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In Vitro Evaluation of Rhizospheric and Endophytic Microflora against *Macrophomina phaseolina* Causing Dry Root Rot of Chickpea

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ABSTRACT

Dry root rot disease caused by *Macrophomina phaseolina* (Tassi) Goid, is a major constraint in chickpea production under drought and high temperature conditions. The present study focused on the isolation and identification of rhizospheric and endophytic microbial antagonists from healthy chickpea plants and their *in vitro* evaluation against *M. phaseolina*. A total of eleven antagonistic isolates were obtained, which included six fungal and five bacterial isolates. These isolates were characterized based on colony morphology and cultural features. Dual culture assays revealed that *T. harzianum* (TH-1) exhibited the highest inhibition of pathogen growth (74.61%), followed by *T. hamatum* (74.09%) and *T. viride* (72.51%). Among bacterial isolates, *Pseudomonas fluorescens* (PF-1) showed maximum inhibition (68.72%), followed by *Bacillus subtilis* (BS-1) (64.36%). The results suggest that native rhizospheric and endophytic isolates of *Trichoderma, Pseudomonas, and Bacillus* possess significant antagonistic potential against *M. phaseolina* and may be exploited in the biological management of dry root rot disease of chickpea.

KEYWORDS: Chickpea, Dry root rot rot, *Macrophomina phaseolina*, Culture media, Bio-agents

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops worldwide, particularly in semi-arid regions of India. Despite its adaptability, chickpea production is severely affected by dry root rot (DRR) disease caused by *Macrophomina phaseolina* (Tassi) Goid., a soil-borne necrotrophic pathogen that thrives under drought and heat stress. As reported by Mitra (1931), dry root rot is prevalent in drought-prone areas, including parts of Western Maharashtra. The pathogen infects roots, leading to cortical shredding, root blackening, and plant death. The increasing incidence of this disease is attributed to changing climatic patterns, including rising soil temperatures and moisture deficits (Pande *et al.*, 2010). Yield losses at full podding and pre-harvest stage due to

dry root rot disease have been estimated to range from 50 % to 70 % in susceptible cultivars (Ahmed and Mohammad, 1986). Current management strategies rely heavily on chemical fungicides. However, these pose risks of environmental contamination, pathogen resistance development and negative impacts on soil microbial diversity. Hence, biological control using beneficial microorganisms such as *Trichoderma, Pseudomonas, and Bacillus* species has emerged as a sustainable alternative. Use of antagonistic microbes or their secondary metabolites is considered to be a practicable technology for the management of plant diseases (Han *et al.*, 2005). These organisms suppress pathogens through mechanisms including mycoparasitism, antibiosis, and induction of systemic resistance (Harman *et al.*, 2004; Sharma *et al.*, 2015).

By considering these aspects, the present study was undertaken to isolate and identify rhizospheric and endophytic microflora associated with healthy chickpea plants and to evaluate their antagonistic potential against *M. phaseolina* under *in vitro* conditions.

MATERIALS AND METHODS

a. Collection of Samples

Symptomless, healthy chickpea were collected from Solapur, Ahmednagar, and Pune districts during *Rabi* 2023–24. Root-adhering soil samples were used for rhizospheric microbial isolation, and surface-sterilized root segments were used for endophytic microbial isolation.

b. Isolation of pathogen and rhizospheric and endophytic microflora

The plants showing typical symptoms of dry root disease were collected from Pulse Improvement Project, MPKV, Rahuri and the pathogen was isolated from diseased plant samples under laboratory condition by following tissue isolation method.

The rhizospheric microflora was isolated from the rhizospheric soil samples by serial dilution technique while the endophytic microflora was isolated from healthy chickpea roots and stems by tissue isolation. The details of the media used are as below:-

Potato Dextrose Agar (PDA): For isolation and subculturing of *Macrophomina phaseolina* and fungal antagonists				
1	Potato Dexrose Agar		Agar agar	20 g
2	Potato (peeled)	200 g	Distilled water	1000 ml
3	Dextrose	20 g		
King's B Medium: For selective isolation of fluorescent *Pseudomonas sp.*				
1	Peptone	20 g	Glycerol	10 ml
2	Dipotassium hydrogen phosphate	1.5 g	Agar	15-20 g
3	Magnesium sulphate	1.5 g	Distilled water	1000 ml
Nutrient Agar (NA): For isolation of bacterial rhizospheric and endophytic microflora				
1	Peptone	5 g	Agar	15 g
2	Beef extract	3 g	Distilled water	1000 ml
3	NaCl	5 g		
Trichoderma Specific Medium (TSM Elad *et al.*, 1981): For isolation of *Trichoderma spp.*				
1	Glucose	20g	Rose Bengal	0.015g
2	Dipotassium phosphate	1.0g	Cholramphenicol	0.25g
3	Magnesium sulphate	0.5g	Pentochloronitrobenzen	0.2g
4	Potassium chloride	0.5g	Agar	20g
5	Ammonium nitrate	1.0g	Distilled water	1000ml

c. Identification

Identification of the fungal pathogen was done on the basis of symptomatology, colony morphology, and microscopic features such as colony type, color, and microsclerotia, which were compared with standard taxonomic descriptions. Fungal antagonists were identified based on colony morphology, colour, and sporulation pattern as per discretion given by Rifai (1969). Bacterial isolates were identified based on colony texture, pigmentation, and fluorescence under UV light on King's B medium (for P*seudomonas*) and dry, opaque growth on NA medium (for Bacillus).

d. *In Vitro* Dual Culture Assay

The antagonistic efficacy of six fungal and five bacterial isolates was evaluated using the dual culture technique (Dennis & Webster, 1971). A five mm mycelial disc of *M. phaseolina* was placed opposite to a five mm disc of the antagonist on PDA plates. Plates were incubated at 28 ± 2°C for 7 day.

The per cent growth inhibition was calculated by applying following formula (Vincent, 1927).

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

Where,
C= growth of the test fungus in control plate (mm),
T= growth of the test fungus in treated plates (mm)

e. Evaluation of chickpea genotypes against *Macrophomina phaseolina* using sick soil method (pot culture)

A pot culture experiment was conducted during Rabi 2023–24 at the Department of Plant Pathology and Microbiology, MPKV, Rahuri, to evaluate fifteen chickpea genotypes against dry root rot caused by *M. phaseolina*. Sterilized sorghum seeds inoculated with a highly virulent isolate were incubated for 10 days at 28 ± 1°C and mixed with sterilized soil (10% w/w) before filling pots. Surface-sterilized seeds (0.1% $HgCl_2$) of each genotype were sown (10 seeds/pot) with two replications and un-inoculated controls. Disease symptoms and incidence were recorded up to 80 DAS, and per cent disease incidence (PDI) was calculated. Disease reactions were rated using the 1–9 scale of Nene et al. (1981).

RESULTS AND DISCUSSION

a. Isolation and Identification of Microbial Antagonists

Fungal microflora

Rhizospheric soil samples collected from the root zone of apparently healthy chickpea plants were subjected to serial dilution plate technique. After 3-5 days of incubation at 28 ± 2°C, morphologically distinct colonies were selected and purified. Out of the recovered isolates, six fungal antagonists were identified as species of *Trichoderma*. Identification was performed based on characteristics such as rapid colony growth, the presence of concentric ring patterns, and profuse conidial production observed under a compound microscope. These included three isolates of *T. harzianum,* two isolates of *T. viride* and one isolate of *T. hamatum.* Colonies showed rapid radial growth, dense sporulation, and typical green pigmentation. Such colony features of *Trichoderma* spp. have

also been described by and confirmed by Dubey and Dwivedi (1988) during their work on fungal antagonists against soil-borne pathogens. The details of the *Trichoderma* isolates obtained are presented in Table 1.

Table. 1 Rhizospheric fungal antagonists isolated from chickpea fields in Western Maharashtra

Sr. No.	Isolate Code	Location	Source of Isolation	Morphological characteristics	Identified as
1	TH-1	Pune	Rhizospheric Soil	Greenish colony, compact growth, white margin	*Trichoderma harzianum*
2	TH-2	Ahilyanagar	Rhizospheric Soil	Fast growing, compact concentric rings	*T. harzianum*
3	TH-3	Satara	Rhizospheric Soil	Green mycelium with pale white edge	*T. harzianum*
4	TV-1	Pune	Rhizospheric Soil	Dark green concentric colony, cottony texture	*T. viride*
5	TV-2	Ahilyanagar	Rhizospheric Soil	Radial green growth with white margins	*T. viride*
6	THa-1	Solapur	Rhizospheric Soil	Dull green colony with irregular margins	*T. hamatum*

Bacterial microflora

Three isolates of *Pseudomonas fluorescens* and two of *Bacillus subtilis* were successfully isolated using Nutrient Agar and King's B medium, respectively. The *Pseudomonas fluorescens* isolates produced smooth, green-pigmented colonies that exhibited characteristic fluorescence under UV light, confirming their identity. In contrast, the *Bacillus subtilis* isolates formed dry, rough, and creamy to yellowish colonies, typical of the species. The details of the bacterial isolates obtained are presented in Table 2.

Table. 2 Rhizospheric and endophytic bacterial antagonists isolated from chickpea fields in Western Maharashtra

Sr. No.	Isolate Code	Location	Source of Isolation	Morphological characteristics	Identified as
1	PF-1	Pune	Rhizospheric Soil	Light yellow colonies, slimy texture	*Pseudomonas fluorescens*
2	PF-2	Pune	Endophytic (Plant)	Circular, pale yellow, smooth edges	*P. fluorescens*
3	PF-3	Sangli	Rhizospheric soil	Creamy white colonies, flat	*P. fluorescens*
4	BS-1	Ahilyanagar	Endophytic (Plant)	Dry, wrinkled, irregular cream-colored colony	*Bacillus subtilis*
5	BS-2	Solapur	Rhizospheric soil	Flat, rough colonies, pale cream to yellowish in color	*B. subtilis*

These findings are consistent with the observations of Gopalakrishnan *et al.* (2011), who reported that endophytic bacteria like *Bacillus spp.* are capable of colonizing internal plant tissues and play a crucial role in disease suppression and enhancing plant health by inducing systemic resistance and competing with pathogens.

The eleven isolates were coded, sub-cultured, and stored for further evaluation through dual culture assay. The diversity observed among both rhizospheric and endophytic isolates highlights the microbial richness of chickpea-growing environments in Western Maharashtra. The dominance of *T. harzianum, T. viride, T. hamatum, P. fluorescens, and B. subtilis* confirms earlier findings by Sreedevi *et al.* (2011).

b. In Vitro Evaluation of fungal and bacterial antagonist against *Macrophomina phaseolina* on PDA by dual culture technique

The *in vitro* dual culture assay involving six *Trichoderma* isolates demonstrated marked differences in their capacity to inhibit the radial growth of *Macrophomina phaseolina*. As presented in Table 3, among the fungal antagonists, *T. harzianum* (TH-1) was most effective, achieving a mean inhibition of 74.61%, followed by *T. hamatum* (THa-1) with 74.09%, *T. harzianum* (TH-2) with 73.71%, *T. viride* (TV-1) with 72.51%, *T. harzianum* (TH-3) with 70.97%, and *T. viride* (TV-2) with 66.92%.

Table. 3 Inhibition of mycelial growth of *Macrophomina phaseolina* by fungal antagonist

Tr. No.	Antagonist	Mean Radial Growth(mm)*	Mean Inhibition (%)
T_1	*T. harzianum* (TH-1)	18.16	74.61
T_2	*T. harzianum* (TH-2)	18.80	73.71
T_3	*T. harzianum* (TH-3)	20.76	70.97
T_4	*T. viride* (TV-1)	23.66	66.92
T_5	*T. viride* (TV-2)	19.66	72.51
T_6	*T. hamatum* (THa-1)	18.53	74.09
T_7	Control	71.53	0.00
	S.Em. (±)	0.10	-
	C.D. at 1 %	0.42	-

*: Average of three replications

Among bacterial antagonists, *Pseudomonas fluorescens* PF-1 and PF-2 recorded mean inhibition of 73.56% and 55.53%, respectively, followed by *Bacillus subtilis* BS-1 (53.39%). The other isolates, *P. fluorescens* PF-3 (52.17%) and *B. subtilis* BS-2 (50.32%) also showed moderate suppression. The control, which received no bio-agent treatment, showed the highest radial growth with no inhibition, confirming the growth-promoting nature of the pathogen without any intervention (Table 4).

Table. 4 Efficacy of bacterial antagonist against *Macrophomina phaseolina* on PDA by dual culture technique

Tr. No.	Antagonist	Mean Radial Growth (mm)*	Mean Inhibition (%)
T_1	*P. fluorescens* (PF-1)	18.91	73.56
T_2	*P. fluorescens* (PF-2)	31.80	55.53
T_3	*P. fluorescens* (PF-3)	34.12	52.17
T_4	*B. subtilis* (BS-1)	33.33	53.39
T_5	*B. subtilis* (BS-2)	35.53	50.32
T_6	Control	71.53	0.00
	S.Em. (±)	0.12	-
	C.D. at 1 %	0.54	-

* : Average of three replications

Fungal isolates belonging to the genus *Trichoderma* were found to be the most effective, with *T. harzianum* and *T. hamatum* exhibiting the highest inhibition levels, reducing pathogen growth by more than 70 per cent. These isolates also showed visible overgrowth over the pathogen, indicating strong mycoparasitic activity. These findings are consistent with earlier studies. Dubey and Dwivedi (1988) who reported *T. harzianum* as strong fungal antagonists against *M. phaseolina*, showing significant inhibition through mycoparasitism. Khan and Gangopadhyay (2008) observed the mycelial growth of *Macrophomina phaseolina* was significantly reduced by the strains of *Pseudomonas flurescens*. Gowdra *et al.* (2012) evaluated antagonist against pathogen causing dry root rot disease and recorded inhibition by *Trichoderma harzianum* strainTh-55 (81.48 %) followed by *Bacillus subtilis* (75.85 %) and *Trichoderma viride* strain TV-27 (74.07 %) compared to control.

Among the bacterial isolates, *Pseudomonas fluorescens* and *Bacillus subtilis* exhibited maximum antagonistic effects, inhibiting the pathogen's growth by 73-50 per cent. Sreedevi *et al.* (2011) also found *Trichoderma spp*. and *Pseudomonas fluorescens* to be effective in reducing pathogen growth *in vitro*. Similarly, Manjunatha *et al.* (2013) reported effectiveness of *Trichoderma viride* and *Pseudomonas fluorescens* against dry root rot pathogen of chickpea, *M. phaseolina*. Smitha *et al.* (2017) suggested rhizospheric Bacillus strain effective for the management of dry root rot disease of chickpea.

These isolates formed distinct inhibition zones, suggesting antibiosis and competitive exclusion as their probable modes of action. The differential performance of the antagonists highlights the diversity in mechanisms employed, ranging from rapid colonization and nutrient competition to antibiotic and enzyme production. The effective antagonists can be considered for further evaluation under greenhouse and field conditions for integrated disease management in chickpea.

The current management strategies for disease control rely heavily on chemical fungicides. However, these pose risks of environmental contamination, pathogen resistance development and negative impacts on soil microbial diversity. Biological control, involving antagonistic microorganisms, offers an ecologically sustainable alternative. Several microbial agents, including *T. viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis*, have demonstrated the ability to suppress *M. phaseolina* through mechanisms such as mycoparasitism, competition, antibiosis and induction of systemic resistance (Han *et al.*, 2005; Gopalakrishnan *et al.*, 2011).

The increasing prevalence of dry root rot disease under stress-prone agroecosystems and the limitations of chemical control underscores the need for effective, eco-friendly alternatives.

Conclusion

A total of eleven microbial antagonists were isolated and identified from the rhizosphere and root tissues of healthy chickpea plants. Among them, *Trichoderma harzianum* (TH-1) and *Pseudomonas fluorescens* (PF-1) exhibited the highest antagonistic activity against *Macrophomina phaseolina in vitro*. The strong inhibitory potential of these isolates highlights their suitability as biocontrol candidates for eco-friendly management of dry root rot in chickpea. Further greenhouse and field evaluations are warranted to confirm their efficacy under natural conditions.

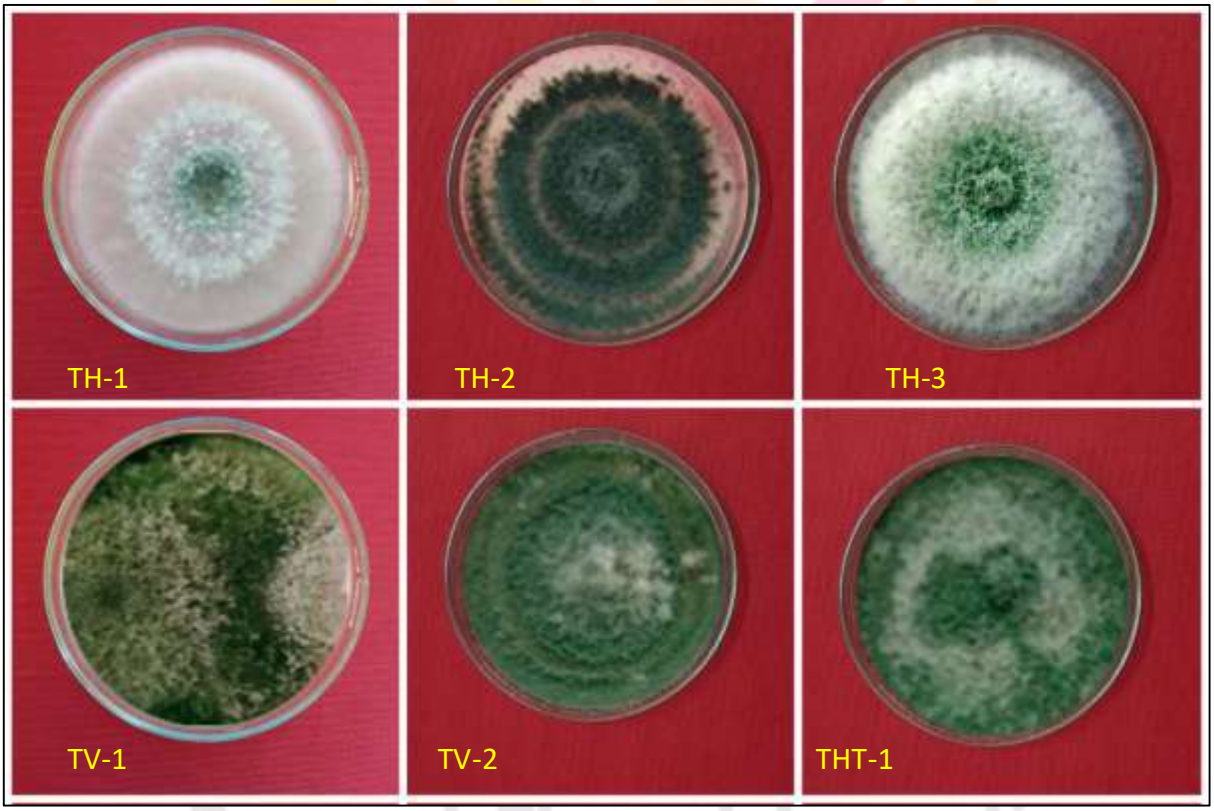

Plate a: Pure culture of fungal antagonists obtained from the soil and plant samples collected from five districts of Western Maharashtra

Plate b: Pure culture of bacterial antagonists obtained from the soil and plant samples collected from five districts of Western Maharashtra

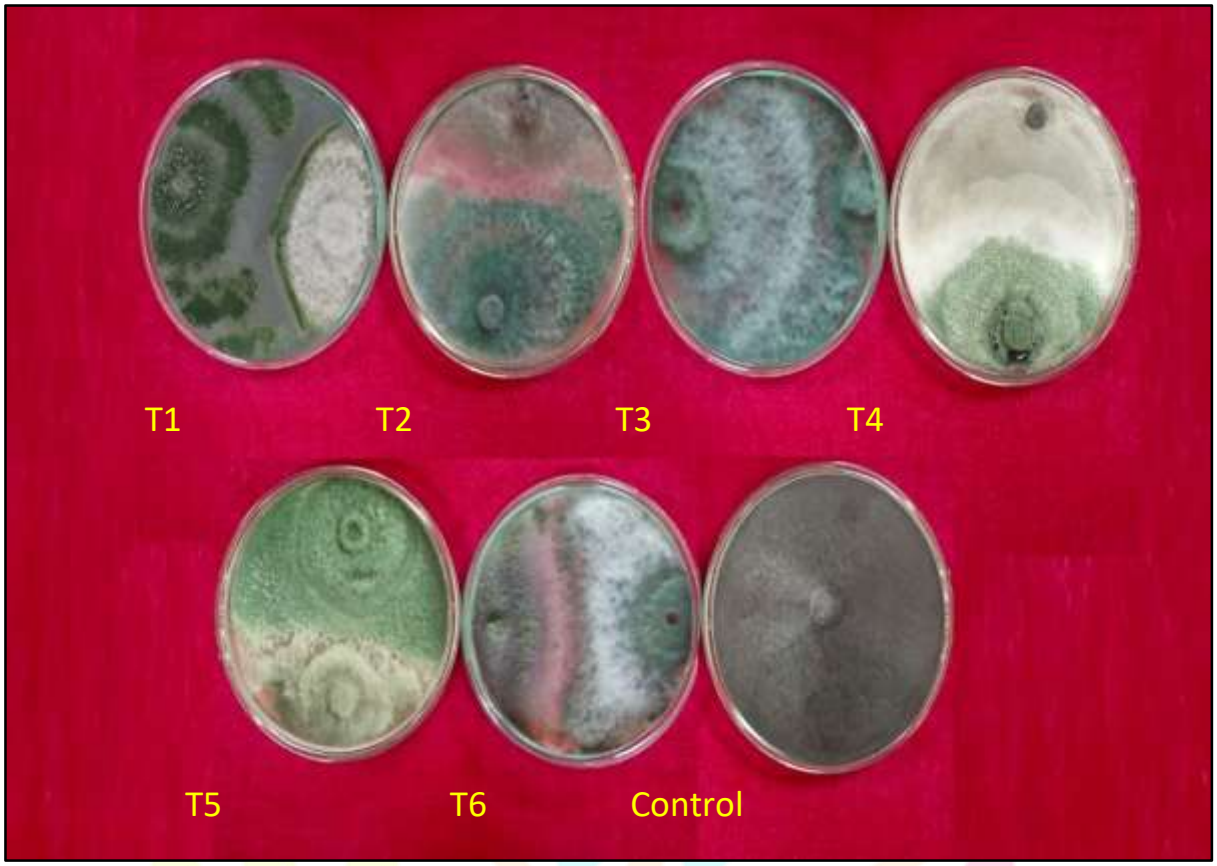

Plate a: *In Vitro* evaluation of fungal antagonists against *Macrophomina phaseolina*

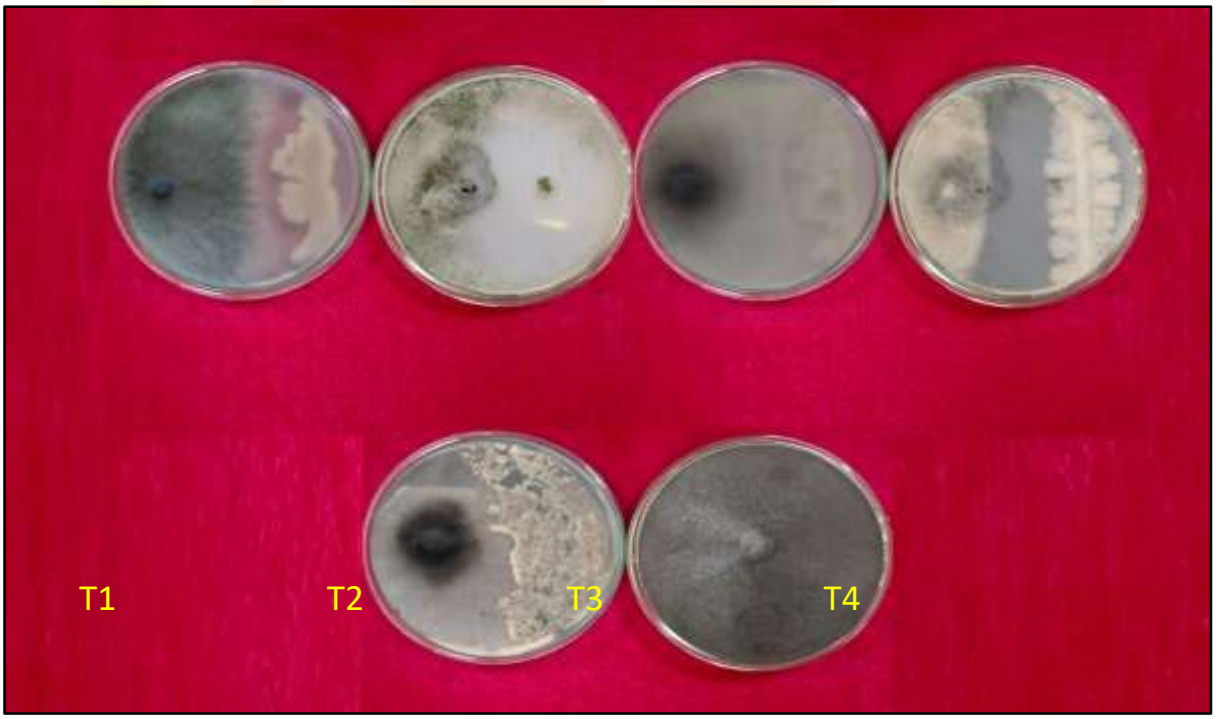

Plate : *In Vitro* evaluation of bacterial antagonists against *Macrophomina phaseolina*

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