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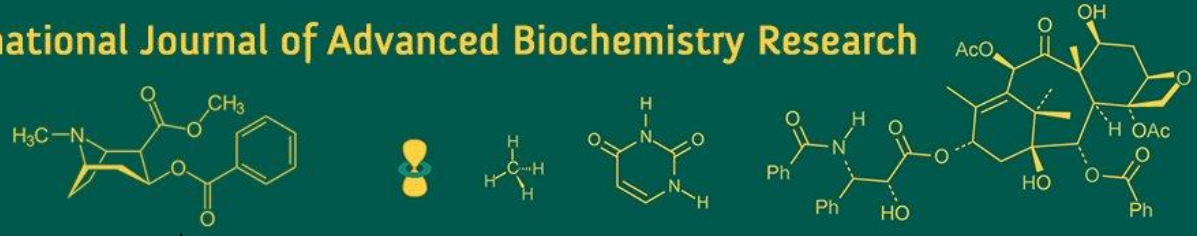

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The potential of bio agents for management of dry root rot of chickpea caused by *Macrophomina phaseolina* (Tassi.) Goid.

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Abstract
Five different bio agents *viz.*, three isolates of *Trichoderma* spp., one isolates of *Pseudomonas* spp. and one isolate of *Bacillus* spp. were tested for their ability to inhibit *Macrophomina phaseolina* causing Dry root rot of Chickpea under *in vitro* conditions. Dual culture of pathogens and bio agents revealed that among the fungal bio agents, *T. viride* (Tv-1) was found best with 47.36% fungal mycelia inhibiton over control. Among all the bacterial bio agents tested *Bacillus subtalis* was found best with 30.74% fungal inhibition over control. Further, these bio agents tested against *M. phaseolina* were categorized on the basis of bells scale of antagonism.

Keywords: Bio agents, *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp., *Macrophomina phaseolina*

1. Introduction
Chickpea (*Cicer arietinum* L.) is one of the major food legume crops grown in over 50 countries of Asia, Africa, America and Oceania in rain-fed environments. In India, Chickpea is cultivated in an area of 10.91 million hectare. Total production is 13.75 million tones with an average productivity of 1260 kg/ha (Anon., 2023) [1, 2] of which the state of Madhya Pradesh contributes 2.03 million hectare area with 3.03 million tonnes production and 1492 kg/ha productivity (Anon., 2023) [1, 2]. Chickpea productivity has been affected greatly due to several soil borne disease and these include susceptibility to diseases including wilt (*Fusarium oxysporum* Schlech tend.: Fr. *f.* sp. *ciceri* (Padwick) Matuo & K. Sato], dry root rot (*Macrophomina phaseolina* (Tassi.) Goidanich), *collar rot* (*Sclerotium rolfsii* Sacc.) and blight (*Ascochyta rabiei* (Passerini) Labrousse). Among the diseases, dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler (Synonym: *Macrophomina phaseolina* (Tassi.) Goid.) has the potential to cause devastation in susceptible cultivars. High day temperature (>30°C) and dry soil conditions at flowering and podding stages exacerbate the disease. The root system of diseased plant shows extensive rotting with most of destroys lateral roots. The rotten roots are brittle with minute sclerotial bodies appeared on the outer surface of the tap root (Gurha *et al.*, 2003) [7]. The total losses caused by the disease ranged from 30 to 40 percent (Mirchandani *et al.*, 2023 and Ghosh *et al.*, 2013) [10, 8].
Soil-borne diseases have a wide host range and can remain in the soil for prolonged periods of time due to resistant resting structures. Chemical management of soil-borne diseases gives a degree of control while also having negative impacts on the ecosystem, including important soil microbes. As a result, biological control of plant diseases has been proposed as a viable control technique in recent years, and the hunt for these biological agents has intensified. *Trichoderma* spp. and *Bacillus* spp. is the most often employed fungal and bacterial biological control agent and has long been acknowledged as a potent opponent of plant pathogenic fungus.
The present research work describes the impact of different fungal and bacterial bio agents tested against *M. phaseolina* using dual culture method under *in vitro* conditions.

2. Materials and Methods
M. phaseolina causing Dry root rot of chickpea was isolated from Chickpea showing typical

Disease symptoms. Different fungal and bacterial bio agents *were* isolated from diverse region following Dilution plate method (Johnson, 1957) [9]. Three isolates of *Trichoderma viz., T. viride* (Tv-1), *T. harzianum* (Th-2), *T. reesei* (Tr-3) and one isolate of *Bacillus subtalis* (Bs-1) and one isolate of *Pseudomonas Fluorescens* (Ps-1) were screened against test pathogens by dual culture method (Dennis and Webster 1971a) using potato dextrose agar (PDA). The radial growth of the pathogen in dual culture and control plates was measured after seven days of incubation at 28±1 °C and the inhibition per cent of pathogen was calculated as described by Vincent and Budge (1990) [13]. The degree of antagonisms between each bio agent and test pathogen in dual culture was scored on scale of 1-5 as proposed by Bell *et al.* (1982) [4].

Table 1: Bell's Scaling of antagonism

Bell's rating	Category
1	Antagonist completely overgrew the pathogen and covered the entire medium surface.
2	Antagonist overgrew at least two third of the medium surface.
3	Antagonist and the pathogen each colonized one half of the medium surface (more than one third and less than two third) and neither organism appeared to dominate each other.
4	The pathogen colonized at least two third of the medium surface and appeared to with stand encroachment.
5	The pathogen completely overgrew the antagonist and occupied the entire medium surface.

3. Results and Discussion

The observation recorded on the inhibition of mycelium of *M. phaseolina* in dual culture test (Table-1) revealed that all fungal and bacterial bio agents were effective in reducing the mycelial growth of test pathogen. Total five bio agents were evaluated for their efficacy against *M. phaseolina* through dual culture techniques (Figure-1). The inhibition of pathogen in dual culture test exhibited that all the five bio agents *viz.*, *Trichoderma harzianum* (Th-1), *Trichoderma viride* (Tv-1), *Trichoderma reesei* (Tr-1), *Pseudomonas Fluorescens* (Ps-1) and *Bacillus subtalis* (Bs-1) tested inhibited the growth of *M. phaseolina*. The perusal of result showed that *Trichoderma viride* (Tv-1) gave maximum growth of inhibition 47.36% among all the fungal bio agents. Among the bacterial bio agents tested *Bacillus subtalis* (Bs-1) gave maximum growth inhibition of 30.74%, followed by *Pseudomonas Fluorescens* (Pf-1) gave 13.03% pathogen growth inhibition. Other fungal bio agent *i.e. Trichoderma harzianum* (Th-1) gave 39.63% inhibition and least fungal growth inhibition was found with *Trichoderma reesei* (Tr-1) as 28.41%. The degree of antagonists between each bio agent and test pathogen in dual culture was scored on scale of 1-5 as proposed by Bell (Bell *et. al.* 1982) [4]. Among fungal and bacterial antagonists according to Bell scale (Table-2, Figure-1), *Trichoderma harzianum* (Th-1) and *Trichoderma reesei* (Tr-1), antagonist and each colonized one half of the medium surface (more than one third and less than two third) and neither organism appeared to dominate each other and were placed in class-3 degree of antagonism. Bio agent *Trichoderma reesei* (Tr-1), *Pseudomonas Fluorescens* (Ps-1) and *Bacillus subtalis* (Bs-1) were categorized into class-4 degree of antagonism where colonized at least two third of the medium surface and appeared to with stand encroachment.

Table 2: Evaluation of different bio agents against *M. phaseolina* using dual culture

Treatment	Treatment Details	Strain	Radial growth (mm) of test pathogen	Dual culture (% inhibition)
T_1	*Trichoderma harzianum*	Th-1 (Jabalpur)	54.33 (47.46)	39.63
T_2	*Trichoderma viride*	Tv-1 (Maharastra)	47.37 (43.47)	47.36
T_3	*Trichoderma reesei*	Tr-1 (Jabalpur)	64.43 (53.36)	28.41
T_4	*Pseudomonas Fluorescens*	Pf-1 (Pondicherry)	78.27 (62.21)	13.03
T_5	*Bacillus subtalis*	Bs-1 (Tamil Nadu)	62.33 (52.12)	30.74
T_6	Control		90.00 (71.53)	
SE(m)±			0.408	
CD (p=0.05)			1.220	

Table 3: Evaluation of bio agents against *M. phaseolina* using dual culture, using Bell's scale*

Treatment	Treatment Details	*Macrophonima phaseolina*
T_1	*Trichoderma harzianum*	3
T_2	*Trichoderma viride*	3
T_3	*Trichoderma reesei*	4
T_4	*Pseudomonas Fluorescens*	4
T_5	*Bacillus subtalis*	4

*Degree of antagonism as proposed by Bell *et al.* (1982) [4]

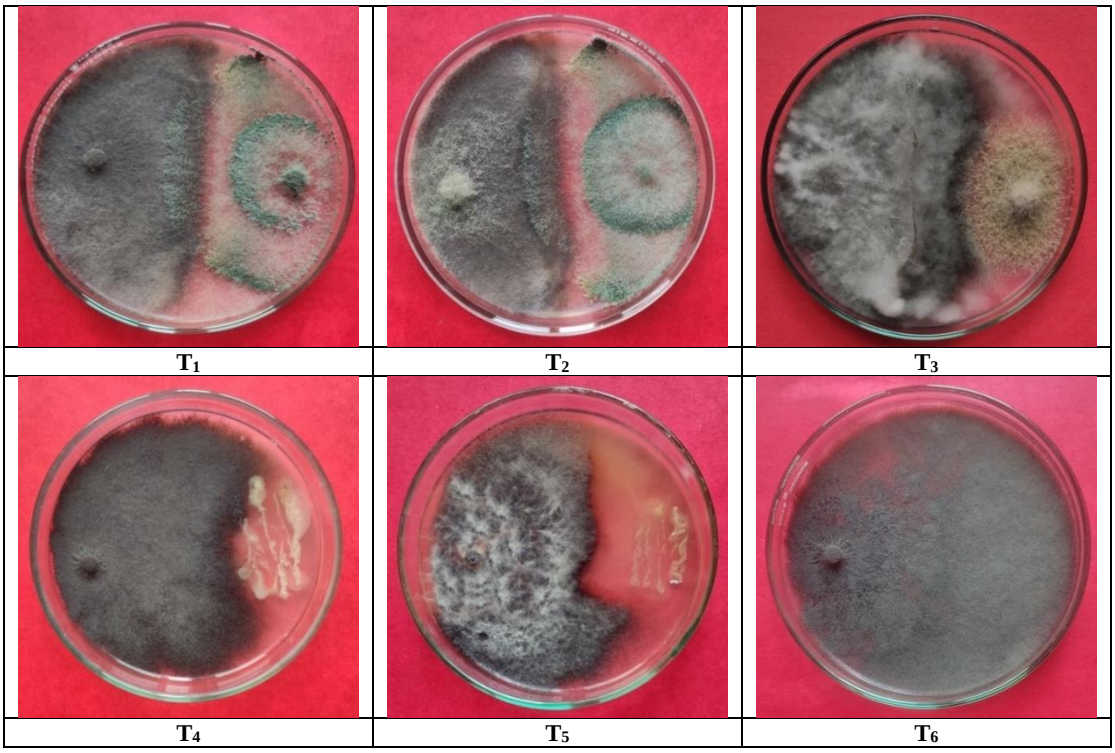

Fig 1: Antagonistic activity of bio agents against test pathogen *M. phaseolina*

The inhibitory activity of *T. harzianum*, *T. viride* and *T. reesei* against soil borne fungal pathogens and *baccilus subtalis*, *Pseudomonas Fluorescens* as bacterial bio agents found here were similar to the findings of (Robert *et al*., 1993; Dohroo *et al*., 1990; Abdollahzadeh *et al*., 2003) [11, 6, 3]. The inhibitory effects observed here were mainly attributed to competition for space, nutrition between the pathogens and antagonists. Antagonists may also affect growth of pathogen either through antibiosis or mycoparasitism. Besides they may also produce antifungal phenolic compounds (Saba Banday *et al*., 2008) [12].

4. Conclusion

Five distinct biological agents, namely three isolates of *Trichoderma* spp., one isolate of *Pseudomonas* spp., and one isolate of Bacillus spp., were evaluated for their capacity to suppress *Macrophomina phaseolina*, the pathogen responsible for dry root rot in chickpea, under *in vitro* conditions. The dual culture of pathogens and bioagents demonstrated that *T. viride* (Tv-1) had the highest efficacy among fungal bioagents, achieving a 47.36% inhibition of fungal mycelia compared to the control. Among all the bacterial bioagents evaluated, *Bacillus subtilis* exhibited the highest fungal inhibition at 30.74% compared to the control. Additionally, the bioagents evaluated against *M. phaseolina* were classified according to the Bell's scale of antagonism.

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