Biological Forum – An International Journal 13(3a): 261-265(2021)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

In vitro Efficacy of Microbial Bioagents Against *Rhizoctonia bataticola* (Taub.) Butler, Inciting Dry Root Rot Disease of Soybean

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(Received 06 July 2021, Accepted 11 September, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Dry root rot of soybean (*Glycine max* (L.) Merril) incited by *Rhizoctonia bataticola* (Taub.) Butler, is one of the most destructive diseases of soybean during recent years causing substantial economic losses (70 to 80%) to soybean growers. The study aimed at minimizing the indigenous usage of chemical fungicides and adopting the strategy of biological control for management of this devastating fungus. In this study, dual culture technique was used to see how effective various bioagents were at managing *R. bataticola*. All the nine bioagents tested *in vitro* had a significant inhibitory impact on the pathogen. *Trichoderma viride* produced maximum mycelial inhibition of the pathogen (86.30%) followed by *T. virens* (82.97%), *Aspergillus niger* (81.48%), *T. atroviride* (79.63%), *T. hamatum* (78.52%), *T. harzianum* (77.03%), *Pseudomonas fluorescens* (73.70%), *Bacillus subtilis* (70.74%), and *T. koningii* (69.63%).

Keywords: bioagents, biological control, dry root rot, *Rhizoctonia bataticola*, soybean.

INTRODUCTION

Soybean [*Glycine max* (L.) Merril; 2n=40], is one of the most happening crops of 21st century (Chauhan and Joshi, 2005) and occupies premier position among Indian oilseed crops. Popularly known as 'Golden bean' or 'Miracle bean', it is the world's most important oil-producing leguminous crop. Soybean has a global production of 385.524 million metric tons, mostly concentrated in the countries *viz*., USA, Brazil, Argentina and China. India occupies 4th position with respect to area under soybean cultivation and 5th position with respect to production at the world level (SOPA, 2021). This widely preferred crop is rich in vitamins, minerals such as calcium and iron and other nutraceuticals like isoflavones, tocopherol, lecithin etc. and have immense health benefits. It contains roughly 40% protein and all of the essential amino acids, as well as 18-20% oil (Raghuvanshi and Bisht, 2010).

Soybean crop has to compete against a plethora of biotic stresses. Plant diseases *viz.*, fungal, bacterial, viral diseases, are one of the major biotic limitations that affect soybean production, negatively impacting yield and quality. Among the fungal diseases, dry root rot caused by necrotrophic sclerotial fungus, *Rhizoctonia bataticola* (Taub.) Butler (Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid) has become a major problem in recent years affecting the profitable production of soybean. High incidence of dry root rot results in severe economic losses upto 80% particularly in areas where temperature ranges from 35 to 40°C during the crop season (Gupta and Chauhan, 2005). In India, *M. phaseolina* infection is responsible for almost 70% of soybean crop loss (Dave *et al*., 2020). Changing weather patterns, particularly longer and more frequent droughts during the growing season has also made this a big issue (Lodha and Mawar, 2010; Gupta *et al*., 2012).

This soil-borne polyphagous pathogen infects the host plant's roots *via* numerous germination hyphae at the seedling stage, and the fungus disrupts the vascular system, affecting water and nutrient transport to the upper portion of the plant. The management strategies of *R. bataticola* remains a challenge due to wider adaptability and longer survivability of the pathogen. Chemical control is posing serious threat to the ecosystem. Chemical pesticides are associated with a number of disadvantages such as environmental contamination, lack of specificity, high toxicity to animals, soil absorption and buildup since they are not biodegradable and finally, these chemicals have a high potential to result in pathogen resistance (Oskay, 2009). Hence, it is of paradigm importance to develop environmentally friendly disease management alternatives for management of the pathogen.

The use of microbial bioagents to control soil borne pathogens is not only a low cost technique but also protects soil health and is a promising option over chemical disease management. Thus, the current research was conducted to establish the efficacy of various bioagents and to develop a viable biocontrol approach for the management of soybean dry root rot incited by *R. bataticola*.

MATERIAL AND METHODS

A. Isolation of Pathogenic Fungi

Dry root rot affected soybean plants were collected in paper bags from the field of Plant Pathology farm, Post Graduate Institute, MPKV, Rahuri and carried to the laboratory for pathogen isolation. Isolation was carried out by tissue isolation technique. First, the diseased roots were rinsed thoroughly under running tap water before being transferred to sterilized blotting paper. Then, keeping half of the healthy portion and half of the diseased portion intact, the affected root portions were sliced into small (5 mm) pieces. These cut pieces were surface sterilized with 1% Sodium hypochlorite for 30 sec in order to eliminate surface contaminants. Immediately wash these pieces in sterile distilled water on Petri plates by sequential transfer to eliminate traces of Sodium hypochlorite and again it is blot dried. Later, the bits were transferred aseptically into previously sterilized Petri dishes containing solidified PDA medium and incubated at 25±1°C in BOD incubator. After 5th day of incubation, hyphal growth was observed from the plated bits which was further purified by hyphal tip method. Following the purification of the fungus, pathogen was identified under microscope based on morphological and cultural characters *viz*., septation, branching pattern of mycelium and formation of special structures like sclerotial bodies. By comparing it to the available standard literature, the pathogen was recognised as *Rhizoctonia bataticola* (Barnett and Hunter, 1972).

B. Sources of microbial bioagents

The Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri provided pure cultures and talc-based formulations of various biocontrol agents. These were maintained and multiplied on suitable culture media and utilised in the current research.

C. In vitro evaluation of bioagents against Rhizoctonia bataticola (Taub.) Butler

Using dual culture approach (Dennis and Webster, 1971) the efficacy of nine biocontrol agents were assessed against *R. bataticola* in the laboratory conditions. The study used cultures of the test bioagents and test pathogen that had been grown on PDA and/or NA for seven days. With the use of a sterilised cork borer, two culture discs (each 5 mm dia.) were cut out, one for the test pathogen and one for the test bioagent and placed at equidistance, exactly opposite to each other on autoclaved and cooled solid surface of PDA medium in Petri plates. Three replications were maintained for each antagonist and incubated at 28±2°C. PDA plates inoculated only with pure culture disc (5 mm) of the test pathogen was maintained as untreated control. *In vitro* test for antagonism against *R. bataticola* by dual culture was carried out with the following experimental details:

(i) Design : Completely Randomized Design (CRD)
(ii) Replications : Three
(iii) Treatments : Ten
(iv) Treatment details: Bioagents as below

Table 1: List of bioagents.

T_1	*Trichoderma viride*
T_2	*T. hamatum*
T_3	*T. harzianum*
T_4	*T. koningii*
T_5	*T. virens*
T_6	*T. atroviride*
T_7	*Aspergillus niger*
T_8	*Pseudomonas fluorescens*
T_9	*Bacillus subtilis*
T_{10}	Control

Percent inhibition of test pathogen by the bio-agents over untreated control was calculated by applying following formula given by (Vincent, 1927).

$$\text{Percent growth inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = growth of the test fungus in (mm) untreated control plates

T = growth of the test pathogen in (mm) treated plates

RESULTS AND DISCUSSION

In vitro efficacy of tested bioagents against *Rhizoctonia bataticola* (Taub.) Butler

The overall results, as shown in Table 2, revealed that all the bioagents tested against *R. bataticola* had a significant antagonistic effect on the mycelial growth of pathogen in dual cultures, when compared to untreated controls. Among the nine bioagents tested *in vitro*, *T. viride* was found the most effective with highest mycelial growth inhibition (86.30%) of test pathogen, followed by *T. virens* (82.97%), *A. niger* (81.48%), *T. atroviride* (79.63%), *T. hamatum* (78.52%), *T. harzianum* (77.03%), *P. fluorescens* (73.70%), *B. subtilis* (70.74%), and *T. koningii* (69.63%). Untreated control recorded maximum growth of *R. bataticola* i.e., 90mm. Competition for space and nutrition, antibiosis, lysis, synthesis of volatile or non-volatile chemicals and synthesis of cellulolytic/pectolytic enzymes by antagonistic organisms are all possible mechanisms for microbial antagonism against *R. bataticola*, which causes soybean dry root rot.

Table 2: *In vitro* efficacy of bioagents against *R. bataticola.*

Tr. No.	Treatments	Mean colony diameter of test pathogen (mm)	Percent mycelial growth inhibition over control
T1	*Trichoderma viride*	12.33	86.30 (68.28)*
T2	*T. hamatum*	19.33	78.52 (62.39)*
T3	*T. harzianum*	20.67	77.03 (61.36)*
T4	*T. koningii*	27.33	69.63 (56.56)*
T5	*T. virens*	15.33	82.97 (65.63)*
T6	*T. atroviride*	18.33	79.63 (63.17)*
T7	*Aspergillus niger*	16.67	81.48 (64.51)*
T8	*Pseudomonas fluorescens*	23.67	73.70 (59.15)*
T9	*Bacillus subtilis*	26.33	70.74 (57.25)*
T10	Control (untreated)	90.00	0.00 (0.00)*
SE(m) ±		0.37	-
CD at 1%		1.09	-

*Figures in parentheses are arc sine transformed values

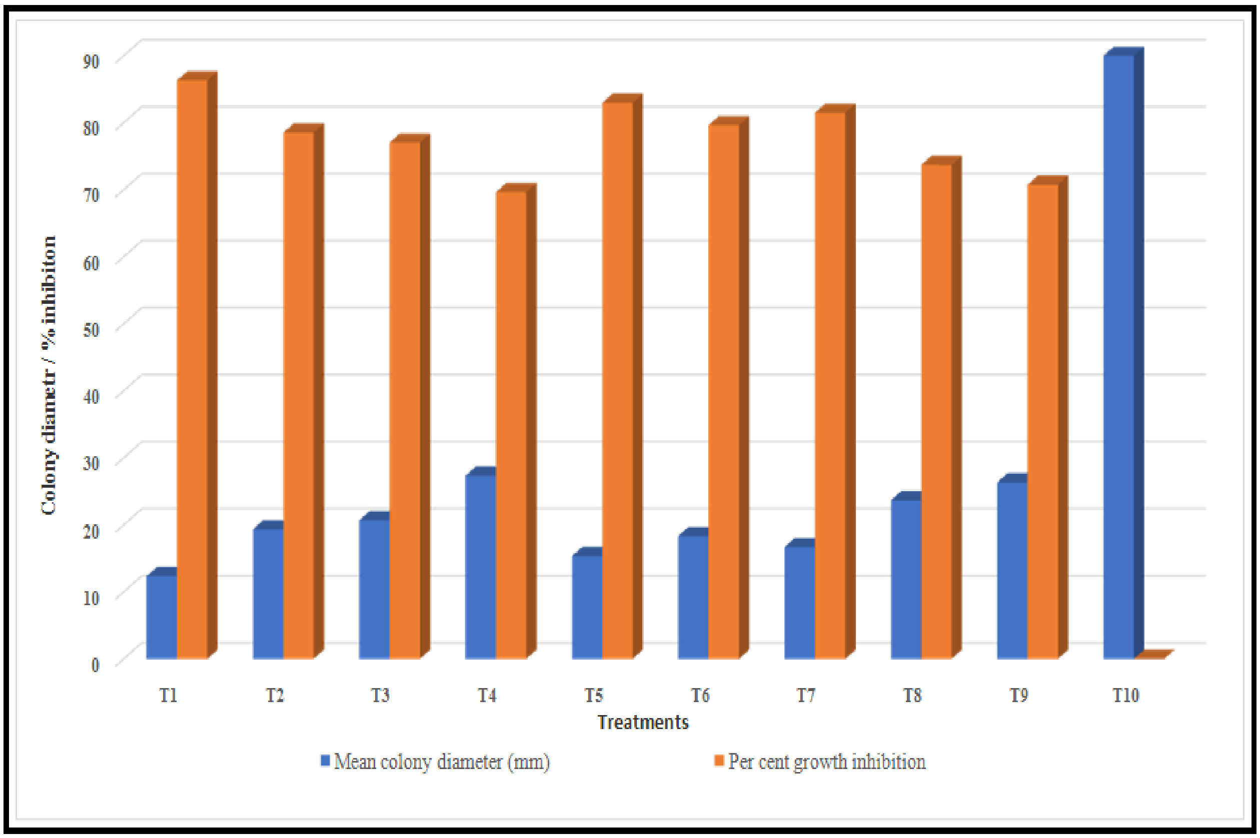

Fig. 1. *In vitro* efficacy of bioagents against *R. bataticola*.

The current results of *in vitro* evaluation were in accordance with the findings of Patil *et al*., (2004); Gaur and Sharma (2010), and many other workers. Doley and Jite (2012) reported that *T. viride* had considerable antifungal efficacy by suppressing the radial mycelial growth of *M. phaseolina* by 71.42 percent. Karthikeyan *et al.* (2015) also studied *in vitro* efficacy and recorded highest mycelial growth inhibition of 77.77% with *T. viride*. Pawar *et al*., (2018) reported *T. viride* as most effective in reducing pathogen's mycelial growth with mycelial inhibition percentage of 86.02 followed by *P. fluorescens* (37.58%) against *R. bataticola*. Tekade *et al*., (2021) also reported *T. viride* and *T. harzianum* as effective antagonists against *R. bataticola*.

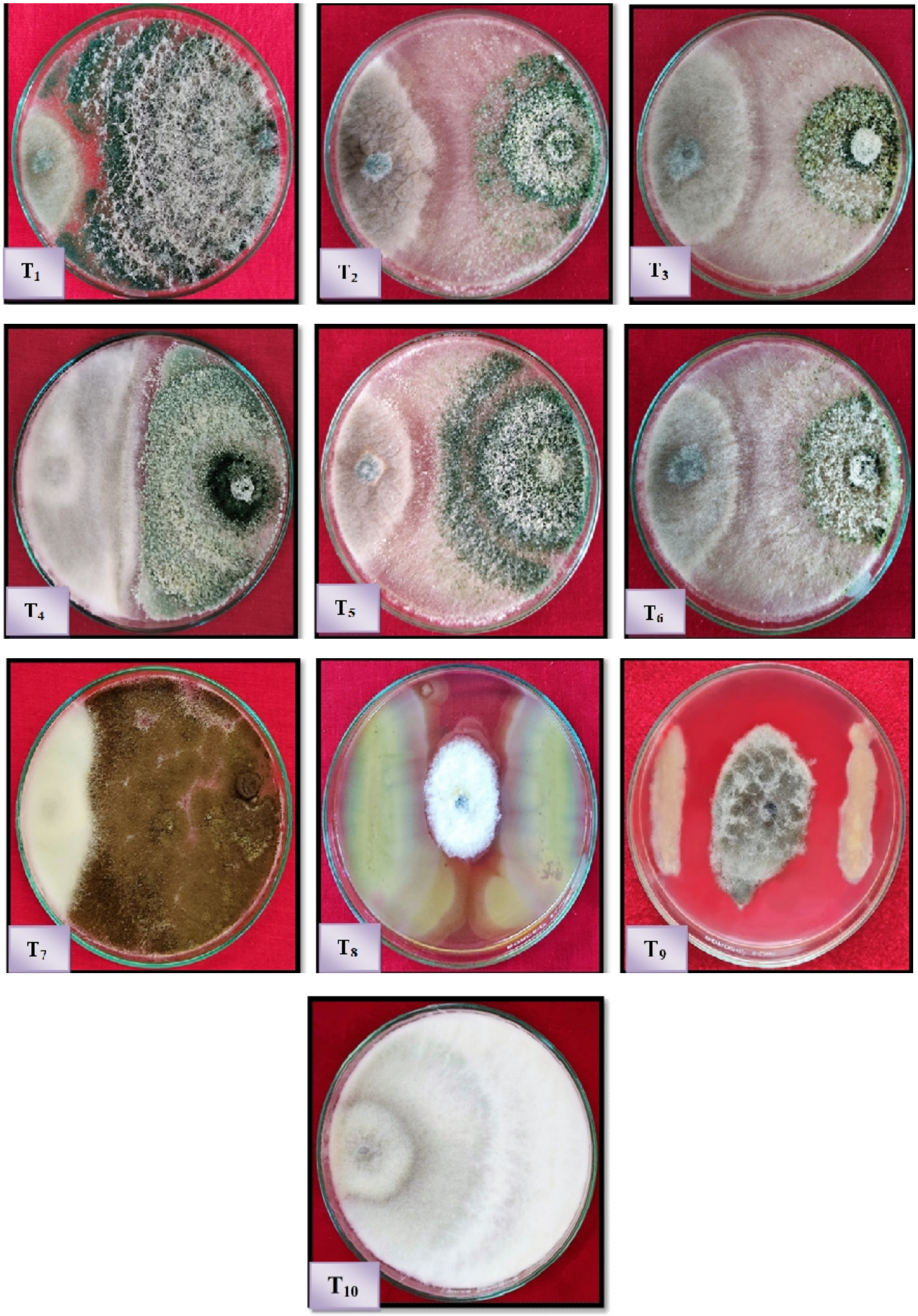

Fig. 2. *In vitro* efficacy of bioagents against *R. bataticola*.

CONCLUSION AND FUTURE SCOPE

The ability of polyphagous, soil inhabiting *R. bataticola* to survive for longer periods as sclerotia in the soil makes its management a difficult chore. However, the current study found that using bioagents aids in the effective management of soybean dry root rot disease. *Trichoderma viride* was found to be the most effective bioagent, followed by *T. virens* with mycelial growth inhibition percentage of 86.30 and 82.97 respectively. *T. koningii*, on the other hand, was shown to be the least effective against pathogen.

Use of bioagents greatly lower the disease incidence. Farmers benefit from the use of bioagents not only in terms of lowering cultivation costs, but also in terms of increasing yields. The current study advises more trials to be undertaken in naturally infected soybean fields to encourage the use of bioagents as an eco-friendly technique for the management of the dry root rot disease and thereby, lower the cost of cultivation by avoiding unsustainable chemical practices.

Acknowledgement. The authors gratefully thank the contributions of anonymous reviewers and the handling editor in revising the manuscript.

Conflict of Interest. None.

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How to cite this article: Ajesh B.R., Narute, T.K., Kolase, S.V., Latake, S.B. and Gaikwad, R.T. (2021). *In vitro* Efficacy of Microbial Bioagents Against *Rhizoctonia bataticola* (Taub.) Butler, Inciting Dry Root Rot Disease of Soybean. *Biological Forum – An International Journal*, *13*(3a): 261-265.