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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(3): 675-678

www.thepharmajournal.com
Received: 05-01-2021
Accepted: 12-02-2021

Pankaj Kishanawat
Department of Plant Pathology, S.K.N. College of Agriculture (S.K.N. Agriculture University, Jobner), Jobner, Rajasthan, India

Jitendra Singh
Department of Plant Pathology, S.K.N. College of Agriculture (S.K.N. Agriculture University, Jobner), Jobner, Rajasthan, India

Mahabeer Singh
Department of Plant Pathology, S.K.N. College of Agriculture (S.K.N. Agriculture University, Jobner), Jobner, Rajasthan, India

Corresponding Author:
Jitendra Singh
Department of Plant Pathology, S.K.N. College of Agriculture (S.K.N. Agriculture University, Jobner), Jobner, Rajasthan, India

Efficacy of different fungicides, plant extracts and bio-agents for the management of root rot of cluster bean incited by *Macrophomina phaseolina*

Pankaj Kishanawat, Jitendra Singh and Mahabeer Singh

DOI: https://doi.org/10.22271/tpi.2021.v10.i3j.5854

Abstract
An investigation was made to minimize root rot of cluster bean incited by *Rhizoctonia bataticola* (*Macrophomina phaseolina*) by use of *Trichoderma*, neem leaf extract and carbendazim. It was observed in Dual Culture Technique, *Trichoderma harzianum* showed highest inhibition of mycelial growth of the test pathogen when compared to *Trichoderma viride* and *Bacillus subtilis*. The extract of five plants part and five fungicides were evaluated against *Rhizoctonia bataticoa* by Poisoned Food Technique. Among these the extract of garlic (10%), neem (10%) and carbedazim were found most effective to inhibiting mycelial growth of test fungus followed by tebuconazole + tryfloxystrobin. Plant extracts, bio-agents and fungicides which were found most effective in *in vitro* studies were tested as seed treatment in pot against *R. bataticola*. Carbendazim, *Trichoderma harzianum* and garlic extract were proved most effective in reducing disease incidence.

Keywords: *Macrophomina*, cluster bean plant extracts, bio-agents, fungicides, *Trichoderma*

Introduction
Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] popularly known as "Gaur" is an important legume crop and mainly grown under rainfed conditions in arid and semi-arid regions of Rajasthan during *Kharif* season. The crop is grown for different purposes such as vegetable, green manure and seed production. Cluster bean is an excellent soil building crop with respect to available nitrogen. It provides nutritional concentrate, fodder for cattle and add to the fertility of soil by fixing considerable amount of atmospheric nitrogen.
One of the important factors which limit the productivity of this crop in Rajasthan is poor health of seeds which takes heavy toll of the crop at all the stages right from sowing to harvesting and also in storage.
The crop suffers from number of phytopathogenic fungal and other diseases. The common fungal diseases observed in cluster bean are Alternaria leaf spot, Anthracnose, Curvularia leaf spot, Charcoal rot/ Damping off/ Dry root rot/Root rot, Myrothecium leaf spot, Powdery mildew and wilt. Out of these diseases, dry root rot or charcol rot caused by *Rhizoctonia bataticola* (*Macrophomina phaseolina*) is a serious disease [1, 2, 3].
At first symptom of the disease starts with yellowing of the leaves which droop in next 2 or 3 days and withers off. The plant may wilt within a week after the appearance of first symptom. When stem is examined closely, dark lesions may be seen on the bark at the ground level. If the plants are pooled from soil, the basal stem and main root may show dry rot symptoms. The tissues are weakened and break off easily in advanced case and sclerotial bodies may be seen scattered on the affected tissues.
Management of this disease mainly depends on fungicides. However, fungicidal applications cause hazard to human health and increase environmental pollution. Therefore, alternative eco-friendly approaches for management of root rot of cluster bean are needed. Diseased can be managed by seed treatment with botanicals and bio control agents. Therefore, keeping in view all these facts, the investigations were carried out.

Materials and Method
Seed samples of cluster bean were collected from farmer's field of Jaipur district. For isolation of pathogen seeds were washed with sterilized water then each seed was surface sterilized with 0.1% sodium hypo chloride solution for one minute followed by three consecutive washing with sterilized water and dried on sterilized blotter paper.

For isolation of *Rhizoctonia* sp. One seed per test tube was placed aseptically on PDA slant then inoculated in BOD incubator at 30 ± 1º C. Purification of the fungus was obtained by hyphal tip cut method in plain agar and pathogenicity test was done for confirmation of pathogen associated with disease.

Bio-agents (*in vitro*)

Screening of bio-agents was done by Dual Culture Technique [4]. The bio-agents used for study *viz*., *Bacillus subtilis*, *Trichoderma harzianum* and *T. viride*. Fifteen ml of PDA medium was poured into sterilized Petri plates and allowed for solidification. Five mm diameter discs from actively growing colony of pathogen was cut with a sterile cork borer and placed near the periphery of PDA plate. Similarly, bio-agents also placed on the other side *i.e*., at an angle of 180º. Plates with no antagonist served as control. The plates were incubated at 30 ± 1º C for seven days. For each treatment three replications were maintained. The extent antagonistic activity by bio-agent was recorded after the incubation period of 7 days by measuring the growth of the test pathogen in dual culture and in control plates. In case of bacterial bio-agents, nutrient agar medium was used in place of PDA.

Botanicals (*in vitro*)

In recent years, many phyto-extracts are being used as fungitoxicant for the management of various plant diseases. The present investigation was carried out using following five natural phyto-extracts to see their antimycotic behaviour on the growth of *Rhizoctonia bataticola* following Poisoned Food Technique [5]. The effect of each plant extract was tested at two concentrations (5 and 10) following the method suggested by [6] with slight modifications. To get these, the required plant part *viz*., leaf of neem (*Azadirachta indica*), datura (*Datura stramonium*), marigold (*Datura stramonium*), tulsi (*Ocimum sanctum*) and clove of garlic (*Allium sativum*) were thoroughly washed with sterilized water and ground separately in electric grinder using equal amount of sterilized distilled water (*i.e*. 1:1 ratio, w/v). The mixture was squeezed with double layered sterilized cheese cloth. The extracts thus obtained were considered as of 100 per cent concentration. Required quantity of each plant extract (*i.e*. Stock solution) was mixed thoroughly in melted PDA, to get desired concentration, just before pouring in sterilized 9 cm diameter glass Petri dishes and was allowed to solidify for 12 hours. Each plate was inoculated with 5 mm disc of mycelial bit taken with the help of cork borer from the periphery of 7 days old culture of *R. bataticola* growing on PDA. The inoculated Petri dishes were incubated at 30± 1º C. Three Petri dishes were used for each treatment serving as three replications. A control was also maintained where medium was not supplemented with plant extract. The experiment was conducted in Completely Randomised Design (CRD). Colony diameter (Two diagonals) was measured at 7th day of incubation. Per cent growth inhibition was calculated by [7] formula as follows:

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

Where:

C = diameter of the colony in check (average of both diagonals)

T = diameter of colony in treatment (average of both diagonals)

Fungicides (*in vitro*)

Efficacy of five systemic and non systemic fungicides *viz*., carbendazim 50% W.P., tebuconazola 50% + trifloxystrobin 25% WG, cyamoxanil 8% + mancozeb 64% WP, thiram 75 WP and propineb 70% WP against *R. bataticola* was tested by Poisoned Food Technique [8]. Three different concentrations *viz*., 100, 300 and 500 ppm of each fungicide was evaluated. Required quantity of each fungicide was added separately to sterilized medium, mixed thoroughly and poured in sterilized 9 cm diameter glass Petri plates and allowed to solidify. Three replications were maintained for each treatment. A control was also maintained in which medium was not suspended with any fungicides. Each plate was inoculated with 5 mm discs taken with help of sterilized cork borer from the edge of the fungal culture and incubated at 30± 1º for 7 day. The linear growth of the test fungus was recorded and per cent growth inhibition was calculated by Vincent's formula mentioned as above.

Through bio-agents, plant extracts and fungicides (*in vivo*)

The bio-agents, plant extracts and fungicides, which proved to be efficacious *in vitro*, were also evaluated by seed treatment (*in vivo*) under pot conditions. Prior to sowing, cemented pots (45 cm diameter) were sterilized + FYM (soil: FYM= 3:1, sterilized at 1.045 kg/cm^2 for 1 hr for consecutive days). These pots were inoculated with 7 days old inoculum, multiplied on sorghum grains @ 20 g/pot. Apparently healthy and surface sterilized cluster bean seeds (var., RGC-936) were treated as per following details. Three replications were maintained for each treatment. Disease incidence was noted at 40 and 60 days after sowing. Per cent disease incidence was calculated as follows:

$$\text{Per disease incidence} = \frac{\text{Number of diseased plant}}{\text{Total number plant}} \times 100$$

Treatment	Dose	Method of seed treatment
Carbendazim 50% W.P.	2g/kg seed	By dry seed dressing
Tebuconazola 50% + Trifloxystrobin 25% W.G.	2g/kg seed	By dry seed dressing
Trichoderma harzianum	4g/kg seed	By dry seed dressing
Trichderma viride	4g/kg seed	By dry seed dressing
Garlic clove extract	100 ml/kg seed (10%)	By dipping of seeds for 30 min.
Neem leaf extract	100 ml/kg seed (10%)	By dipping of seeds for 30 min.

Results and Discussion

Management through bio-agents

Efficacy of *Bacillus subtilis*, *Trichoderma harzianum* and *T.viride* were tested against *R. bataticola* using Dual Culture Technique). After 7 days of incubation at 30 ± 1º C, the mycelial growth inhibition was recorded. Result (Table 1) indicated that all the bio-agents *viz*., *Bacillus subtilis*, *T. harzianum T. viride* were antagonistic to the growth of *R.*

bataticola. Maximum mycelial growth inhibition (70.67%) of the pathogen was recorded with *T.harzianum* followed by *T. viride* (63.25%) and minimum mycelia growth inhibition was recorded with *Bacillus subtilis* (44.22%). All bio-agents were found significantly superior with each other. Results are in agreement with findings of [9] who reported *Trichoderma viride* and *T. harzianum* as an effective in inhibition the mycelia growth of *Macrophomina phaseolina* inciting root rot of cluster bean *in vitro* as well as *in vivo* condition [10]. also reported that, *T.harzianum* and *T. viride* inhibited mycelia growth of *M. Phaseolina* incited groundnut root rot disease.

Management through plant extract (*in vitro*)

The efficacy of five plant extracts (Table 2) was tested *in vitro* at two concentrations viz., 5 and 10 per cent against *R. bataticola* on PDA by Poisoned Food Technique. Among five plant extracts, garlic cloves extract was found most effective in inhibiting mycelial growth (52.35 and 71.25%) of *R. bataticola* at 5 and 10 per cent respectively, followed by neem (44.52 and 64.32%) over control. Extracts of datura (42.39 and 54.25%), tulsi (35.12 and 50.39%) and marigold (12.67 and 37.55%) were found least effective in inhibiting mycelial growth of *R. bataticola* over control. All the concentrations (5 and 10%) of all the tested plant extracts were found significantly superior with each other [11]. have been reported inhibition of mycelial growth of *Rhizoctonia solani* causing sheath blight of rice by using 10% *Allium sativum* extract. Similar results have also been observed by [12] while working with *R. solani* under *in vitro* conditions.

Through fungicides *in vitro*

The efficacy of five fungicides (Table 3) was tested *in vitro* at three concentrations *viz*. 100, 300 and 500 ppm against *R. bataticola* on PDA by Poisoned Food Technique. Among five fungicides, carbendazim was found most effective in inhibiting mycelia growth (100%) of *R. bataticola* at 100, 300 and 500 ppm respectively followed by tebuconazole + trifloxystrobin (79.25, 92.34 and100 %) and thiram (68.00, 89.28 and 100%) over control. Fungicides like cymoxanil + mancozeb (59.32, 65.20 and 70.67%) and propineb (63.10, 69.78 ad 6.00%) were found least effective in inhibiting mycelial growth of test pathogen over control. All the concentrations (100, 300 and 500 ppm) of tested fungicides were found significantly superior with each other. Similar observations were also made by [13] who found that carbendazim and thiram were highly effective against *Macrophomina phaseolina* in laboratory as well as in field condition [14]. also reported that sclerotia production of *M. Phaseolina* was completely inhibited by using carbendazim and thiram, which again support the investigation.

Management through bio-agents, pat extracts and fungicides *in vivo*

Bio-agents, plant extracts and fungicides were found effective in *in vitro* were also tested as seed treatment under pot conditions against *R. bataticola* and these were garlic, neem, *T. harzianum*, *T. viride*, carbedazim and tebuconazole + tryfloxystrobin.

The resulted in Table 4 revealed that all plant extracts, bio-agents in regarding per cent disease control at 40 and 60 days after sowing. Minimum per cent disease incidence was recorded with carbendazim (13.20 and 15.25%) followed by tebuconazole + tryfloxystrobin (15.34 and 18.59%). *T. harzianum* (22.20 and 29.12%), *T. viride* (24.20 ad 32.13%) over control (43.83 and 62.86%) at 40 and 60 days after sowing, respectively.

Maximum disease control over check was recorded with carbendazim (69.92 and 75.74%), followed by tebuconazole + tryfloxystrobin (65.04 and 70.43%), *T. harzianum* (49.41 and 53.67%), *T. viride* (44.85 and 48.89%) and neem (30.63 and 39.34%) over control at 40 and 80 days after sowing, respectively. These observations are in line with those recorded by [15], who have been screened carbendazim, tebuconazole thiophanate methyl, captan, mancozeb and thiram against *M.phaseolina* causing root rot of cluster bean both *in vitro* and *in vivo*. They observed minimum emergence rot, post emergence seedling rot disease incidence and higher seed yield with carbendazim [16]. Evaluated inhibitory effect of *T. viride* against charcoal rot (*M. Phaseolina*) on cucumber under pot house condition [17]. Have also been reported the effectiveness of many plant extracts, bio-agents and fungicides in controlling with root rot of sage (*Selvia officinallis*) caused by *R. bataticola* and *Fusarium solani* in field as well as in laboratory.

Table 1: Efficacy of bio-agents against *Rhizoctonia bataticola* by Dual Culture Technique after 7th day at 30± 1 ºC

Bio-agents	Inhibition of mycelial growth (%)*
Bacillus subtilis	44.22 (41.68)
Trichoderma harzianum	70.67 (57.21)
Trichoderma viride	63.25 (52.68)
Control	0.00 (0.00)
SEm± CD (p=0.05)	0.88 2.70

* Average of three replications

Figures given in parentheses are angular transformed values

Table 2: Fungitoxicity of plant extracts against *Rhizoctonia bataticola* by poisoned food technique after 7th day at 30± 1 ºC

Common name of plant	Scientific name plant	Part used plant	Per cent mycelia growth inhibition at different concentrations*		
			5%	10%	Mean
Marigold	*Tegetus eracta*	Leaf	12.67 (20.85)	37.55 (37.79)	25.11
Neem	*Azadirachta indica*	Leaf	44.52 (41.85)	64.32 (53.32)	54.42
Garlic	*Allium sativum*	Clove	52.35 (46.35)	71.25 (57.58)	61.80
Tulsi	*Ocimum sanctum*	Leaf	35.12 (36.34)	50.39 (45.22)	42.76
Datura	*Datura stramonium*	Leaf	42.39 (40.62)	54.25 (47.44)	48.32
Control	-	-	0.00 (0.00)	0.00 (0.00)	
	P Con. P x Con.	SEm± 0.58 0.65 1.3	CD (p=0.05) 1.63 1.82 3.65		

* Average of three replications

Figures given in parentheses are angular transformed values

Table 3: Efficacy of fungicides against *Rhizoctonia bataticola* by poisoned food technique after 7th day at 30± 1 ºC

Fungicide	Per cent mycelia growth inhibition at various concentrations*			
	100ppm	300ppm	500ppm	Mean
Carbendazim	100 (90.00)	100 (90.00)	100 (90.00)	100
Tebuconazola + Trifloxystrobin	79.25 (62.90)	92.34 (73.93)	100 (90.00)	90.53
Cyamoxanil + Mancozeb	59.32 (50.37)	65.20 (53.85)	70.67 (57.21)	65.06
Thiram	68.00 (55.55)	89.28 (70.79)	100 (90.00)	85.76
Propineb	63.10 (52.59)	69.78 (56.65)	86.00 (68.03)	72.96
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	F C F x C	SEm± 1.17 1.30 2.61	CD (p=0.05) 3.23 3.61 7.23	

* Average of three replications
Figures given in parentheses are angular transformed values

Table 4: Efficacy of bio- agents, plant extracts andfungicides against root rot of clustrbean applied through seeds (in pots)

Treatments	Disease incidence (%)		Disease control (%)	
	40 DAS	60 DAS	40 DAS	60 DAS
Carbendazim	13.20(21.30)	15.25(22.99)	69.92	75.74
Tebuconazola + Trifloxystrobin	15.34(23.06)	18.59(25.54)	65.04	70.43
Trichoderma harzianum	22.20(28.11)	29.12(32.66)	49.41	53.67
Trichoderma viride	24.2(29.47)	32.13(34.53)	44.85	48.89
Garlic clove extract	28.56(32.30)	37.56(37.80)	34.91	40.25
Neem leaf extract	30.44(33.49)	38.13(38.14)	30.63	39,34
Control	43.88(41.48)	62.86952.45)	0.00	0.00
SEm± CD (p=0.05)	0.20 0.63	0.26 0.81		

* Average of three replications
Figures given in parentheses are angular transformed values

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