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Standardisation of *Trichoderma asperellum* as seed treatment in the management of root rot disease of sesame

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Abstract
Root rot caused by *Macrophomina phaseolina* (Tassi) Goid is the destructive disease in sesame. The effect of *Trichoderma asperellum* talc based formulation as seed treatment in managing the root rot disease and enhancing the rhizosphere population in sesame was investigated. Seed treatment with T. a*sperellum* @ 10 g/kg seed was effective in managing the root rot disease and increasing the seed yield under field conditions. In addition, seed treatment with *T. asperellum* @ 10 g/kg of seed recorded the maximum population of *T. asperellum* when compared to other dosages of seed treatment both under pot culture and field conditions. The rhizosphere population was gradually increased from 0 DAS and reached the maximum at 30 DAS and then gradually declined.

Keywords: Sesame, root rot, *Trichoderma asperellum*, seed treatement

Introduction
Sesame (*Sesamum indicum* L.), is one of the oldest oilseed crop grown widely under tropical and subtropical regions in India. Sesame seed is a rich source of protein (20%), edible oil (50%), oleic acid (47%) and linolenic acid (39%). Although sesame is widely used for different purposes, it has low productivity due to non-availability of high-yielding varieties, resistant variety to biotic and abiotic stresses, low harvest index, seed shattering and indeterminate growth habit (Buldeo and Rane, 1978) [4].
Among the diseases, root rot caused by *Macrophomina phaseolina* (Tassi) Goid is the most serious one affecting the crop at the later stages of growth. It is the destructive disease in all sesame growing areas and causes about 5–100% yield loss. Maiti *et al*. (1988) [8] reported an estimated yield loss of 57% at about 40% of disease incidence. The most common symptom of the disease is the sudden wilting of growing plants mainly after the flowering stage, the stem and roots become black due to severe infection. The pathogen survives as sclerotia in the soil and crop residues and has also been reported to be seed-borne, characteristics that make it difficult to control. The disease is both seed and soil borne and usually infects the crop under dry and warm conditions. Sesame is mostly grown as a rainfed crop and under this situation, the crop is exposed to sufficient soil moisture during its initial growth stages (up to 30-35 days), while subsequently the crop is maintained as a dry crop. High temperature and water stress during growing season favours the pathogen's incidence (Chattopadhyay and Kalpana Sastry, 1998) [5]. About one per cent increase in disease intensity reduces its yield by 8.36 kg/ha (Maiti *et al*., 1988) [8].
Although chemical control, sometime achieves considerable results, though it causes negative impact to environment and human health. This enforces operators to work hard to avoid these toxic Although chemical control, sometime achieves considerable results, though it causes negative impact to environment and human health. This enforces operators to work hard to avoid these toxic Chemical control, sometimes achieves considerable results, though it causes negative impact to environment and human health. This enforces operators to work hard to avoid these toxic chemicals and replace them with safe products. Biological control is one of the most promising and safe measure in this respect. Biocontrol agents work through different modes of action. This helps biocontrol agent to be more durable, more effective and without chemical residues in human food chain. In addition, the bioagent, *Trichoderma* also acts as inducer for resistance in treated plants against certain pathogens (Harman, 2006) [7] and can grow with wide range of temperature and other environmental conditions (Singh *et al*., 2010)

[14]. *Trichoderma* spp. is known to act through different mechanisms ranging between mycoparasitic or through production of antifungal substances such as trichodermin (Balode, 2010) [3].

Although some biological control agents may protect seeds from soil borne pathogens, they do not proliferate in the rhizoplane and rhizozphere (Papavizas, 1985) [5]. Integrating functional and ecological knowledge on microbial populations in soil will be a prerequisite in developing novel management strategies for sustainable agriculture for which the population abundance of soil microbiome is an important component.

The objective of the research reported here was to investigate the possibility of *Trichoderma asperellum* in the management of root rot disease of sesame and to establish the rhizosphere population of *T. asperellum*.

Materials and Methods

Isolation of pathogen

The pathogen *M.phaseolina* was isolated from the infected roots of sesame and maintained on potato dextrose agar (PDA) slants (Rangaswami, 1972) [12].

Effect of seed treatment with *Trichoderma asperellum* on the management of root rot of sesame

Field eperiment was conducted in the New Farm, Regional Research Station, Vridhachalam during *kharif* 2018 to standardize the dosage of T. *asperellum* for the management of root rot disease of sesame under sick plot conditions. The field experiment was conducted in a plot size of 2.4x3.0 m with seven treatments and three replications in RBD using the susceptible variety VRI-1 for the management of root rot of sesame. The seeds of sesame were treated with *T. asperellum* talc formulation at different dosages *viz*., 4 g, 10 g, 15 g, 20 g, 25 g, 30 g per kg of seed before sowing. Seeds not treated with bioagent served as the control. The germination percentage was assessed on 30 days after sowing. The root rot disease incidence was recorded at the time of physiological maturity (90 days after sowing) by counting the number of infected plants and total number of plants. Seed yield was also recorded for each treatment and the data were statistically analyzed. Per cent disease incidence was calculated using the formula

$$\text{Disease Incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Assessment of rhizosphere population of *Trichoderma asperellum* in sesame with different dosages of seed treatment (Pot culture)

A pot culture experiment was laid out with seven treatments and three replications to estimate the population of *T. asperellum* in the rhizosphere of sesame at different time intervals with different dosages of seed treatment with *T. asperellum.* The population density of *T. asperellum* in rhizosphere soil was calculated by serial dilution plate count method (Papavizas, 1985 [9]. Ten grams of soil sample was taken separately and suspended in 100 ml of sterile water and stirred well to get 1:10 dilution (10^{-1}). One ml from this was transferred to test tube containing 9 ml of sterile distilled water to get 1:100 (10^{-2}) dilution. Likewise the dilution of the sample was prepared up to $10^{-5.}$ One ml of the final dilution of each soil sample was aseptically pippetted out into each sterile Petri plate separately to which a quantity of 20 ml of sterilized and cooled Trichoderma selective medium (TSM) was poured and gently rotated for uniform mixing. The plates were incubated at 27±1 ºC for five days and colonies were counted and the population was estimated and expressed as colony forming units (cfu) per gram of soil.

Assessment of rhizosphere population of *T. asperellum* in sesame with different dosages of seed treatment (field conditions)

A field experiment was laid out during *rabi* 2018 with seven treatments and three replications to estimate the population of *T. asperellum* in the rhizosphere of sesame at different time intervals when treated with different dosages of *T. asperellum.*

Results and Discussion

The results of the field experiments conducted on the management of root rot disease of sesame revealed that all the treatments were found to be superior over control. Seed treatment with *T. asperellum* at the rate of 10 g/kg of seed recorded the maximum germination percentage of 93.4% as against the minimum germination percentage of 81.7% in the control. The highest plant survival percentage and improved yield components were observed by *T. asperellum* in strawberry (Pastrana *et al.*, 2016) [10].

Among the dosages tested for *T. asperellum,* seed treatment with *T. asperellum* at the rate of 10 g/kg of seed recorded the minimum root rot disease incidence of 22.7%. Seed treatment with *T. asperellum* at the rate of 4 g/kg of seed ranked next in managing root rot disease with 23.1% disease incidence. The maximum root rot disease incidence of 46.3% was observed in the control. Pastrana *et al.* (2016) [10] reported that *T. asperellum* was the most effective in reduction of crown and root rot caused by *Fusarium solani* (up to 100% in greenhouse and 81% under field conditions). Fiki and Farouk (2017) [6] reported maximum inhibition of soil borne pathogens *viz*., *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum* and *M. phaseolina* by *T. harzianum*. In addition to disease reduction, the maximum seed yield of 598 kg/ha was recorded in seed treatment with *T. asperellum* at the rate of 10 g/kg of seed; while in the control seed yield of 464 kg/ha was observed (Table 1).

Anandaraj and Sarma (2003) [1] reported that the application of *T. harzianum* (MTCC 5179) resulted in enhanced growth in black pepper with increased number of nodes, and consequently the number of cuttings. Sibi (2013) [13] also showed the positive influence of *T. harzianum* (MTCC 5179) on the improvements in the formation of fresh root and shoot, followed by increase in the dry weight of root and shoot in black pepper. Treatment with *T. harzianum* (MTCC 5179) individually imparted better growth promotion and disease suppression than that of a consortia of plant growth-promoting rhizobacteria alone or in combination with *T. harzianum* (MTCC 5179) (Sibi, 2013) [13]. These studies indicated growth promotion and the organism was recommended as a component of integrated disease management and without a clear understanding of other mechanisms.

Plants contribute to the establishment of specific ecological niches of microbes in the rhizosphere by playing key role as ecosystem engineers. The microbial community at the rhizosphere reflects its functional specificity at the level of plant–microbe interactions. It suggests that taxonomically-contrasted plant growth promoting strains may coexist in soil and colonize the same rhizosphere. Under pot culture set up, among the different dosages of seed treatment with *T.*

asperellum, seed treatment with *T. asperellum* @ 10 g/kg of seed recorded maximum rhizopsphere population of *T. asperellum*. In all the dosages of seed treatment with *T. asperellum*, the rhizopsphere population of *T. asperellum* was found to be increased gradually and reached the maximum population at 30 days after sowing and then gradually declined (Table 2). Rajan *et al*. (2002) [11] showed the biocontrol and disease suppression activities of *T. harzianum* (MTTC 5179) in black pepper against foot rot disease at field conditions; which was found to be efficiently proliferating in the soil and remained in the soil for long time, apart from imparting protection to the root system against *P. capsici*.

The results of field experiment laid out on the assessment of rhizosphere population of *T. asperellum* with different dosages of seed treatment revealed that seed treatment with *T. asperellum* @ 10 g/kg of seed treatment recorded the maximum population of *T. asperellum* when compared to other dosages of seed treatment. The rhizosphere population was gradually increased from 0 DAS and reached the maximum at 30 DAS and then gradually declined (Table 3). Asha (2013) [2] observed that population of *Trichoderma* isolates was maximum at 15th day after sowing and the lowest population was observed in natural soil. The population of *Trichoderma* isolates differed significantly from the control. The population dynamics and functional richness of rhizosphere ecosystem in black pepper influenced by the treatment with *T. harzianum* provides the ecological importance of *T. harzianum* in the cultivation of black pepper (Umadevi *et al*., 2018) [15].

Table 1: Effect of seed treatment with *T. asperellum* on management of root rot of sesame

Treatments (ST with *T. asperellum*)	Germination (%)	Root rot disease incidence (%)	Yield (kg/ha)
ST - 4 g/kg	88.6	23.1	593
ST - 10 g/kg	93.4	22.7	598
ST - 15 g/kg	85.7	25.6	586
ST - 20 g/kg	91.2	29.4	524
ST - 25 g/kg	86.3	27.6	531
ST - 30 g/kg	88.1	27.1	516
Control	81.7	46.3	464
CD (P=0.05)	4.6	3.2	14.7

Table 2: Effect of seed treatment with *T. asperellum* on population of *T. asperellum* (Pot culture)

Treatments (ST with *T. asperellum*)	Cfu of *T. asperellum*			
	0 Day ($x10^5$)	15 DAS ($x10^5$)	30 DAS ($x10^5$)	45 DAS ($x10^5$)
ST - 4 g/kg	12	24	28	18
ST - 10 g/kg	14	28	33	26
ST - 15 g/kg	12	21	29	16
ST - 20 g/kg	12	22	24	19
ST - 25 g/kg	14	23	27	17
ST - 30 g/kg	9	17	21	14
Control	6	4	6	5
CD (P=0.05)				

Mean of three replications

Table 3: Effect of seed treatment with *T. asperellum* on population of *T. asperellum* (Field condition)

Treatments (ST with *T. asperellum*)	Cfu of *T. asperellum*			
	0 Day ($x10^5$)	15 DAS ($x10^5$)	30 DAS ($x10^5$)	45 DAS ($x10^5$)
ST - 4 g/kg	15	22	31	24
ST - 10 g/kg	13	34	38	31
ST - 15 g/kg	16	24	36	27
ST - 20 g/kg	12	21	28	24
ST - 25 g/kg	14	26	22	17
ST - 30 g/kg	12	21	24	16
Control	10	8	6	6
CD (P=0.05)				

Mean of three replications

Conclusion

The present study indicated the usefulness of *T. asperellum* formulation for the management of root rot disease in *S. nigrum*. Hence, this approach can be exploited as it is natural, safe, effective, persistent and durable alternative to chemical pesticides for controlling plant diseases. It was inferred that seed treatment with *T. asperellum* at the rate of 10 g/kg of seed was found to be the best in enhancing the rhizosphere population. Seed treatment with *T. asperellum* is an effective delivery system to achieve biocontrol, increase growth response and enhancing the rhizosphere population.

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