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Original Research Article

In-Vitro Evaluation of Fungicides, Bioagents and Plant Extracts against *Alternaria* sp. infecting Pigeonpea

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A B S T R A C T

Alternaria leaf blight of pigeonpea was earlier considered a minor disease in Jharkhand but in course of time, this disease has assumed alarming proportions in some pockets of Jharkhand in late and medium varieties due to climate change. Evaluation of fungicides, bioagents, plant extract against Alternaria leaf blight of pigeonpea were carried out in laboratory of Plant pathology department of Birsa Agricultural University, Ranchi during 2016-17to determine their efficacy. The systematic investigation revealed that Alternaria leaf blight was found to be infected with *Alternaria* spp. i.e., *Alternaria alternata* and *Alternaria tenuissima.* Out of six different fungicides tested *in vitro*, Tilt (Propiconazole) at all concentrations completely inhibited (100%) the mycelial growth and sporulation of *Alternaria* spp. In biocontrol studies, rate of mycoparasitism was faster in *Trichoderma viride* (Pusa isolates) providing 80.36% inhibition of growth of *Alternaria alternata* whereas *Trichoderma viride* (Pusa isolates) provided 74.27% inhibition of growth in case of *Alternaria tenuissima*.. Among all the botanicals evaluated against *Alternaria* spp. *in vitro*, Garlic clove extract at 10% was found most effective giving 84.31% inhibition against *Alternaria alternata* whereas 82.18% inhibition of mycelial growth was observed in case of *Alternaria tenuissima* followed by onion and Neem.

Keywords

Bioefficacy, *Alternaria* spp., Botanicals, Fungicides, Biocontrol agents

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp] commonly known as 'Arhar' or 'Tur' is a major grain legume of the tropics and subtropics worldwide. In India among different pulses, the highest growth rate was observed in chickpea production (5.89%) followed by pigeonpea (2.61%). Pigeonpea is grown in the area of 5.13 million ha with production of 4.23 million tonnes during 2016-17 in India (Anon 2016-17) with productivity of 824 kg/ha. Pigeonpea is being grown as food crop (Dried peas, flour or green vegetables peas) and a forage/ cover crop. India is the largest producer and consumer because pigeonpea plays an important role in food security, balanced diet and alleviation of poverty (Rao *et al.* 2002). India contributes over 90% of the pigeonpea production in the world where it is mostly consumed as dehusked splits or dhal. It is grown as sole crop or intercrop with urdbean, mungbean, castor, sorghum,

soybean, cotton, maize and groundnut in different states like Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Gujarat, Jharkhand, Rajasthan Odisha, Punjab and Haryana. Pigeonpea make a well- balanced human food because it is an important source of protein (22%), carbohydrates (57.6%), vitamin B, and certain minerals (3.5%). It provides a superior protein to the vegetarian population of India. However, the current production of pigeonpea in India cannot meet the domestic demand leading to a decrease in per capita availability of pigeonpea from 70 gm to 35 gm. Despite the fact that a large number of high yielding varieties and have been released, productivity in the crop remains stagnant around 700 kg ha-1as compared to its potential yield (2500-3000 kg ha-1). This gap may be attributed to several biotic and abiotic factors. Since it is mainly a rainfed crop, unfavorable rainfall (delayed, erratic, improper distribution) leads to terminal drought or heavy down pour.

Non adoption of improved management practices and lack of proper research and commercial perspective for the crop influence the low productivity to a greater extent. Pigeonpea crop is vulnerable to be infecting by bacterial, fungal, viral disease. Among all fungal diseases Alternaria blight was considered as minor disease in Jharkhand but now days, blight symptoms on pigeonpea are being observed in alarming proportion in some of the pockets of Jharkhand.

The systematic investigation revealed that Alternaria leaf blight was found to be infected with *Alternaria* spp. In present investigation, the biocontrol agents, plant extracts and chemicals were tested for their efficacy against the pathogens for safe and eco-friendly global developments.

Materials and Methods

Evaluation of effect of seven different fungicides viz., Ridomil-MZ Gold (Metalaxyl), Saaf (Carbendazim 12% + Mancozeb 63%), Indofil M-45 (Mancozeb), Folpet (Folpet), Kavach (Chlorothalonil) and Tilt (Propiconazole) @ 0.1%, 0.2% and 0.3% were tested *in vitro* against the pathogen by using poison food technique (Nene and Thapliyal, 1982). The growth inhibition in per cent was calculated by the formula given by Vincent (1927)

The leaf extracts of the Tulsi (*Ocimum sanctum* L.), Neem (*Azadirachta indica* L.), Garlic (*Allium sativum* L.), Onion (*Allium cepa* L.*)*, Calotropis (*Calotropis procera*) and Eucalyptus (*Eucalyptus globules Labil*) were evaluated in vitro against *Alternaria* spp. at 5 and 10% concentrations through poisoned food technique.

The procedure for crude plant extracts are collect fresh plant materials and washed first in tap water and then in distilled water. 100 grams of fresh sample was chopped and then crushed in a surface sterilized mortar and pestle by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution. To study the anti fungal mechanism of plant extracts poisoned food technique was used (Nene and Thapliyal, 1973). Five and ten ml. of stock was mixed with 95 and 90 ml of sterilized molten PDA medium, respectively so as to get 5 and 10 percent concentration. The medium has thoroughly shaken for uniform mixing of extracts. Twenty ml of medium was poured into sterile petriplate, totally six plant extracts were evaluated at two concentrations (5% and 10%). Suitable control plates were maintained by growing the test pathogen on PDA slants the growth inhibition in per cent was calculated.

Six bio-control isolates viz., *Trichoderma harzianum* 1 (Delhi isolates), *Trichoderma harzianum* 2 (Ranchi isolates), *Trichoderma harzianum* 3 (ANGRAU isolates), *Trichoderma viride* (Pusa, Bihar isolates), *Pseudomonas fluorescens* 1 *and Pseudomonas fluorescens* 2 were tested for their efficacy on PDA. Radial growth of the test fungus as well as antagonist measured after 72 hours. Pathogen alone inoculated on PDA plate served as control. Per-cent inhibition of pathogen was calculated based on the growth in control and dual culture plates.

Results and Discussion

All the six fungicides evaluated, were found inhibitory for both species when compared with check. Tilt (propiconazole) was proved to be most effective even at low concentration (0.1%) which completely inhibited (100%) mycelial growth and sporulation of both species *Alternaria alternata* and *Alternaria tenuissima* followed by SAAF and Indofil M-45. Raja and Reddy (2008) who reported that propiconazole showed (100%) inhibition at 0.0125 per cent concentration. In case of *Alternaria alternata* SAAF showed colony diameter of 16.53 mm with 78.69 per cent inhibition of mycelial growth while in *Alternaria tenuissima* SAAF showed colony diameter of 18.96 mm with 74.55 per cent inhibition of mycelium. Mathivanan and Prabavathy (2007) proved that SAAF completely (100%) inhibited the mycelial growth of *A. helianthi* (Table 1 and 2).

Among *in-vitro* studies of six plant extracts, Garlic (clove) extract (*Allium sativum*) is effective at both concentration 5 & 10 per cent. *Alternaria alternata* (12.66 mm) showed minimum colony diameter with 84.31 per cent growth inhibition of species. And *Alternaria tenuissima* showed minimum colony diameter (14.13 mm) with 82.18 per cent growth inhibition of species followed by onion and Neem. Raja (2010) reported that maximum reduction of 62.1% disease intensity was observed in pot culture experiments after spraying with garlic clove extract at 10% (v/v) concentration.

Table.1 *In-vitro* assay of fungicides on the inhibition of mycelial growth of *Alternaria alternate*

Fungicides		*Colony diameter (mm)			Growth inhibition (%)		
		0.1%	0.2%	0.3%	0.1%	0.2%	0.3%
T_1	Ridomil Gold (Metalaxyl –M 4%+ Mancozeb 64% WP)	32.3	27.2	18.36	58.95	65.12	76.34
T_2	SAAF(Carbendazim 12% + Mancozeb 63% WP)	25.5	21.9	16.53	67.59	71.92	78.69
T_3	Indofil M- 45 (Mancozeb 75% WP)	32.6	22.63	17.93	58.57	70.98	76.89
T_4	Folpet (Folpet50% WP)	37.46	27.73	22.53	52.40	64.48	70.96
T_5	Kavach (Chlorothalonil 75% WP)	39.26	35.96	33.80	50.11	53.89	56.44
T_6	Tilt (Propiconazole 25 % EC)	0	0	0	100	100	100
T_7	Control	78.7	78.0	77.6	-	-	-
		S Em ±	CD at 5%				
Fungicide (F)		1.05	3.01				
Concentration (C)		0.69	1.94				
F × C		1.82	5.22				

***** Average of 3 replications

Table.2 *In-vitro* assay of fungicides on the inhibition of mycelial growth of *Alternaria tenuissima*

Fungicides		*Colony diameter (mm)			Growth inhibition (%)		
		0.1%	0.2%	0.3%	0.1%	0.2%	0.3%
T_1	Ridomil Gold (Metalaxyl –M 4%+ Mancozeb 64% WP)	36.3	25.96	23.3	51.93	65.41	68.72
T_2	SAAF (Carbendazim 12% + Mancozeb 63% WP)	24.40	20.63	18.96	67.69	72.51	74.55
T_3	Indofil M- 45 (Mancozeb 75% WP)	30.40	24.6	22.80	59.75	67.22	69.39
T_4	Folpet (Folpet50% WP)	32.30	28.23	27.03	57.23	62.39	63.73
T_5	Kavach (Chlorothalonil 75% WP)	43.36	40.33	36.20	40.96	46.26	51.42
T_6	Tilt (Propiconazole 25 % EC)	0	0	0	100	100	100
T_7	Control	72.53	75.06	74.50	-	-	-
		S Em ±	**CD at 5%**				
		1.05	3.00				
	Fungicide (F)	0.69	1.97				
	Concentration (C)	1.82	5.21				
	F × C						

***** Average of 3 replications

Table.3 *In-vitro* assay of plant extracts on the inhibition of mycelial growth of *Alternaria alternata*

Treatments		Plant parts used	*Colony diameter (mm)		Growth inhibition (%)	
			5%	10%	5%	10%
T_1	Tulsi (*Ocimum sanctum* L.)	Leaf	35.13	32.56	56.48	59.65
T_2	Neem (*Azadirachta indica* L.)	Leaf	25.46	21.86	68.46	72.92
T_3	Garlic (*Allium sativum* L.)	Clove	19.86	12.66	75.39	84.31
T_4.	Onion *(Allium cepa* L.)	Bulb	32.56	22.93	59.66	71.59
T_5	Calotropis (*Calotropis procera)*	Leaf	68.93	46.70	14.61	42.15
T_6	Eucalyptus (*Eucalyptus globules Labil*)	Leaf	44.96	30.76	44.30	61.89
T_7	Control	-	80.73	80.73	-	-
			S Em ±	**CD at 5%**		
			0.59	1.73		
	Plant extracts (E)		0.31	0.92		
	Concentration (C)		0.84	2.45		
	(E × C)					

***** Average of 3 replications

Table.4 *In-vitro* assay of plant extracts on the inhibition of mycelial growth of *Alternaria tenuissima*

Treatments		Plant parts used	*Colony diameter (mm)		Growth inhibition (%)	
			5%	10%	5%	10%
T_1	Tulsi (*Ocimum sanctum* L.)	Leaf	33.33	31.33	57.98	60.50
T_2	Neem (*Azadirachta indica* L.)	Leaf	42.83	23.43	46.01	70.46
T_3	Garlic (*Allium sativum* L.)	Clove	27.8	14.13	64.95	82.18
T_4.	Onion *(Allium cepa* L.)	Bulb	36.66	22.33	53.78	71.85
T_5	Calotropis (*Calotropis procera)*	Leaf	70.90	59.83	10.62	24.58
T_6	Eucalyptus (*Eucalyptus globules Labil*)	Leaf	45.86	32.66	42.19	58.83
T_7	Control	-	79.33	79.33	-	-
			S Em ±	**CD at 5%**		
		Plant extracts(E)	0.63	1.84		
		Concentration (C)	0.34	0.98		
		(E x C)	0.90	2.61		

***** Average of 3 replications

Table.5 *In-vitro* assay of biocontrol agents on the mycelial growth inhibition of *Alternaria alternata*

Treatments		*Colony diameter of antagonist (mm)	*Colony diameter of pathogen (mm)	Growth inhibition over control %
T_1	*Trichoderma harzianum 1 +A. alternata*	64.00	17.00	74.96
T_2	*T. harzianum 2+A. alternata*	60.43	20.43	69.91
T_3	*T. harzianum*3 + *A.alternata*	54.60	26.93	60.33
T_4	*T. viride + A. alternata*	74.00	13.33	80.36
T_5	*Pseudomonas fluorescens* 1+ *A.alternata*	30.66	27.50	59.49
T_6	*Pseudomonas fluorescens* 2+ *A. alternata*	18.76	23.56	65.30
T_7	*Alternaria alternata*	-	67.9	-
	S Em ±	1.09	0.69	
	CD at 5%	2.38	2.16	
	CV %	2.99	4.74	

***** Average of 3 replications

Table.6 *In-vitro* assay of biocontrol agents on the mycelial growth inhibition of *Alternaria tenuissima*

Treatments		*Colony diameter of antagonist (mm)	*Colony diameter of pathogen (mm)	Growth inhibition over control %
T_1	*Trichoderma harzianum1+A. tenuissima*	63.13	18.00	67.68
T_2	*T. harzianum 2+A. tenuissima*	60.33	21.43	61.52
T_3	*T. harzianum* 3 + *A. tenuissima*	55.60	25.26	54.64
T_4	*T. viride + A. tenuissima*	75.00	14.33	74.27
T_5	*Pseudomonas fluorescens* 1+ *A. tenuissima*	32.66	27.50	50.62
T_6	*Pseudomonas fluorescens* 2+ *A. tenuissima*	21.66	22.50	59.60
T_7	*Alternaria tenuissima*	-	55.7	-
	S Em ±	1.04	0.48	
	CD at 5%	2.27	1.49	
	CV %	2.92	3.15	

***** Average of 3 replications

In *Alternaria alternata*, Neem (leaf) extracts @ 10 per cent recorded colony diameter of fungi (21.86 mm) inhibition of growth of fungus over control of 72.92 per cent as compare to at 5% it showed 25.46 mm colony diameter with 68.46 per cent inhibition of fungal growth, which was at par with Onion (bulb) extracts @ 10 per cent which recorded colony diameter (22.93 mm) and inhibition (71.59%) (Table 3 and 4).

In dual culture studies minimum colony diameter of *Alternaria alternata* (13.33 mm) was observed in the plate grown with *Trichoderma viride* which differed significantly with the colony diameter (17.00 mm) grown in *Trichoderma harzianum* 1 (Delhi isolates). The growth inhibition was highest (80.36%) in *Trichoderma viride* followed by 74.96 per cent in *Trichoderma harzianum* 1 while minimum colony diameter of *Alternaria tenuissima* (14.33 mm) was observed in the plate grown with *Trichoderma viride* which differed significantly with the colony diameter (18.00 mm) grown in *Trichoderma harzianum* 1 (Delhi isolates) followed by test pathogen growth (21.43 mm) in *Trichoderma harzianum* 2 (Ranchi isolates) plate. Balai *et al.* (2011) reported that *Trichoderma harzianum* and *Trichoderma viride* were superior antagonists of *Alternaria alternata* in brinjal. Lal and Upadhyay (2002) evaluated *Trichoderma viride, Gliocladium virens* and *T. harzianum* against *Alternaria tenuissima in- vitro*. In dual culture *Trichoderma viride* overgrew the colony of *Alternaria tenuissima* within 72 hr of incubation followed by *Gliocladium virens* and *Trichoderma harzianum*. Ambuse *et al*. (2012) worked on influence of *Trichoderma spp.* against *Alternaria tenuissima* in Sorrel (*Rumex acetosa*) showed 80 per cent antagonistic activity (Table 5 and 6).

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