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Ecofriendly Management of Dry Root Rot in Black Gram Caused by *Macrophomina phaseolina*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In the present study *Trichoderma asperellum* (ECK), *Trichoderma asperellum* (TNAU), *Streptomyces* sp (native) and *Pseudomonas fluorescens* (TNAU) were tested *in vitro* by dual culture technique. Among the six treatments *Trichoderma asperellum* (ECK) and *Streptomyces* sp (native) *was* found to be more effective as compared to other bio-control agents and inhibited maximum fungal growth (100 %) of *Macrophomina phaseolina* followed by *Trichoderma asperellum* (TNAU) (81.6 %). The disease incidence controlled by all other isolates was ranged from 67.72 to 81.66%. The growth promoting activity of various bio-control agents and chemical was assessed

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based on seedling vigour index by standard Roll Towel Method (ISTA,1993). The treated seeds and controls are placed on the pre-soaked germination paper. After one-week various growth parameters like root length, shoot length, vigour index are determined by using the formulae. The seeds treated with *Trichoderma asperellum* (ECK) and *Streptomyces* sp (native) exhibited increased yield parameters like increased germination percentage, root length, shoot length, and Seedling vigour index 2562.0. Under field conditions the seeds are treated with different biocontrol agents and carbendazim. The various growth parameters like root length, shoot length and seedling vigour index observed under field conditions. *Streptomyces* sp (native) was found to be most effective in reducing pre and post emergence mortality and also it enhances the yield than other treatments. In field trail among the six treatments, treatment containing seed treatment of *Streptomyces* sp (native) was found to be most effective in reducing pre and post emergence mortality with germination of 87.0 %. *Streptomyces* sp (native) was found to be more effective as compared to other bio-control agents in increasing the yield parameters like root length, shoot length, and seedling vigour and also it enhances the yield *(322.8 Kg/ha)* compared to other treatments. *Streptomyces* sp (native) needs further species level characterization and other biotrophic and tritrophic interaction studies for developing commercial formulation.

Keywords: Biological control; macrophomina phaseolina; pseudomonas fluorescens; streptomyces sp.; trichoderma asperellum.

1. INTRODUCTION

Black gram is one of the most important pulse crops. Black gram popularly known as urdbean or mash, is a grain legume domesticated from *V. mungo var silvestris*. Blackgram, is the fourth important pulse crop cultivated in India [1,2]. India is the world's largest producer and consumer of black gram. In India 1.5 to 1.9 million tons of black gram annually from about 3.5 million hectares of area with a average productivity of 500 kg/ha and India's total pulse production is about 10%. In Tamilnadu the average area of cultivation 2.88 lakh hectare, production of 1.41 lakh tonnes and productivity of 463.67kg per hectare in black gram. The production is constrained by higher incidence of disease and pest attack. Black gram is affected by several fungal diseases among which dry root rot is the major fungal disease in black gram. Root rot is caused by virulent and destructive pathogen *Macrophomina phaseolina. Macrophomina* is primarily soil borne in nature with heterogeneous host specificity. The dry root rot symptoms are most commonly observed in chickpea during post-flowering stage which include drooping and chlorosis of petioles and leaflets, initially confined to top leaves of the plant. Leaves and stems of affected plants are usually straw coloured and in some cases, the lower leaves and stems are brown [3-5]. This infection is due to high soil temperature and low soil moisture, particularly the regions with warm and dry weather conditions challenging severe yield loss during their growing season. It causes 28.6 % yield loss in black gram. The pathogen was isolated from the affected portion of the diseased plants collected from different places [6,7]. The pathogen was isolated from the diseased plants separately purified and maintained. Present study was planned to formulate a suitable black gram root rot management technology through isolation of location specific biocontrol agents and to investigate unexplored microorganisms for root rot management as an alternate for existing biocontrol agents.

2. METHODOLOGY

2.1 Isolation of Pathogen

The pathogen was isolated from the affected portion of the diseased plants collected from different places. The infected plant with root rot symptom were pulled out and infected portions were cut into small pieces of 1cm to 1.5 cm which is surface sterilized by using 0.1% mercuric chloride for 1min and washed in sterile distilled water thrice and cut portions placed on the solidified petri dish (9mm) containing Potato dextrose agar (PDA) medium. These plates were incubated at room temperature of 28 ± 2°C for five days and observed for the radial growth of the fungi after 2-3 days.

2.2 Dual Culture Technique

In vitro efficacy of four resident fungal and bacterial antagonists are TNAU *Trichoderma asperellum,* ECK *Trichoderma asperellum, Pseudomonas fluorescence, Streptomyces sp* was tested using this method. Transfer of 15-

20ml Autoclaved PDA to each sterilized petri plates under aseptic condition and allowed to solidify. A 7mm disc of actively growing pathogenic fungal culture is placed on one side at one cm away from the edge of the petri plate and incubated under laboratory conditions. 48 hrs later, actively growing 48 hrs old culture of test antagonist is streaked on the medium at the opposite side of the plate. The PDA petri plates inoculated with the pathogen alone served as control. Inoculated petri plates were incubated at 25 ±1oC in the incubator for 7 days. For each treatment 3 replication taken. The radial growth of the pathogen and the antagonist was measured and the percent inhibition was recorded after 7 days of incubation.

Percent inhibition over control (%) = Dc-Dt/Dc*100

Dc - Average radial growth of the pathogen in control (cm).
Dt - Average radial growth of the pathogen in treatment (cm).

2.3 Poisoned Food Technique

A known quantity of test fungicide dissolved in 100 ml of sterile molten PDA medium to get a final concentration of 0.05, 0.1, 0.2% and poured into sterile Petri plates under aseptic conditions and allowed to solidify. Then mycelial discs of 7 mm diameter test pathogen are cut from 7 days old culture plate and place it in the Centre of petri plates containing PDA medium amended with fungicide. The PDA medium (without fungicide) inoculated with test fungus alone served as control. The plates are incubated at room temperature for 7 days until the control plate completely covered by test fungus. Each treatment is replicated for three times. After the incubation period depending upon the pathogen, the radial growth of the fungus is measured separately. The percent inhibition of growth of the test pathogen was calculated by using the formula.

Percent inhibition over control = C-T/C*100

Where,

C = growth of fungus (cm) in control
T = growth of fungus (cm) in treatment

2.4 Roll Towel Method

The growth promoting activity of various bio-control agents and chemical was assessed based on seedling vigour index by standard Roll Towel Method (ISTA,1993). The treated seeds T1, T2, T3, T4, T5, and control are placed on the pre-soaked germination paper. The seeds are rolled up along with the polythene sheet and incubated in growth chamber for one week. After one-week seedling vigour index are determined by using the below formulae.

Seedling vigour index = (Average root length+ Average shoot length) *germination (%)

TREATMENT DETAILS

T1-TNAU *Trichoderma sp*
T2-ECK *Trichoderma sp*
T3-*Pseudomonas fluorescens*
T4-*Streptomyces sp*
T5-Carbendazim
T6-Control

3. RESULTS AND DISCUSSION

Present study was planned to formulate a suitable black gram root rot management technology through isolation of location specific biocontrol agents and to investigate unexplored microorganisms for root rot management as an alternate for existing biocontrol agents. The results of this study are discussed hereunder in the present study *Trichoderma asperellum ECK, Trichoderma asperellum TNAU*, *Streptomyces* sp and *Pseudomonas fluorescens* were tested *in vitro*. Among the six treatments *Trichoderma asperellum ECK was* found to be more effective as compared to other bio-control agents and inhibited maximum fungal growth (100%) of *Macrophomina phaseolina* followed by *Trichoderma asperellum TNAU* (81.6 %) followed by *Streptomyces* sp (native) (68.0 %).

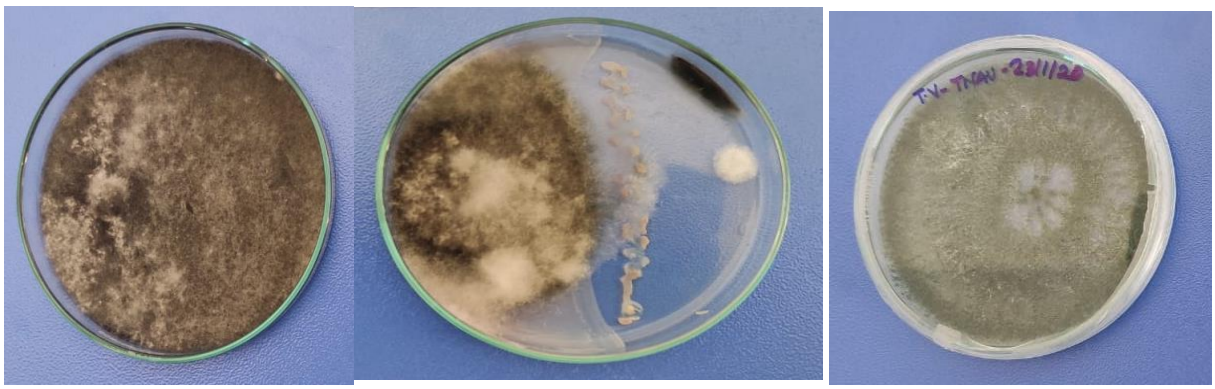
In field trail among the six treatments, treatment containing seed treatment of *Streptomyces* was found to be most effective in reducing pre and post emergence mortality by (15.0 %), *Streptomyces* sp *was* found to be more effective as compared to other bio-control agents in increasing the yield parameters like root length, shoot length, and seedling vigour and also it enhances the yield (*322.8 Kg/ha)* compare to other treatments.

Biological control is one of the sustainable and ecofriendly methods for suppressing plant diseases. Seed treatment and soil application of *Trichoderma* species has been reported as most effective for the management of dry root rot

disease in black gram. Most of these studies were carried out in the laboratories to evaluate the antagonistic effects of *Trichoderma* (biocontrol fungi) to inhibit growth of root rot pathogens, *Macrophomina*, few are in the glasshouse and field. In the field study, [8] examined that mixed application of vermicompost (10%) + bavistin (0.1%) + *T. harzianum* (4%) reduced seedling mortality caused by *M. phaseolina* where 5.74 and 5.04% mortality of seedlings were reported during pre and post emergence, respectively [9]. In greenhouse, application of *T. harzianum* (4 g/kg seeds) with 25 g/kg of phosphate solubilizing bacteria (PSB) as seed dresser reduced 26% incidence of dry root rot. *T. viride* and *T. harzianum* reduced the mycelial growth (42.33 and 44.25 mm, respectively) of *Macrophomina* in dual culture method and combined application of *T. harzianum* at 4 g kg-1 + PSB at 25 g/kg seed as seed dresser with FYM in field trials had minimum dry root rot incidence in seedlings (26.0%) [10].

Table 1. Mycelial growth of the pathogen

Measurements Treatment	**Mycelial growth of the pathogen(cm)**			
Control	R1	R2	R3	PI (%)
T1	1.5	1.3	2.0	81.66
T2	2.5	2.2	2.7	100.0
T3	2.9	3.0	2.8	67.72
T4	2.6	3.0	2.9	68.00
T5	3.1	3.6	4.2	59.33
T6	9cm	9cm	9cm	00.0



Macrophomina *Pseudomonas fluorescens Pf1* *Trichoderma asperellum ECK*

Plate 1. Screening of streptomyces against macrophomina by dual culture technique

Table 2. Seedling vigour index for different treatments

Measurement Treatment	Average Root length (cm)	Average Shoot length (cm)	Germination (%)	Seedling Vigour Index
Control	8.5	13.0	60	1290.0
T1	10.0	15.5	76	1938.0
T2	12.5	18.0	84	2562.0
T3	9.3	14.6	68	1002.1
T4	8.5	12.0	64	1312.0
T5	8.0	13.3	56	1192.8

Table 3. Yield attributes for different treatments

Measurement Treatments	Average Root length (cm)	Average Shoot length (cm)	SEEDLING VIGOUR	Germination (%)	Disease incidence (%)	YIELD (Kg/ha)
T1	13.75	8.25	1650.0	85	*18.85*	*296.4*
T2	13.25	7.00	1440.5	83	*20.55*	*291.6*
T3	12.5	8.25	1431.75	80	*22.23*	*264.0*
T4	13.75	9.25	1805.25	87	*15.00*	*322.8*
T5	13.5	8.5	1298.0	78	*28.33*	*256.8*
T6	12.75	6.5	1212.75	73	*63.00*	*234.0*

4. CONCLUSION

The results revealed that *Streptomyces* sp (native) was found to be most effective in reducing pre and post emergence mortality and also it enhances the yield than other treatments. In field trail among the six treatments, treatment containing seed treatment of *streptomyces* sp (native) was found to be most effective in reducing pre and post emergence mortality with germination of 87.0 %. *Streptomyces* sp (native) was found to be more effective as compared to other bio-control agents in increasing the yield parameters like root length, shoot length, and seedling vigour and also it enhances the yield *(322.8 kg/ha)* compared to other treatments. *Streptomyces* sp (native) needs further species level characterization and other biotrophic and tritrophic interaction studies for developing commercial formulation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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