*Original Research Article*

# Potential of native microflora of Banana+Chilli+Garlic intercropping system for bio-control of *Rhizoctonia solani* causing root rot of chilli


## ABSTRACT

Root rot disease caused by *Rhizoctonia solani* is a serious problem in chili pepper production, leading to significant crop loss and reduced yield. The chemical control method for controlling this soil borne disease is not preferred due environmental concerns and harmful effect on beneficial soil microflora. So there is an increasing demand for safer and sustainable approaches. In the present investigation which was conducted in laboratory of the Department of Plant Pathology and Nematology, RPCAU, Pusa, Bihar during the year 2021-23, the microflora isolated from the rhizosphere of Banana+Chilli+Garlic intercropping system were evaluated for their effectiveness against *Rhizoctonia solani* causing root rot in Chilli. In course of study the isolates of *Trichoderma* and *Bacillus* significantly reduced the radial growth of *Rhizoctonia solani*. . The maximum growth inhibition was recorded by the *Trichoderma* isolate - GITr5 causing 71.40 % inhibition of the pathogen growth , This was followed by CITr4 with 67.41% and BITr1 with 62.86 %inhibition of the pathogen Among the bacterial isolates, The isolate- CIRB1 was found to inhibit the target pathogen by 71.22% followed by BIRB8 causing 66.92% inhibition of the pathogen.



***Key words:*** *Bacillus, Chili, Rhizoctonia solani and Trichoderma*


## 1. INTRODUCTION

Chili peppers, also known as *Capsicum annuum* are an important crop in agriculture with global production reaching over 34 million tons annually. This crop is widely cultivated and consumed in many parts of the world due to its culinary and medicinal properties. Chili pepper is rich in vitamin C, vitamin A, and other essential nutrients, making it a vital component of a balanced diet. Additionally, they are used in the production of spices, sauces, and other food products, contributing significantly to the food industry's economic value. The chilli crop is also grown as companion crop in different intercropping system.

Like other crops, chili pepper production is also threatened by various diseases caused by fungal pathogens, including *Rhizoctonia solani,* which causes root rot disease, leading to significant crop losses and reduced yield. *Rhizoctonia solani* is a soil-borne fungal pathogen that causes significant damage to chili pepper plants, resulting in reduced crop yield and quality. This pathogen is commonly found in soil and can survive for several years. It can cause up to 33.2 percent disease incidence of the seedling in greenhouse condition and in main field 40.2 percent [1]. This pathogen is commonly found in soil and identified as both as seed and soil-borne pathogen [2]. It attacks chili pepper plants at the root level, causing root rot disease and stunted growth. Symptoms of the disease include wilting, yellowing of leaves, and root decay, and leading to plant death in severe cases.

Traditional control methods for controlling this disease involve the use of chemical pesticides, which is known to pose serious threat to the environment and human health. Therefore, there is an increasing demand for safer and sustainable approaches to manage this disease. Hence the isolates of various micro-organism obtained from crop rhizosphere, have been largely explored for their bio-control potential against various soil borne pathogens. These microorganisms can colonize the root zone and provide protection against soil-borne pathogens by either producing antimicrobial compounds or inducing systemic resistance in the host plant.

In the present investigation the isolates of *Trichoderma* and *bacteria* obtained from the crop rhizosphere of Banana+Chilli +Garlic intercropping system were evaluated for their bio-control potential against Rhizoctonia solani causing root of chilli.

## 2. Materials and methods

### 2.1 Isolation of Pathogen

*Rhizoctonia solani*, obtained from infected chilli roots/seedlings was isolated as per the methodology outlined [3]. The samples obtained from affected plants were utilized for isolation. The roots were thoroughly washed with tap water to remove soil. Small pieces of about 0.5 cm length were surface sterilized with 0.1 per cent mercuric chloride solution for 2 minutes, then three washings with sterilized distilled water, were aseptically transferred to potato dextrose agar (PDA) medium. The plates were then incubated at a temperature of 25 ± 2°C to facilitate mycelial growth. Purification of the cultures was achieved using the hypal tip method as described [4]. The subsequent analysis involved a comparison of various cultural and morphological characteristics of the isolated pathogen with the descriptions provided [5].

### 2.2 *In-vitro* experiment

Different Isolates of *Trichoderma* and *bacteria,* obtained from the rhizosphere of Banana+Chilli +Garlic intercropping system, were evaluated for their antagonistic effect on *Rhizoctinia solani* (pathogen of chillli root rot) by dual culture technique [6] using potato dextrose agar (PDA) medium. 7 days old mycelia culture (5 mm) of target pathogen and test biocontrol agents were plated on 90 mm Petri plates containing PDA, near periphery about 60 mm apart. The growth of the pathogen alone on PDA plate was utilized as a control. The

experiment comprised three replicates for each treatment, and the entire setup followed a completely randomized design. The plates were incubated at 25 ± 1°C for 7 days. Radial growth was recorded on 7th day of incubation and mycelia inhibition calculated [7].

$$\text{Percentage Growth Inhibition} = \frac{(C-T)}{C} \times 100$$

Where,

C = Radial growth of pathogen (mm) in check

T = Radial growth of pathogen (mm) in treatment

## 2.2 Single hyphal tip isolation technique

To maintain the pure culture of *Rhizoctonia solani,* single hyphal tip isolation technique was adopted. Using a microscope at low magnification (10X), a single hyphal segment was identified and delineated, then carefully severed with a mechanical cutter. Each hyphal fragment was transferred to potato dextrose agar (PDA) slants by using an inoculating needle. The inoculated slants were placed in a BOD Incubator and allowed to grow at 26 ± 1°C for a duration of 5 days. This process resulted in the establishment of purified cultures for pathogenic isolates obtained from diseased chilli fields. These cultures were maintained through periodic transfers onto PDA slants and were subsequently utilized for further investigations.

### 2.2.1 Dual culture method

#### *2.2.1.1 Evaluation of different Trichoderma isolates for their antagonistic potential against Rhizoctonia solani*

The Trichoderma isolates were evaluated for their antagonistic potential *in vitro* against *Rhizoctonia solani* by dual culture method [8]. 5 mm mycelial discs of the test pathogen from a 7-day old culture and *Trichoderma* isolate from an actively growing culture were aseptically placed into 90 mm Petri plates containing PDA media, opposite to each other with 60 mm distance between them. The plates were incubated at 28± 2$^0$C. Three replications of each isolate were maintained. The unchallenged pathogen was maintained as control.

#### *2.2.1.2 Evaluation of different Bacterial isolates for their antagonistic potential against Rhizoctonia solani*

The bacterial isolates were evaluated for their antagonistic potential *in vitro* against *Rhizoctonia solani* [9 ] . A 5mm sample of the test pathogen from a seven-day old culture was placed at the centre of a 90 mm Petri plate containing PDA medium. Four different bacterial isolates from respective 24-hour old cultures were streaked on four sides of the Petri plate opposite to each other and at 1 cm from the periphery. These plates were incubated at 26± 1 $^o$C. A plate inoculated with only *Rhizoctonia solani* was maintained as control. The inhibition of mycelial growth of the test pathogen was observed. Radial growth of *R. solani* was recorded and percent inhibition was calculated by using following [6].

$$\text{Percentage Growth Inhibition} = \frac{(C-T)}{C} \times 100$$

Where: C = Radial growth of in *R. solani* control (mm)

T= Radial growth of *R. solani* against antagonist (mm)

## 3. RESULTS AND DISCUSSION

### 3.1 Antagonistic effect of *Trichoderma* isolates on *Rhizoctonia solani*

The antagonistic activity of *Trichoderma* isolates were evaluated against the target pathogen-*Rhizoctonia solani* by dual culture method. Percentage inhibition was calculated based on observations of the radial growth of the antagonist and the target pathogen and the results presented (Table 1). The results revealed that all the test isolates of *Trichoderma* were significantly effective in inhibiting the growth of target pathogen. The maximum growth inhibition was recorded in GITr5 with 71.40% growth inhibition followed by CITr4 with 67.41% and BITr1 with 62.86% inhibition of target pathogen respectively, after 5 days of incubation. However, eight days later *R. solani* was completely overgrown by *Trichoderma spp.* This discovery aligns with numerous reports that have asserted that *T. harzianum , T. virens* and *T. hamatum* exhibit high efficacy in suppressing the mycelial growth of soil-borne, seed borne, phyllosphere and storage plant pathogens on PDA [ 10, 11]. *Trichoderma* strains were reported to secrete many cell wall degrading enzymes during the mycoparasitic interaction with its hosts. In this respect, Chitinases and β -1,3-glucanases have been found to be directly involved, which allow them to bore holes into its host fungi and extract nutrients for their own growth. In addition, it strongly inhibited sclerotia production and suppressed sclerotia germination of pathogen [12]. *Trichoderma* isolates are renowned for their ability to produce diverse bio-products that exhibit toxicity against phyto-pathogenic fungi. Among the metabolic byproducts, pyrones, koninginins, viridian, gliovirin, gliotoxin, peptaibols, and various others have been documented [11]. The inhibition of pathogen may also be attributed to the production of secondary metabolites by antagonists such as glioviridin, viridin and gliotoxin. Similar observations were made regarding the suppression of mycelial growth of *R. solani* pathogenic to chilli and certain other host plants by different microbial antagonist like *Trichoderma* spp. by various workers[13, 14,].

**Table: 1 *In vitro* effect of *Trichoderma* spp. on radial growth of *Rhizoctonia solani***

| Sl. No. | *Trichoderma* spp. | 72Hrs | | 120Hrs | |
|---|---|---|---|---|---|
| | | Radial Growth (mm) | Inhibition (%) | Radial Growth (mm) | Inhibition over control (%) |
| 1 | BITr1 | 16.13±0.58 | 62.99 | 16.99±0.47 | 62.86 |
| 2 | CSTr2 | 19.33±0.64 | 55.65 | 20.56±0.64 | 55.06 |
| 3 | BSTr3 | 18.83±1.34 | 56.81 | 19.08±0.88 | 58.29 |
| 4 | CITr4 | 13.78±0.56 | 68.38 | 14.91±0.48 | 67.41 |
| 5 | GITr5 | 12.51±0.55 | 71.29 | 13.08±0.49 | 71.40 |
| 6 | BSTr6 | 22±0.88 | 49.53 | 23.58±0.71 | 48.45 |
| 7 | CITr7 | 29.81±0.87 | 31.61 | 31.29±0.69 | 31.59 |
| 8 | CSTr8 | 25.58±0,71 | 41.31 | 27.17±0.71 | 40.62 |
| 9 | BSTr9 | 32.27±0.97 | 25.98 | 33.99±1.06 | 25.70 |
| | Control | 43.59±0.71 | | 45.75±0.73 | |
| | C.D. at 5 (%) | 2.68 | | 2.46 | |
| | SE(m) ± | 2.21 | | 1.78 | |

| | | | | | |
|---|---|---|---|---|---|
| | C.V. (%) | 6.20 | | 5.24 | |

**B: Banana, C: Chilli, G: Garlic, I: Intercrop, S: Sole, Tr: *Trichoderma* spp.,**

### 3.2 Antagonistic effect of the *Bacterial* bio-agents on *R. solani*

In the present study, the bacterial isolate (CIRB1) was found to record maximum growth reduction of *R. solani* showing 71.22 percent inhibition in dual culture, which was followed by bacterial isolates (BIRB8), which recorded the growth reduction by 66.92 percent of the pathogen (Table-2). The other isolates were not so effective in suppression of the pathogen growth. Earlier also various bacteria have been reported to exhibit strong antagonistic effect on the pathogen. *B. subtilis* might also act on pathogenic fungi by either producing antifungal substances or colonizing microsites faster than the surface fungi. *Bacillus subtilis* has been reported to produce lip peptides belonging to the iturin and surfactin in the late phase of growth that inhibit *R. solani* growth [17]. Similar observations were made regarding the suppression of mycelial growth of *R. solani* pathogenic to chilli and certain other host plants by different microbial antagonist like *P. fluorescens*, *B. subtilis* etc. by various workers [ 15, 16].

**Table: 2 *In vitro* effect of rhizobacteria on radial growth of *Rhizoctonia solani***

| Sl.No. | Rhizobacteria | 72hrs | | 120hrs | |
|---|---|---|---|---|---|
| | | Radial Growth (mm) | Inhibition (%) | Radial Growth (mm) | Inhibition (%) |
| 1 | CIRB1 | 12.25± 0.54 | 73.02 | 13.50± 0.54 | 71.22 |
| 2 | BIRB2 | 17.42±0.65 | 61.65 | 18.50± 0.42 | 60.56 |
| 3 | BIRB3 | 26.1±0.49 | 42.52 | 27.10± 0.41 | 42.23 |
| 4 | CIRB4 | 23.07±0.48 | 49.20 | 24.08± 0.44 | 48.66 |
| 5 | CIRB5 | 25.03±0.46 | 44.87 | 26.03± 0.43 | 44.50 |
| 6 | BSRB6 | 31.07±0.48 | 31.56 | 32.08± 0.42 | 31.62 |
| 7 | BSRB7 | 22.02±0.48 | 51.52 | 23.34± 0.71 | 50.24 |
| 8 | BIRB8 | 14.27±0.60 | 68.58 | 15.52± 0.58 | 66.92 |
| 9 | BIRB9 | 33.17±0.51 | 27.07 | 34.37± 0.60 | 26.74 |
| 10 | CSRB10 | 37.47±0.71 | 17.60 | 39.17± 0.94 | 16.51 |
| 11 | CSRB11 | 26.17±1.06 | 42.38 | 28.50± 1.17 | 39.25 |
| 12 | CSB12 | 36.23±0.58 | 20.21 | 37.71± 0.76 | 19.62 |
| | Control | 45.47±0.71 | -0.01 | 46.92± 0.76 | -0.01 |
| | C.D. at 5 (%) | 2.58 | | 2.86 | |
| | SE(m) ± | 1.27 | | 1.78 | |
| | C.V. (%) | 4.48 | | 4.95 | |

**B: Banana, C: Chilli, G: Garlic, I: Intercrop, S: Sole, R: Rhizosphere, B: Bacteria**

## 4. CONCLUSION

In the present investigation, eight *Trichoderma* isolates and nine bacterial isolates ,obtained from the rhizosphere Banana+Chilli +Garlic intercropping system were evaluated i*n vitro* for their bio-control potential against the fungus- *Rhizoctonia solani* , the root rot pathogen of chillii. Among various isolates*, the Trichoderm isolate*- GITr5 was most effective in reducing radial growth of the pathogen-*Rhizoctonia solani* (71.40% inhibition ) followed by bacterial isolates CIRB1(71.22% inhibition ). These isolates may be further evaluated under field condition for evolving eco-friendly tool for management of root rot disease of chilli caused by *Rhizoctonia solani.*

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