copper hydroxide and acombi-products of carbendazim 12 % + mancozeb 63 % recorded maximum inhibition of (100 %) mycelial growth at all concentrations (0.10 %, 0.20 % and 0.30 %) among contact fungicides. Carbendazim, benomyl, thiophanate methyl, carbendazim 25 % + mancozeb 50 %, zineb 68 % + hexaconazole14 % and tricyclazole 18 % + mancozeb 62 % showed 100 per cent inhibition at all concentrations (0.05, 0.10 and 0.20 %) among systemic fungicide. *T. harzianum* was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (41.86 %).

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