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Evaluation of different bio control agents against root rot incidence of castor incited by *Macrophomina phaseolina* (Tassi) Goid

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Abstract
Castor (*Ricinus communis* L.) is one of the most important non-edible oilseed crop in the world and it is affected by number of diseases, of which the root rot is the one serious disease caused by *Macrophomina phaseolina.* Experiment was conducted in *Kharif* 2021 at Department of Plant Pathology, College of Agriculture, JAU, Junagadh. In this study, six fungal bio control agents and bacterial bio control agents, respectively were evaluated for their antagonistic ability to suppress the growth of *Macrophomina phaseolina* in laboratory condition. Among the tested fungal antagonists, *Trichoderma asperellum* showed maximum mycelial growth inhibition (89.11 per cent) of the pathogen, followed by *Trichoderma harzianum* (87.22 per cent) and among bacterial bio control agents, *Bacillus subtilis*-isolate 1 showed maximum mycelial growth (86.66 per cent) of the pathogen, followed by *Pseudomonas fluorescens*-isolate 1 (84.44 per cent) under *in vitro.*

Keywords: Castor, root rot, *Macrophomina phaseolina*, fungal bio control agents, bacterial bio control agents

1. Introduction
"Castor" name is given by English traders who confused it with the oil of another shrub, *Vitex agnus-castus.* The Scientific name *Ricinus communis* L. in which *Communis* means common in Latin, *Ricinus* means tick in Latin. The genus *Ricinus* is considered to be monotypic and *Ricinus communis* is the only species, which may include many polymorphic types. It is a species of flowering plant in the spurge family, Euphorbiaceae. It is native of Ethiopian region of tropical east Africa (Weiss, 2000) [16]. Castor is a hardy crop which survives in a wide range of ecology and its range cannot easily be defined. Castor is short-lived small tree or shrub with soft wood and hollow stems which can grow to 5 m or more. The *Ricinus communis* plant's seeds contain more than 45 per cent oil that is rich in triglycerides, primarily ricinolein oil. In traditional medicine, the leaves and seeds are used as a laxative, for wound dressing, against rheumatism and mental illness (Singh and Geetanjali, 2015) [13]. A vast variety of value- added products are made from it due to the presence of a hydroxyl fatty acid known as ricin oleic acid. Due to high protein content (12–15%), the cake is used as a binder in the production of plywood, matchboxes and packing boxes. The area, production and productivity in India during 2019-20 were 10.46 lakh hectares, 18.42 lakh tones and 1761kg/ha, respectively (Anonymous, 2020) [1]. The productivity of castor in India is high as compared to other countries. Gujarat state ranks first position in the country with respect to area, production and productivity *i.e.* 7.36 lakh hectares, 14.31 lakh tones and 1944kg/ha, respectively (Anonymous, 2020) [1]. In Gujarat, castor is grown in districts of Mehsana, Sabarkantha, Banaskantha, Kutch, Ahmedabad, Kheda, Vadodara, Rajkot, Junagadh, Jamnagar and Gandhinagar. The pathogen attacks on all parts of plant like, root, stem, branches, petioles, leaves and seeds. The leaves progressively wilt, turning yellow and then brown. The wilting leaves typically remain attached to the leaf stalk. The outer stem tissue underneath the epidermis is typically peppered with small black specks (microsclerotia). In root, the tap root shows sign of drying and root bark shreds-off easily. Rotting spreads partly above the ground.

2. Materials and Methods
2.1 Evalution of fungal bio control agents by dual culture method (*in vitro*)
Screening of six fungal bio control agents was done by using dual culture technique (Morton and Stroube, 1955) [10] in CRD with four repetitions. Twenty milliliters of sterilized melted

PDA were poured aseptically in each 90 mm Petri plates and were allowed to solidify. Mycelial disc of five-millimeter diameter of each antagonist and test fungus was cut with the help of sterilized cork borer from the edges of actively growing culture and were placed by keeping 1 cm distance from distal ends of PDA containing Petri plates. The plates were then incubated at 28±2 ºC for five days. After incubation the growth of antagonist and the test fungus was measured by linear measurement. Per cent growth inhibition of test fungus by Growth inhibition (%) antagonist was calculated using the formula suggested by Arora and Upaddhyay (1978) [2].

$$\frac{\text{Colony growth in control plate} - \text{Colony growth in control plate intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Table 1: List of fungal bio control agents tested against *Macrophomina phaseolina*

Tr. No.	Antagonists
T1	*Trichoderma viride*
T2	*Trichoderma harzianum*
T3	*Trichoderma virens*
T4	*Trichoderma koningii*
T5	*Trichoderma hamatum*
T6	*Trichoderma asperellum*
T7	Control (Test Pathogen only)

2.2 Evaluation of bacterial bio control agents by dual culture method (*in vitro*)

Screening of six bacterial bio control agents was done by using dual culture technique (Morton and Stroube, 1955) [10] in CRD with four repetitions. Twenty milliliters of PDA were poured aseptically in each petri plates and allowed to solidify. Mycelial disc of four mm diameter of test fungus was placed by keeping 1 cm distance from distal ends of PDA containing Petri plates and bacterial antagonist was streaked at one side of Petri plate. The plates were then incubated at 28±2 ºC for five days. After incubation the growth of antagonist and the test fungus was measured by linear measurement. Percent growth inhibition of test fungus by antagonist was calculated. Index of antagonism has been determined in each treatment by following standard formula suggested by Arora and Upaddhyay (1978) [2] as mentioned earlier.

Table 2: List of bacterial bio control agents tested against *Macrophomina phaseolina*

Tr. No.	Antagonists
T1	*Pseudomonas fluorescens*–isolate 1
T2	*Pseudomonas fluorescens*–isolate 2
T3	*Bacillus subtilis*–isolate 1
T4	*Bacillus subtilis*–isolate 2
T5	*Bacillus thuringiensis*–isolate 1
T6	*Bacillus thuringiensis*–isolate 2
T7	Control (Test Pathogen only)

3. Results and Discussion

3.1 Evaluation of fungal bio control agents (*in vitro*)

Results (Table 3 and Fig. 1) indicated that all fungal bio control agents were antagonistic to the growth of *Macrophomina phaseolina*. Among six different fungal bio control agents tested, maximum inhibition over control was recorded in *T. asperellum* (89.11%) which are statically at par with *T. harzianum* (87.22%). While *T. hamatum* (83.88%) was found next best followed by *T. koningii* (76.94%) and *T. virens* (67.22%) were moderately effective to inhibit fungal growth. The least inhibition was recorded in *T. viride* (42.17%) under laboratory condition. The results are in harmony with earlier workers, Indra and Tribhuvanmala (2002) [4], Kannan *et al.* (2003) [5], Sreedevi *et al.* (2011) [14] and Arya *et al*. (2017) [3] who reported *T. harzianum* as a strong antagonist against *Macrophomina phaseolina* in dual culture technique. Similar observations were also noticed by Khaledi and Taheri (2016) [6] while working with different isolates of *T. harzianum*. Lokesha and Benagi (2007) [8] also observed and reported that *T. virens* was effective in mycelial growth inhibition of *Macrophomina phaseolina*.

Table 3: *In vitro* evaluation of fungal bio control agents

Sl. No.	Fungal bio control agents	Percent inhibition over control* (%)
1.	*Trichoderma viride*	40.49 (42.17)
2.	*Trichoderma harzianum*	69.07 (87.22)
3.	*Trichoderma virens*	55.07 (67.22)
4.	*Trichoderma koningii*	61.32 (76.94)
5.	*Trichoderma hamatum*	66.35 (83.88)
6.	*Trichoderma asperellum*	70.80 (89.11)
	S.Em. ±	0.65
	C. D. at 5%	1.94
	C.V. %	2.15

*Data outside the parentheses are arcsine transformation whereas, inside are re-transformed values

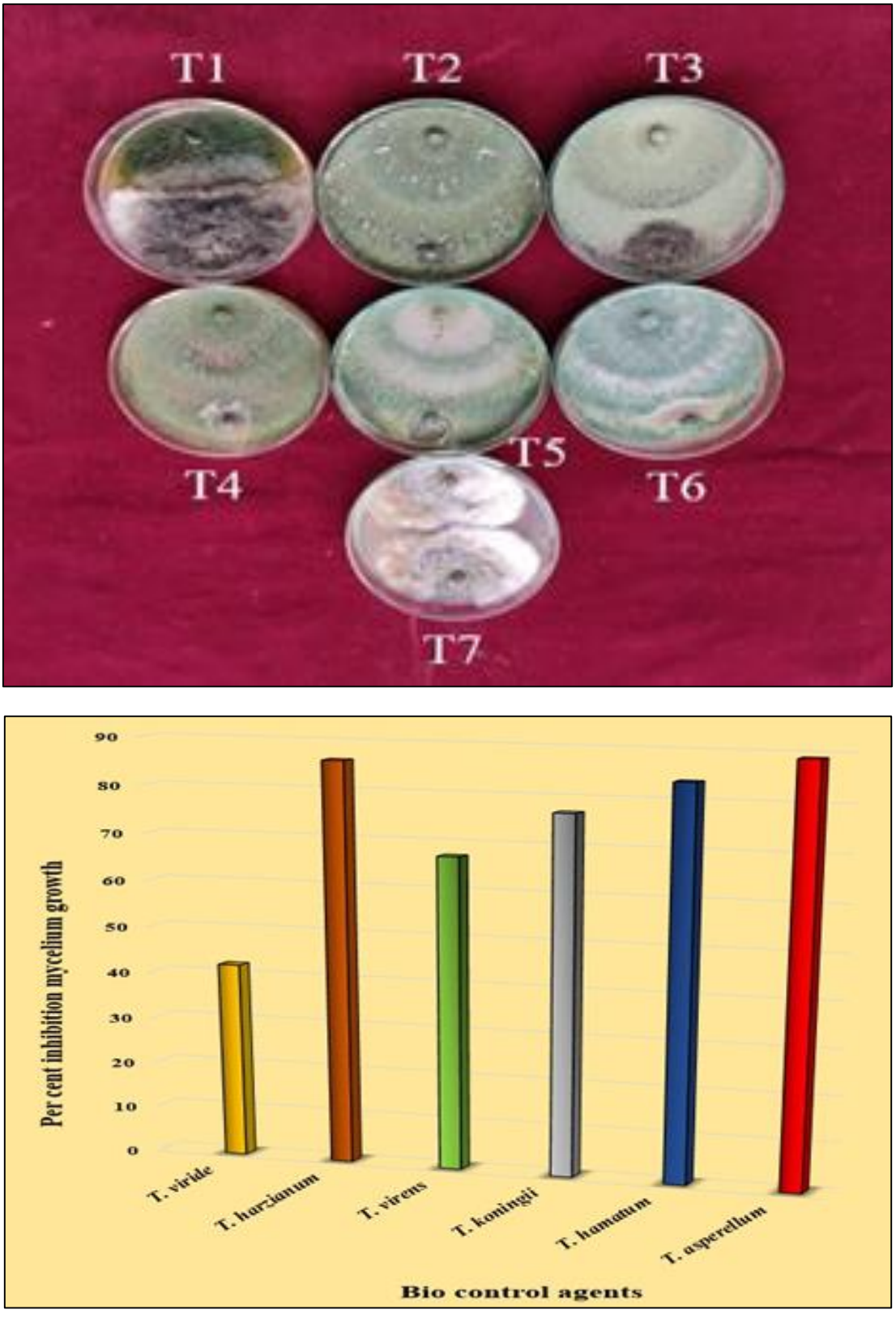
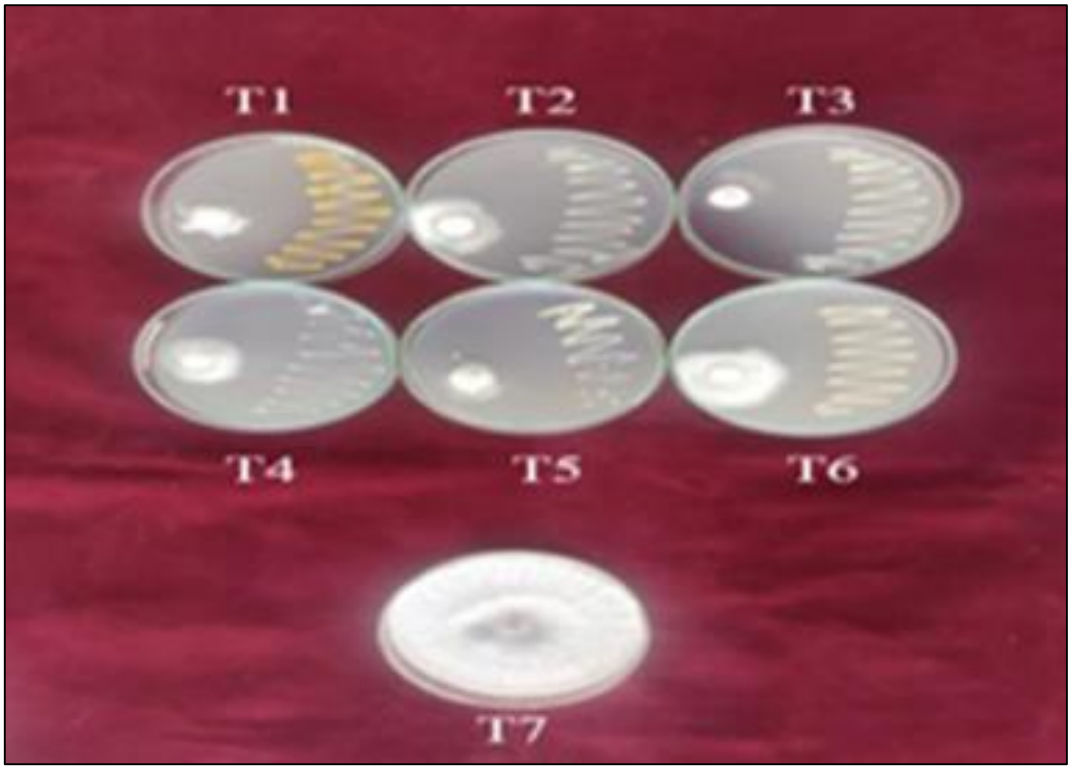


Fig 1: Evaluation of fungal bio control agents against *Macrophomina phaseolina* by dual culture technique

3.2 Evaluation of bacterial bio control agents (*in vitro*)

Results (Table 4 and Fig. 2) indicated that all bacterial bio control agents were antagonistic to the growth of *Macrophomina phaseolina*. Out of six antagonists tested, maximum inhibition over control was recorded in *B. subtilis*–isolate 1 (86.66%) which is statically at par with *P. fluorescens*-isolate 1 (84.44%). At the same time *B. thuringiensis*-isolate 1 (78.33%) was found next best followed by *P. fluorescens*-isolate 2 (73.61%) and *B. subtilis*-isolate 2 (70.55%) were moderately effective to inhibit fungal growth. Whereas, *B. thuringiensis*-isolate 2 was found least effective bacterial bio control agent inhibiting the mycelial growth of test pathogen (63.61%).



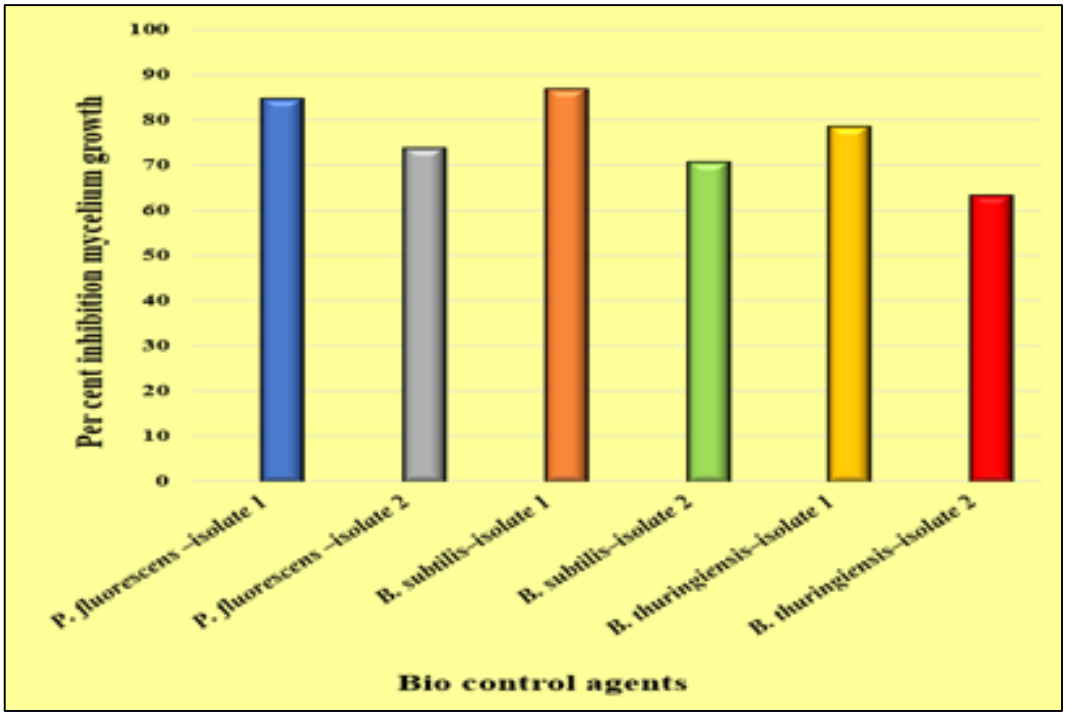


Fig 2: Evaluation of bacterial bio control agents against *Macrophomina phaseolina* by dual culture technique

Table 4: *In vitro* evaluation of bacterial bio control agents

Sl. No.	Bacterial bio control agents	Percent inhibition over control* (%)
1.	*Pseudomonas fluorescens*- isolate 1	66.80 (84.44)
2.	*Pseudomonas fluorescens*- isolate 2	59.09 (73.61)
3.	*Bacillus subtilis*- isolate 1	68.62 (86.66)
4.	*Bacillus subtilis*- isolate 2	57.14 (70.55)
5.	*Bacillus thuringiensis*- isolate 1	62.26 (78.33)
6.	*Bacillus thuringiensis*- isolate 2	52.90 (63.61)
	S.Em. ±	0.64
	C. D. at 5%	1.92
	C.V. %	2.12

*Data outside the parentheses are arcsine transformation whereas, inside are re-transformed values

The present findings are supported with the records of Rani *et al*. (2009) [11], Yalda *et al*. (2013) [17] and Kumar *et al*. (2015) [7] who reported the antagonistic potential of *Bacillus subtilis* against charcoal rot pathogen *Macrophomina phaseolina* under *in vitro*. Similar observations were commented for *P. fluorescens* over *Macrophomina phaseolina* by Kannan *et al.* (2003) [5], Lokesha and Benagi (2007) [8], Manoj (2018) [9] and Vinothini *et al*. (2020) [15] from dual culture technique. The results overlapped with the findings of Reetha and Mohan (2015) [12] while working with different isolates *P. fluorescens* and *B. subtilis,* observed and reported that both were effective in mycelial growth inhibition of *Macrophomina phaseolina.*

4. Conclusion

Biological control is of much significant in view of hazards caused by toxic chemicals where pathogens develop resistance to fungi toxicants. It can be concluded considering the results that the among all fungal bio control agents, *T. asperellum* followed by *T. harzianum* and out of all bacterial bio control agents, isolate 1 of *P. fluorescens*, *B. subtilis* found as a potent antagonist against *Macrophomina phaseolina*.

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