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Efficacy of botanicals and bioagents against root rot of cowpea incited by *Rhizoctonia bataticola*

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ABSTRACT

Cowpea [Vigna unguiculata (L.) Walp], an annual legume, is subject to root rot, a major disease incited by the fungus Rhizoctonia bataticola (Macrophomina phaseolina). This infection leads to leaf yellowing, rapid defoliation, wilting, and necrosis, as the pathogen blocks the plant's vascular bundles and secretes toxins. Infected stems and leaves become dry, brittle, and straw-colored. An experiment to address this disease was conducted at the farm of SKNCOA, Jobner, during Kharif 2023. Cowpea root rot, a major disease, leads to symptoms like leaf yellowing, rapid defoliation, wilting, necrosis, and dry, brittle stems/leaves due to vascular blockage. An experiment conducted in Kharif 2023 at SKNCOA, Jobner to tested the management strategies against this pathogen. Six botanicals were tested at 5, 10, and 15% concentrations by the poisoned food technique, among these garlic (86.43, 93.96 and 97.16%), was found most effective in inhibiting mycelial growth, followed by neem (84.76, 90.40 and 94.93%) leaves extract. Similarly, under field conditions using seed treatment, garlic (28.03%) was the most effective botanical, followed by neem (34.56%). Among four bioagents tested via the dual culture technique, Trichoderma harzianum (79.87%) was the most effective in inhibiting mycelial growth, followed by Trichoderma viride (71.75%). Field application as a seed treatment confirmed these results, with Trichoderma harzianum (26.46%) being the most effective bioagent against the root rot pathogen, followed by Trichoderma viride (33.80%).

Keywords: *Botanicals, Bioagents, Root Rot, Rhizoctonia bataticola.*

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] (diploid, 2n=22) belongs to the *Fabaceae* family and originated in Africa [2].

The name "Cowpea" probably derived from its use as an important livestock feed for cows in the United States. Known as "hungry-season crop", it is crucial because its harvest occurs first, preceding the cereal crops [3]. Due to its high nutritional value and early maturity, it is also known as "poor man's meat".

Cowpea is an important and versatile component of cropping systems, valued for its quick growth, high yield potential and ability to function as a catch crop, mulch crop, intercrop, mixed crop and green manure crop. Its roles as cover crop also significantly aids in soil conservation.

The crop contains high amount of protein (23.4%), carbohydrate (60.3%), calcium (76 mg/100 gm), iron (57 mg/100 gm) and vitamins such as thiamine (0.92 mg/100 gm), riboflavin (0.18 mg/100 gm) and nicotinic acid (1.9 mg/100 gm).

Cowpea as a self-pollinated, nitrogen fixing crop, cowpea enhances soil fertility, leading to higher cereal yields in rotation. *Bradyrhizobiuim* sp., symbiotic nodular bacteria fix the atmospheric nitrogen within the nodules and increases the nitrogen level at the rate of 40-80 kg N per ha [4].

Root rot caused by *Rhizoctonia bataticola* (Taub.) Butler (*Macrophomina phaseolina*) plays a significant role in reducing cowpea yields in India due to biotic and abiotic factors [7]. Incidence of root rot of cowpea ranging from 5 to 39 per cent [5].

The fungus capable of living as a facultative parasite on dead organic tissue, primarily from its natural host, and reproduces asexually by developing pycnidia. When atmospheric temperature is above 30 °C and the pycnidiospores remain viable for over a year since the fungus attack wide range of plant species [1].

The infected stems and leaves become dry, brittle and straw colored. The characteristic symptom of root rot is leaf yellowing, followed by defoliation within two to three days. The plants show wilting and necrosis symptoms resulting from the pathogen's vascular bundles blockage and secretion of toxic substances [4].

The expense and environmental risk associated with fungicides make them a less preferred control strategy. However wider use of fungicides is hazardous to nature and human health. Therefore, management with botanicals, bioagents, ecofriendly and economical approaches are employed to suppressing disease.

MATERIALS AND METHODS

Root rot infected plants of cowpea were collected from farmer's field near by vicinity of Jobner. Six botanicals *viz*., Tulsi, Garlic, Giloy, Neem, Aloevera, Clove and four bio-agents *viz*., *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens, Bacilllus subtilis* were used against *Rhizoctonia bataticola* under *in vitro and in vivo condition.*

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Efficacy of botanicals (poisoned food technique) and bio-agents (dual culture technique) were evaluated against *Rhizoctonia bataticola* mycelial growth, and field application as seed treatment and seed coating.

Efficacy of botanicals against *Rhizoctonia bataticola* (*in vitro* and *in vivo*)

Preparation of the botanicals

Plant materials such as fresh leaves/cloves of botanicals under test were harvested and thoroughly washed with tap water. Hundred grams from each plant was collected and washed 2-3 times with water and allowed to dry at room temperature (25±1°C) for six hours. Before extraction, leaves of each plant (100g) were crushed separately with 100 ml sterilized distilled water. The extract was filtered through muslin cloth and centrifuged at 5000 rpm for 15 min. The extracts were then sterilized by passing them through a filter using a Whatman filter. The supernatant obtained was considered as 100 per cent and diluted accordingly and stored at 5^0C for further use.

***In vitro* condition**

The present investigation was carried out using six different natural botanicals to see their antimycotic behaviour on the growth of *Rhizoctonia bataticola in vitro*. The extract of each plant species was diluted in order to achieve three concentrations *viz.,* 5, 10 and 15 per cent. Petri plates containing PDA supplemented with different botanicals, each with three concentrations and replicated three times. Plates were inoculated with 7 days old culture (5 mm diameter disc). A suitable check was also maintained. Fungal colony was measured after 7 days of incubation at 25±1°C. The linear growth of test fungus was recorded and per cent growth inhibition was calculated.

***In vivo* condition**

The field trials were conducted during *kharif* 2023 at instructional farm, SKN College of Agriculture, Jobner in randomized block design with three replications in 1.2 m x 1.5 m plots. The seeds of cowpea were soaked with the botanicals for 30 minutes and dried under shade. Untreated seeds sown under similar conditions served as control. Inoculum multiplied on sorghum grains was added in the row at the time of sowing. Per cent disease incidence was calculated using the formula given by Wheeler (1969)

$$PDI = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Efficacy of bio-agents against *Rhizoctonia bataticola* (*in vitro* and *in vivo*)

***In vitro* condition**

Four bio-control agents *viz*. *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtills* and *Pseudomonas fluorescens* were evaluated using "Dual Culture Technique" under CRD design and percent inhibition of mycelial growth were recorded (*in vitro*). Using dual culture plate method, 20 ml of autoclaved PDA was poured into in each sterilized petri plate and allowed for solidification after 3 hours of pouring, these plates were inoculated with 5 mm dia mycelial bit taken from 7 days old culture of *Rhizoctonia bataticola* and antagonistic agents both were placed separately at equal distance on the periphery of petri plates.

PDA petri plates inoculated with pathogen alone served as check. Inoculated petri plates were incubated at 25±1°C in BOD incubator for 7 days. Linear growth of pathogen as well as biocontrol agent was measured and per cent growth inhibition was recorded after 7 days of incubation, for each treatment three replications were taken.

***In vivo* condition**

Bioagents were also be tested under field condition (*in vivo*) as a seed treatment following RBD and per cent disease incidence was recorded at 50 days after sowing. Commercial formulations of bio-agents were tried alone against root rot disease, by applying as seed treatment. Four replications of each bio-agent were maintained. Before applying bio-agent on the surface of the seeds, seeds were moistened with five per cent gum solution applied at 10 ml/kg of seeds. The bio-agents were used as seed treatment 6 g/kg seeds with bio-agents. Inoculum multiplied on sorghum grains was added @ 20 g/m row at the time of sowing.

RESULT AND DISCUSSION

Efficacy of botanicals in inhibition of mycelium growth of *Rhizoctonia bataticola* by poisoned food technique

The efficacy of six botanicals (Table 1, Fig 1 and Plate 1) were tested *(in vitro)* at three concentrations *viz*., 5, 10 and 15 per cent against *Rhizoctonia bataticola* on PDA by Poisoned Food Technique.

Among tested six botanicals, Garlic was found most effective in inhibiting mycelial growth (86.43, 93.96 and 97.16%) of *Rhizoctonia bataticola* at 5, 10 and 15 per cent, respectively followed by neem (84.76, 90.40 and 94.93%), tulsi (42.90, 49.13 and 54.26%) and giloy (24.73, 33.95 and 61.83%) over control. Botanicals like aloe vera extract (17.23, 31.46 and 36.33%) and clove extract (12.16, 26.73 and 31.80%) were found least effective in inhibiting mycelial growth of *Rhizoctonia bataticola* over control.

Efficacy of botanicals against root rot of cowpea under field condition applied as seed treatment

An experiment was conducted to evaluate the management strategy of root rot of cowpea (Table 2 and Fig 2) revealed that all botanicals were found significantly superior over control @10% in reducing per cent disease control at 50 days after sowing.

Among tested botanicals, Garlic was found most effective in reducing per cent disease incidence (28.03%) over control followed by neem leaves extract (34.56%), tulsi (38.96%), giloy (42.73%), aloevera (44.60%), clove (45.16%) was least effective over control. Maximum per cent disease control was recorded with garlic (48.78%) followed by neem (36.85%) over control.

Efficacy of bio-agents in inhibition of mycelium growth of *Rhizoctonia bataticola* by dual culture technique

The efficacy of four bioagents (Table 3, fig 3, and Plate 2) *viz Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens and Bacillus subtilis* were tested against *Rhizoctonia bataticola* through dual culture technique.

Among four tested bioagents *Trichoderma harzianum* was found most effective in inhibiting mycelial growth (79.87%) of *Rhizoctonia bataticola* followed by *Trichoderma viride* (71.75%) over control. *Pseudomonas fluorescens* (52.65%), *Bacillus subtilis* (45.37%) was found least effective in inhibiting mycelial growth of *Rhizoctonia bataticola* over control.

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Efficacy of bio-agents against root rot of cowpea applied as seed treatment

A field experiment was conducted to evaluate the management strategy of root rot of cowpea (Table 4 and Fig 4) revealed that all bio-agents were found significantly superior over control in reducing per cent disease control at 50 days after sowing with seed application @ 6g/kg seed.

Trichoderma harzianum was found most effective in reducing per cent disease incidence (26.46%) over control followed by *Trichoderma viride* (33.80%). *Pseudomonas fluorescens* (39.05%), *Bacillius subtilis* (45.27%) was least effective over control. Maximum per cent disease control was recorded with *Trichoderma harzianum* (48.13%), followed by *Trichoderma viride* (33.75%) over control.

Table 1: Efficacy of botanicals in inhibition of mycelium growth of Rhizoctonia bataticola by poisoned food technique

S. NO.	Name of plant	Botanical Name	Plant part used	Per cent mycelial growth inhibition at different concentrations*			
				5%	10%	15%	Mean
1	Tulsi	*Ocimum tenuiflorum*	Leaves	42.90 (40.90)	49.13 (44.48)	54.26 (47.43)	48.76 (44.27)
2	Garlic	*Allium sativum*	Cloves	86.43 (68.39)	93.96 (75.87)	97.16 (80.42)	92.52 (74.89)
3	Giloy	*Tinospora cordifolia*	Stem	24.73 (29.80)	33.95 (35.61)	61.83 (51.83)	40.17 (39.08)
4	Neem	*Azadirachta indica*	Leaves	84.76 (67.02)	90.40 (71.97)	94.93 (77.04)	90.03 (72.01)
5	Aloevera	*Aloe barbadensis miller*	Leaves	17.23 (24.47)	31.46 (34.10)	36.33 (37.04)	28.34 (31.87)
6	Clove	*Syzygium aromaticum*	Flower buds	12.16 (20.36)	26.73 (31.11)	31.80 (34.31)	23.56 (28.59)
7	Control	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
			S.Em±	**CD (p=0.05)**			
		Botanicals (B)	0.47	1.34			
		Conc. (C)	0.30	0.88			
		B x C	0.81	2.33			

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

Table 2: Efficacy of botanicals against root rot of cowpea under field condition applied as seed treatment

Treatments	Plant part used	Concentration (%)	Per cent disease incidence*	Per cent disease over control
Tulsi	Leaves	10	38.96 (38.60)	28.81
Garlic	Cloves	10	28.03 (31.93)	48.78
Giloy	Stem	10	42.73 (40.80)	21.92
Neem	Leaves	10	34.56 (35.99)	36.85
Aloevera	Leaves	10	44.60 (41.88)	18.50
Clove	Flower buds	10	45.16 (42.20)	17.48
Control	-	-	54.73 (47.70)	-
S.Em±			1.15	
CD (p=0.05)			3.58	

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

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Table 3: Efficacy of bio-agents in inhibition of mycelium growth of Rhizoctonia bataticola by dual culture technique

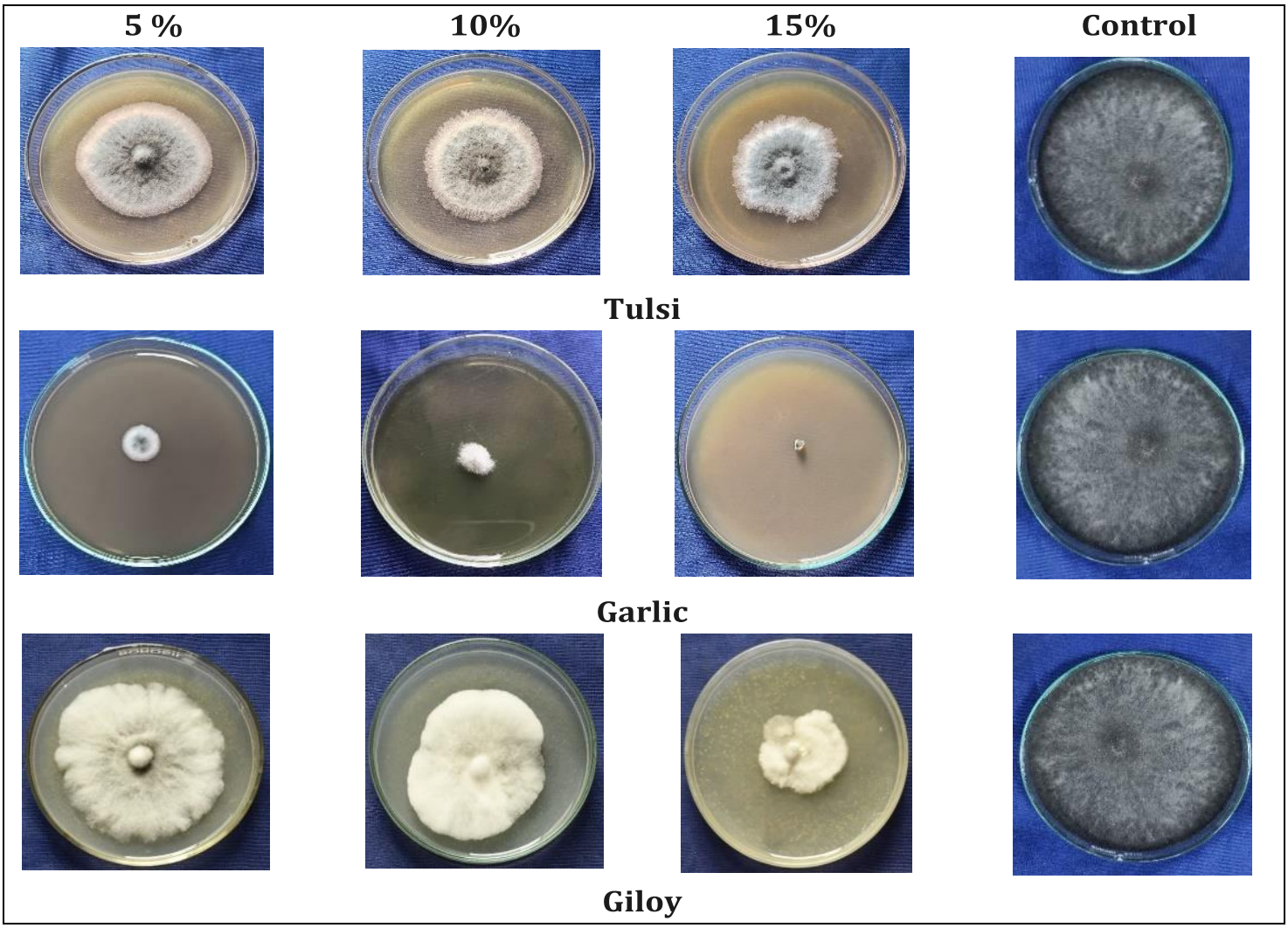
S.No.	Treatments	Inhibition of mycelial growth (%) *
1	*Trichoderma harzianum*	79.87 (63.32)
2	*Trichoderma viride*	71.75 (57,93)
3	*Pseudomonas fluorescens*	52.65 (46.50)
4	*Bacilllus subtilis*	45.37 (42.32)
5	Control	0 (0)
	S.Em±	0.77
	C.D. (p=0.05)	2.35

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

Table 4: Efficacy of bio-agents against root rot of cowpea applied as seed treatment

S.No.	Treatments	Concentration	Per cent disease incidence*	Per cent disease control
1	*Trichoderma harzianum*	6 gm/kg seed	26.46 (30.92)	48.13
2	*Trichoderma viride*	6 gm/kg seed	33.80 (35.52)	33.75
3	*Pseudomonas fluorescens*	6 gm/kg seed	39.05 (38.64)	23.46
4	*Bacillius subtilis*	6 gm/kg seed	45.27 (42.26)	11.27
5	Control		51.02 (45.56)	-
	S.Em±		0.79	
	CD (p=0.05)		2.47	

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



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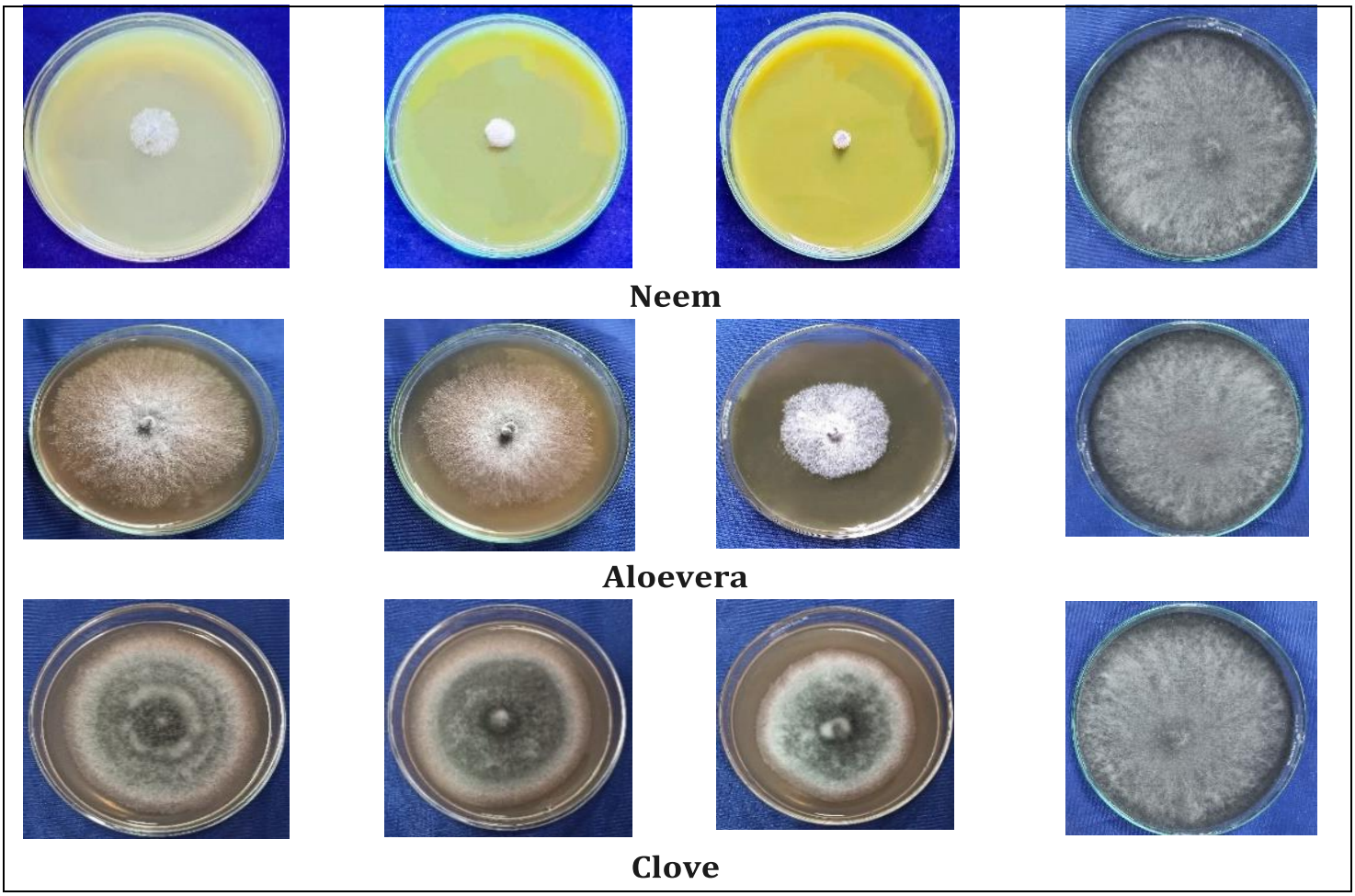

Plate: 1 Efficacy of botanicals in inhibition of mycelium growth of Rhizoctonia bataticola by poisoned food technique

Trichoderma harzianum | *Trichoderma viride* | *Pseudomonas fluorescens* | *Bacillus subtilis* | *Control*

Plate:2 Efficacy of bio-agents in inhibition of mycelium growth of Rhizoctonia bataticola by dual culture technique

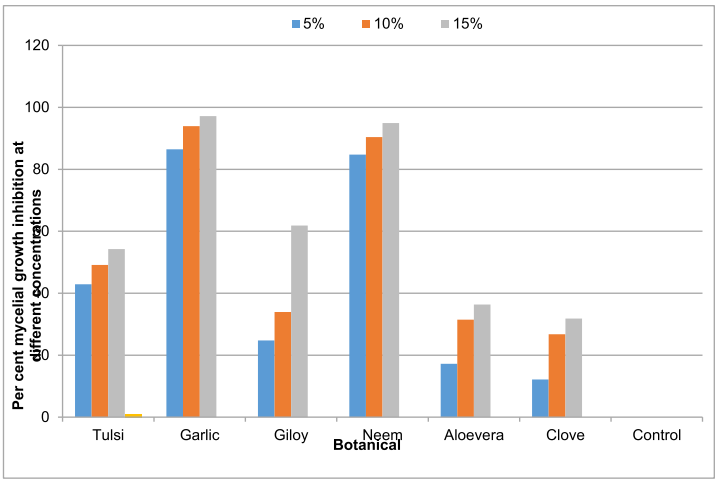

Fig 1: Efficacy of botanicals in inhibition of mycelium growth of Rhizoctonia bataticola by poisoned food technique

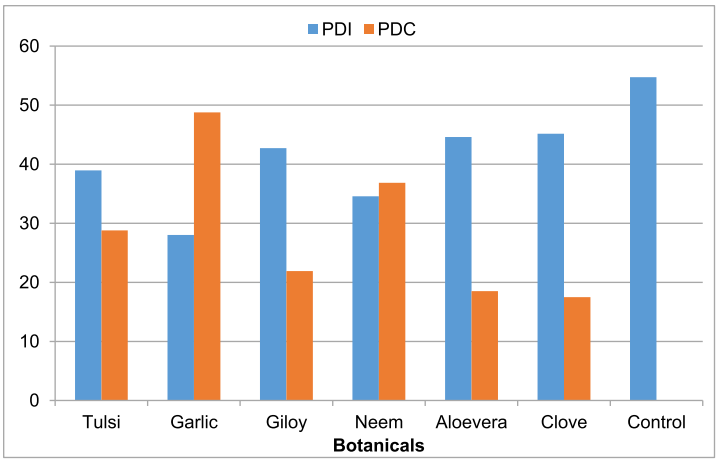

Fig 2: Efficacy of botanicals against root rot of cowpea under field condition applied as seed treatment

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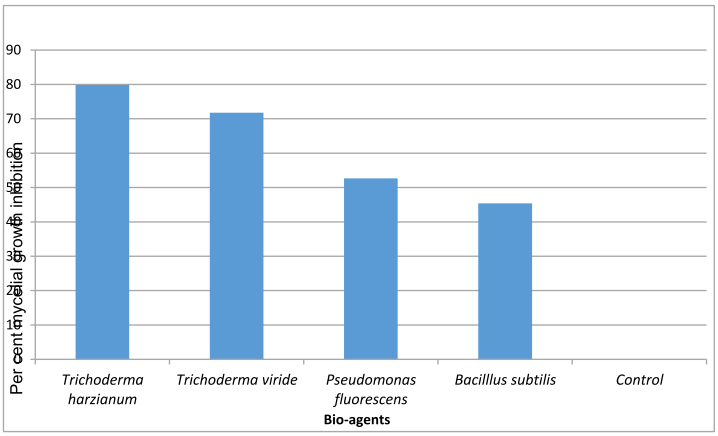

Fig 3: Efficacy of bio-agents in inhibition of mycelium growth of Rhizoctonia bataticola by dual culture technique

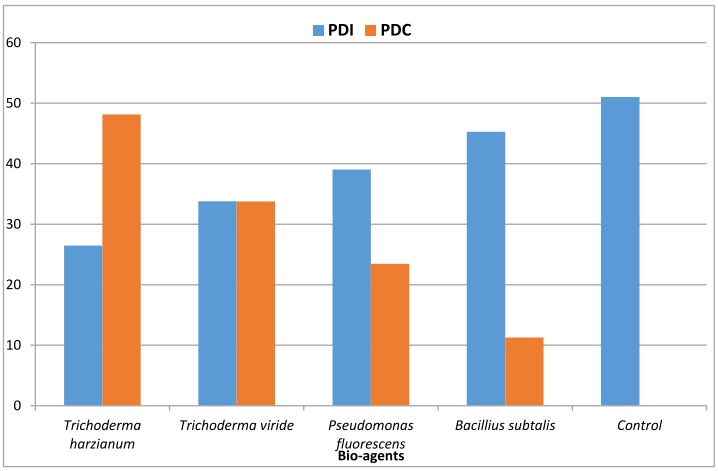

Fig 4: Efficacy of bio-agents against root rot of cowpea applied as seed treatment

CONCLUSION

Garlic extract (@15%) was found highly inhibitory against *Rhizoctonia bataticola* and (@10%) effective in control of root rot of cowpea followed by neem. *Trichoderma harzianum* was found highly inhibitory and (@6g/kg) effective in disease control followed by *Trichoderma viride.*

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