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Evaluation of Bio-control Agents and Organic Amendments for Managing Root Rot (*Rhizoctonia solani*) of Clusterbean (*Cyamopsis tetragonoloba*)

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ABSTRACT

Background: Root rot of clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] caused by *Rhizoctonia solani* is an important menace and causes significant economic losses in India and chemical pesticides are mostly used to overcome this problem. As per environment and health issues and demand of organic produce, the current study aimed to find the most effective control measure of this dreaded disease through eco-friendly approaches.

Methods: The present field-laboratory investigations were conducted during 2018, to evaluate four bio-agents *in vitro* and *in vivo* (*Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens)* and five organic amendments *in vivo* namely wool waste (@ 50 q/ ha), human hair (@ 50 q/ ha), mustard cake (@ 5 q/ ha), castor cake (@ 6 q/ ha) and neem cake (@ 5 q/ ha) were evaluated.

Result: Our investigations *in vitro* with bio-agents depicted that *T. harzianum* was highly inhibitory (62.65 %) followed by *T. viride* (48.52%). Seed-cum-soil application (6g/kg seed + 6kg/ha) of *T. harzianum* was found most superior in reducing disease incidence (74.03%) followed by *Trichoderma viride* (69.83%) while in organic amendments, neem cake (5 q/ha) was found highly effective (70.07%) followed by castor cake (64.40%), mustard cake, wool waste and least effective was human hair. Though, wool waste and human hair least effective in disease management but preliminarily results indicated encouraging response with dual action, one in reducing disease and another in increasing plant biomass that open the future scope of further more sustainable experimentations. The findings of this study can be utilized to manage the disease effectively and eco-friendly.

Key words: Bio-agents, Clusterbean, Organic amendments, *Rhizoctonia solani*, Root rot.

INTRODUCTION

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] is also known by various names like "*Guar*" or "*Guwar*" and it belongs to family *Fabaceae* of kingdom *Plantae.* It is an important legume crop and mainly grown under rainfed conditions of arid and semi arid regions of tropical India during *Kharif* and *Zaid* seasons. It is considered as one of the most drought tolerant grain legumes and very valuable within crop rotation cycle as it lives in symbiotic association with nitrogen fixing bacteria and also used as vegetable, green fodder, green manuring, production of seed and for endospermic gum (30-35 per cent). Green pods of clusterbean are nutritionally rich in protein (3.2 g), fat (1.4 g), carbohydrate (10.8 g), vitamin-A (65.31 IU), vitamin-C (49 mg), calcium (57 mg) and iron (4.5 mg) per 100 g of edible portion (Kumar and Singh, 2002). In Rajasthan, clusterbean as a vegetable crop is cultivated throughout the state for its green pods (immature pods) occupying an area 694 hectares with production of 976 metric tonnes (Anonymous, 2016). For seed production, it is grown in arid and semi-arid regions mainly during rainy season while, for vegetable purpose during *Zaid* and rainy seasons. As vegetable crop, it produces green pods continuously for a long time, thus it needs regular feeding along with much care from pests especially from diseases. Though, it is a hardy crop, but some important diseases like root rot, Alternaria blight, bacterial blight, powdery mildew *etc.* severely damage the crop. Among these, root rot caused by *Rhizoctonia solani* is one of the major diseases occurs in Rajasthan. Lodha *et al.* (1986) and Lodha (1998) observed 31.0 per cent root rot incidence of clusterbean with 32.11 per cent yield loss in arid regions of Rajasthan and appeared at any stage of the crop from pre- emergence to maturity.

Among the initial symptoms of the disease, yellowing of leaves is a first symptom which in next 2 or 3 days leaves droop and wither off. Infected plants may wilt within a week

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and dark lesions can be observed on the bark near ground level. On pulling, the basal stem along with main root show rotting and in advanced cases sclerotial bodies seen scattered on the affected roots. The fungus is mainly a soil dweller and spreads from plant to plant through irrigation water and implements and cultural operations. The sclerotia and pycniospores may also become air borne and cause further spread of the pathogen (Rangaswami and Mahadevan, 2008). In soil, long saprophytic survivability of the pathogen makes chemical control and crop alternation unsuccessful while use of resistant sources is an economical approach for managing dry root rot diseases of pulses caused by *Macrophomina phaseolina* (Manjunath and Saifulla, 2018). Lakhran and Ahir (2018) evaluated bio-control agents, plant extracts and oil cakes against *Macrophomina phaseolina* causing dry root rot of chickpea and observed *Trichoderma viride,* garlic extract and neem cake as highly effective in controlling disease while wool waste and goat manure was the least effective.

Exclusive dependency on pesticides for managing diseases has resulted in residue and environmental disturbances. Consequently, in modern era, efforts are being diverted to employ bio-agents and organic amendments for integrated disease management because they do not cause bio-accumulation in eatables and environmental pollution. Disease management through bio-agents and organic amendments is also an important segment in present era looking to the organic produce, with reference to hazards resulted by toxic chemicals or being developed resistance in pathogens to fungicides. In lieu of this, four bio-agents and five organic amendments were applied for managing the disease under field conditions.

MATERIALS AND METHODS

Isolation and identification of the pathogen

The experiments were conducted in laboratory and field in 2018 at Department of Plant Pathology, S.K.N. College of Agriculture, Jobner, Jaipur (Rajasthan). Jobner, is situated at latitude 26° 05′ N, longitude of 75° 28′ E and altitude of 427 meters above MSL (mean sea level). This region falls under semi-arid eastern plain (Agro Climatic Zone- lll A) of Rajasthan. The isolated fungus was identified on the basis of morphological characters and further was also got confirmed from ITCC, Division of Plant Pathology, IARI, New Delhi and identified as *Rhizoctonia solani* with ID No. 10.659.17.

In vitro evaluation of bio-control agents

Evaluation of four bio-control agents (*Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens)* was done by dual culture technique (Dennis and Webstar, 1971). Petri plates containing potato dextrose agar (PDA) were inoculated with 5 mm diameter bits of *Rhizoctonia solani* and antagonistic agents both were placed separately at equal distance on the periphery of Petri plates and incubated at 30$\pm$1°C in BOD incubator and regularly watched till full growth in check upto 7 days. Linear growth of pathogen as well as bio-control agent was measured and per cent growth inhibition was recorded 7 days after incubation. For efficacy of bacterial bio-control agents (*Pseudomonas fluorescens* and *Bacillus subtilis*) sterilized Petri plates containing 20 ml of nutrient agar were first inoculated with 7 days old culture of *Rhizoctonia solani* and incubated at 30$\pm$1°C for 24 hrs. These plates were again inoculated with 5 mm disc of sterilized four filter paper dipped in suspension of bacterial bio-control agent. Four such discs were placed at equal distance and incubated at 30$\pm$1°C for 7 days. The per cent growth inhibition was measured after 7 days of incubation. For each treatment four repetitions were maintained. Per cent inhibition of mycelial growth was calculated as per formula given by Vincent (1947).

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

Where, C =Diameter of the colony in check (average of both diagonals), T = diameter of colony in treatment (average of both diagonals).

Evaluation of bio-control agents under field conditions

Four bio-agents *(Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens)* were tested in the field by applying through seed, soil and seed-cum-soil methods with four replications under randomized block design (RBD). In all the field experiments, crop was sown in first week of March and inoculum, multiplied on sorghum grains was applied @ 20 g/m row at the time of sowing in plots (2×1 m^2). Fungus inoculated plots without any treatment served as check. Ordinary agronomical practices were followed in preparation of the field to raise the crop. Observation on root rot incidence was recorded at 40 and 60 days after sowing (DAS). Per cent disease incidence (PDI) and per cent disease control in various experiments were calculated as follows:

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

Disease control (%) =

$$\frac{\text{Dsease incidence in inoculated control (\%)} - \text{Disease incidence in treatment (\%)}}{\text{Disease incidence in inoculated control (\%)}} \times 100$$

Bio-agents applied through seeds

Before applying of bio-agents on the surface of seeds, the seeds of vegetable clusterbean (var. M-83) were moistened with 5 per cent gum solution (10 ml/kg seeds). Then, the seeds were treated with commercially available formulation of bio-agents @ 6g/kg seeds.

Bio-agents applied through soil

Prior soil application, the commercially available formulations of bio- agents (6kg/ha) were multiplied on fully decomposed and moistened FYM for 10 days under shade then enriched FYM was incorporated into the field at the time of sowing.

Bio-agents applied through seed and soil

The seeds were treated with bio- agents (6 g/kg seeds) and then bio-agents (6kg/ha) also applied into soil with enriched FYM.

Evaluation of organic amendments under field conditions

Six treatments including control with four replications in 2x1 m^2 plot size were evaluated. Soil was amended with five organic amendments *viz.*, wool waste (@ 50 q/ ha), human hair (@ 50 q/ ha), mustard cake (@ 5 q/ ha), castor cake (@ 6 q/ ha) and neem cake (@ 5 q/ ha) prior 2 week of sowing. A light irrigation was applied after incorporating of amendments. The inoculum multiplied on sorghum grains was added @ 20 g per meter row in each plot and mixed thoroughly up to 5-7 cm depth in the plots at the time of sowing. Plots without amendment served as check. Moderately susceptible vegetable clusterbean variety M-83 was sown. Observation on per cent disease incidence was recorded at 40 and 60 DAS.

RESULTS AND DISCUSSION

Evaluation of bio-agents (*in vitro*)

Results (Table 1) indicated that all the evaluated bio-control agents *viz., T. harzianum*, *T. viride, B. subtilis* and *P. fluorescens* were found statistically significantly superior over control in inhibiting the growth of *Rhizoctonia solani.* Maximum mycelial growth inhibition (62.65%) of the pathogen was recorded with *T. harzianum* followed by *T. viride* (48.52%) and minimum mycelial growth inhibition was recorded with *Pseudomonas fluorescens* (22.10%) and *Bacillus subtilis* (10.05%). Our findings are in agreement with the result of Deshmukh and Raut (1992) who observed *T. harzianum* and *T. viride* as an effective in inhibiting the mycelial growth of *M. phaseolina* and reducing the disease incidence. *Trichoderma harzianum* and *T. viride* have also been recorded effective in growth inhibition by Manczinger *et al.* (2002) and Meena and Pandey (2015) against *M. phaseolina* and *R. solani*.

Table 1: Efficacy of bio-agents against *Rhizoctonia solani* by dual culture technique after 7 days of incubation at 30 ± 1°C.

Bio-agent	Per cent inhibition of mycelial growth*
Trichoderma harzianum	62.65 (52.33)
Trichoderma viride	48.52 (44.15)
Pseudomonas fluorescens	22.10 (28.04)
Bacillus subtilis	10.05 (18.48)
Control	0 (0.00)
SEm±	0.72
CD (p=0.05)	2.21

*Average of four repetitions, Figures given in parentheses are angular transformed values.

Efficacy of bio-agents applied through seed treatment.

A perusal of data (Table 2) revealed that all the treatments were found statistically significantly superior over check. Maximum disease reduction over check was observed with *T. harzianum* (57.77 and 55.55%) followed by *T. viride* (50.05 and 49.29%) at 40 and 60 days after sowing, respectively. Minimum per cent disease control was recorded in *Bacillus subtilis* (34.16 and 31.12%) followed by *Pseudomonas fluorescens* (40.75 and 38.02%).

Efficacy of bio-agents applied through enriched FYM in soil

The result (Table 3) indicated that all the treatments were found statistically significantly superior over check. The highest disease reduction was observed with *T. harzianum* (64.39 and 62.57%) followed by *T. viride* (58.14 and 56.85%) over control at 40 and 60 days after sowing, respectively. The lowest disease reduction was recorded in *Bacillus subtilis* (36.39 and 32.89%).

Efficacy of bio-agents applied through seed-cum- soil treatment

A perusal of data (Table 4) showed that all the treatments were found statistically significantly superior over check. Maximum disease reduction was observed with *T. harzianum* (76.53 and 74.03%) followed by *T. viride* (72.70 and 69.83 %) over control at 40 and 60 days after sowing, respectively

Table 2: Efficacy of bio-agents against root rot of vegetable clusterbean applied through seeds.

Bio-agents	Dose	Per cent disease incidence*		Per cent disease control	
		40 DAS	60 DAS	40 DAS	60 DAS
Trichoderma harzianum	6g /kg	23.25 (28.83)	27.20 (31.44)	57.77	55.55
Trichoderma viride	6g /kg	24.50 (29.67)	31.03 (33.85)	50.05	49.29
Pseudomonas fluorescens	6g /kg	32.62 (34.83)	37.93 (38.02)	40.75	38.02
Bacillus subtilis	6g /kg	36.25 (37.02)	42.15 (40.48)	34.16	31.12
Control	-	55.06 (47.90)	61.20 (51.47)	0.00	0.00
SEm±	-	0.86	0.95	-	-
CD (p=0.05)	-	2.66	2.94	-	-

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

while minimum with *Bacillus subtilis* (49.71 and 29.25%) followed by *Pseudomonas fluorescens.*

Merely dependency on pesticides for managing diseases has resulted in residue and environmental disturbances. Consequently, in recent years, efforts are being diverted to employ bio-agents as a tool for integrated disease management because they do not cause bio-accumulation in eatables and environmental pollution. Disease management through bio-agents is also an important segment in present era looking to the organic produce, with reference to hazards resulted by toxic chemicals or being developed resistance in pathogens to fungicides. Our observations are in agreement with the findings of Deshmukh and Raut (1992) who observed *T. harzianum* and *T. viride* as an effective in inhibiting the mycelial growth of *M. phaseolina* and reducing the disease incidence. Manczinger *et al.* (2002) also noted that *Trichoderma harzianum*, *T. viride* and *T. polysporum* have a strong antagonistic action against soil borne pathogens.

Evaluation of organic amendments under field conditions

It is evident from the data (Table 5) that all the organic manures tested during experimentation reduced root rot incidence of clusterbean significantly over check. Neem cake was rated most effective over all other treatments and resulted in maximum disease reduction (79.15 and 70.07%) followed by castor cake (70.47 and 64.40%) and mustard cake (57.08 and 51.78%) at 40 and 60 days after sowing, respectively. Wool waste and human hair were found least effective in reducing root rot incidence at 40 and 60 days after sowing, respectively.

Incorporation of organic manures into the soil, improves structure and texture of the soil. These exert positive impact on plant growth by changing aeration, porosity temperature and water holding capacity of the soil which results in rapid root extension, balance availability of nutrients and better plant vigour. All these changes indirectly reduce the incidence and intensity of plant diseases. Their effect may include germination of pathogen propagules followed by starvation, microbial lysis and increase general fungistatis. Our results of field experiment indicated that all the organic manures tested, reduced root rot incidence significantly over check. Neem cake was most superior in exhibiting minimum root rot incidence followed by castor cake. Our findings are also in line with the results of Lodha (1993) and Lodha *et al.* (2002) who reported that the population density of *Macrophomina phaseolina* was reduced when soil amended with compost that ultimately resulted in decreasing dry rot incidence and increasing yield of clusterbean. Tiyagi and Alam (1995) tested efficacy of oil cake of neem against density of soil inhabiting fungi in mung bean. The frequency of pathogenic fungi like *Macrophomina phaseolina* including *Rhizoctonina solani*, *Phylosticta phaseolina* and *Fusarium oxysporum* f.sp. *ciceri* were significantly declined. Kumar *et al.* (2005) recorded inhibition of germination of conidia of *Arthrobotrys dactyloides* in soil duly amended with neem cake. It was due to the toxic principle (azadirachtin) present in the neem cake. Karcho *et al.* (2015) evaluated organic amendments, bio agents and fungicides on population dynamics of fungi in chickpea field.

Table 3: Efficacy of bio-agents against root rot of vegetable clusterbean applied in soil through enriched FYM.

Bio-agents	Dose	Per cent disease incidence*		Per cent diseasecontrol	
		40 DAS	60 DAS	40 DAS	60 DAS
Trichoderma harzianum	6 kg/ha	19.24 (26.02)	23.59 (29.06)	64.39	62.57
Trichoderma viride	6 kg/ha	22.62 (28.40)	27.20 (31.44)	58.14	56.85
Pseudomonas fluorescens	6 kg/ha	30.00 (33.21)	36.57 (37.21)	44.48	41.98
Bacillus subtilis	6 kg/ha	34.37 (35.89)	42.30 (40.57)	36.39	32.89
Control	-	54.04 (47.32)	63.04 (52.56)	0.00	0.00
SEm±		0.85	0.99	-	-
CD (p=0.05)		2.61	3.04	-	-

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

Table 4: Efficacy of bio-agents against root rot of vegetable clusterbean applied through seed –cum- soil method.

Bio-agent	Dose (seed+ soil application)	Per cent disease incidence*		Per cent disease control	
		40 DAS	60 DAS	40 DAS	60 DAS
Trichoderma harzianum	6 g/kg + 6 kg/ha	12.25(20.49)	15.63 (23.29)	76.53	74.03
Trichoderma viride	6 g/kg + 6 kg/ha	14.25 (22.18)	18.16 (25.22)	72.70	69.83
Pseudomonas fluorescens	6 g/kg + 6 kg/ha	25.75 (30.49)	36.78 (37.33)	50.67	38.90
Bacillus subtilis	6 g/kg + 6 kg/ha	26.25 (30.82)	42.59 (40.74)	49.71	29.25
Control	-	52.20 (46.26)	60.20 (50.89)	0.00	0.00
SEm±		0.85	0.98		
CD (p=0.05)		2.61	3.02		

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

Table 5: Efficacy of organic amendments against root rot of vegetable clusterbean applied through soil.

Organic amendments	Dose	Per cent disease incidence*		Per cent disease control	
		40 DAS	60 DAS	40 DAS	60 DAS
Neem cake	5 q/ha	12.14(20.39)	18.26(25.30)	79.15	70.07
Castor cake	5 q/ha	17.20(24.50)	22.15(28.08)	70.47	64.40
Mustard cake	6 q/ha	25.00(30.00)	30.00(33.21)	57.08	51.78
Wool waste	50 q/ha	32.62(34.83)	37.57(37.80)	44.00	39.61
Human hair	50 q/ha	35.62(36.64)	44.54(41.87)	38.84	28.41
Control	-	58.25(49.75)	62.22(52.07)	0.00	0.00
SEm±		0.87	0.94		
CD (p=0.05)		2.67	2.90		

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

Though, wool waste and human hair were least effective in disease control but their additional effect in providing sound growth to the crop has observed that ultimate resulted in increased biomass and yield production. Good plant vigour and slight reduction in disease incidence might be due to increase in microbial population of rhizospheric area, good aeration and nutrient availability to the plants. Zheljazkov (2005) has reported that human hair is one of the highest nitrogen containing (16%) organic materials in nature, because it is predominantly made up of (nitrogen-containing) proteins. In addition, it also contains sulfur, carbon and 20 other elements for plants. Our results also have the support by the findings of Sharma *et al.* (2011) who reported that soil fungus (*Chrysosporium indicum*) have the ability to degrade keratin of human hair and animal hair in soil and release chemicals that might be harmful for the pathogens and beneficial for plants. This is the first type of study, where wool waste and human hair included in disease management field trials that preliminarily indicated encouraging response with dual action, one in reducing disease and another in increasing plant biomass that open the future outlook of further more altered and sustainable experimentations.

CONCLUSION

It can be concluded that seed-cum-soil application of *T. harzianum* culture (6g/kg seed + 6kg/ha) is most superior in managing disease while in case of organic amendments, neem cake (5 q/ha) is most effective in reducing disease incidence (70.07%). Though, wool waste and human hair least effective in disease management but preliminarily results indicated encouraging response with dual action, one in reducing disease and another in increasing plant biomass that open the future scope of further more sustainable experimentations.

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