



POTENTIAL OF *TRICHODERMA* SPP. AS BIOCONTROL AGENTS AGAINST *RHIZOCTONIA BATATICOLA* CAUSING DRY ROOT ROT OF CHICKPEA

G Amrutha Veena¹, N P Eswara Reddy², B V Bhasakara Reddy³ and L Prasanthi⁴

^{1&2}Department of Plant Pathology, S V Agricultural College, Tirupati-517 502., A.P., India.

^{3&4}Institute of Frontier Technology, Tirupati-517 502., A.P., India.

ABSTRACT: Ten *Trichoderma* spp were isolated from chickpea rhizosphere and root endophytic region by using serial dilution technique and purified by single hyphal tip method. Out of the ten isolates tested against *Rhizoctonia bataticola*, *Trichoderma* isolate-7 showed highest inhibition percentage (83.33). In compatibility tests with commonly used fungicides, *Trichoderma* isolate-7 showed highest compatibility with validamycin (72.22%) followed by copper oxychloride (66.66%).

Key words: *Trichoderma*, Biocontrol, *Rhizoctonia bataticola*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important food legume crop. In India, it is grown over an area of 8.74 m ha with an annual production of 7.35 million tonnes and productivity of 841 kg ha⁻¹ [2]. In Andhra Pradesh, it is grown in an area of 5.84 lakh ha with an annual production and productivity of 7.19 lakh tonnes and 1233 kg ha⁻¹ respectively [1]. Dry root rot by *Rhizoctonia bataticola* (Taub.) Butler cause considerable yield losses in chickpea which may be as high as 50 to 71 per cent. Effective and practical chemical control is not feasible. Biological control appears to be the only solution for long-term sustainability and effective management of soil borne diseases. Different *Trichoderma* species are effective against *Rhizoctonia*. Testing the compatibility of efficient biocontrol agents with commonly used fungicides against the disease was important for integrated disease management studies.

MATERIALS AND METHODS

Isolation of native antagonistic mycoflora from root endophytes

For isolation of endophytes, five g of root was surface sterilized for 5 min with 70 per cent ethanol and homogenized in 20 ml of sterilized phosphate buffer (0.2M Na₂HPO₄ + 0.2M NaH₂PO₄) pH 7.0 using mortar and pestle. Appropriate dilutions (10⁻⁴ for fungi) of these suspensions were plated on RBA/PDA for the isolation of *Trichoderma* / fungi. The plates were incubated for 72 h at 28 ± 2°C [7]. Three days old colonies of mycoflora were picked and purified by hyphal tip method.

Identification of potential biocontrol agents

The efficacy of antagonistic mycoflora from rhizosphere and root endophytes was determined by dual culture technique [8] under *in vitro* conditions.

Dual culture technique

To test the efficacy of antagonistic fungus, twenty ml of sterilized melted PDA was plated in Petri plates (9 cm) and allowed to solidify. Mycelial discs measuring six mm diameter from three day old cultures of both fungal antagonist and the test pathogen were placed at equidistant on sterile Petri plate containing PDA medium. The petri plates with pathogen inoculated at one end alone, served as control. The petri plates were then incubated at 28 ± 2°C. Three replications were maintained in each treatment. Growth of antagonists and pathogen were measured after recording full growth of the pathogen in control plate. Per cent inhibition of mycelial growth of test pathogen was calculated by the formula:

$$I = \frac{C - T}{C} \times 100$$

where,

I = Per cent inhibition in growth of test pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment.

Identification of Compatibility of Potential Biocontrol Agents with Fungicides *In Vitro*

Potential biocontrol agents were tested for their compatibility with the fungicides *viz.*, copper oxychloride (0.25%), captan (0.25%), hexaconazole (0.2%), tebuconazole (0.1%) and validamycin (0.1%). The compatibility of antagonistic fungi with different fungicides were tested by poisoned food technique [10] under *in vitro* conditions.

Poisoned Food Technique

To 50 ml of sterilized distilled water, required quantity of fungicide was added and mixed thoroughly. This solution was added to 50 ml of sterilized cool molten double strength PDA medium, mixed thoroughly and poured into Petri plates. Six mm discs of four days old culture of pathogen were inoculated at the centre of Petri plates and then incubated at $28 \pm 2^\circ\text{C}$. Three replications were maintained for each fungicide. Medium without fungicide was kept as control. Per cent inhibition of the growth of the fungus over the control was calculated using the formula:

$$I = \frac{C - T}{C} \times 100$$

where,

I = Per cent inhibition in growth of test pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment.

RESULTS AND DISCUSSION

Isolation and identification of native antagonistic mycoflora from rhizosphere soil and root endophytic region of chickpea against *R. bataticola*

Antagonistic mycoflora from rhizosphere soil and roots of healthy chickpea plants were isolated. The mycoflora were isolated on Rose Bengal Agar (RBA) medium. The fungal antagonists were purified by single hyphal tip method and were maintained on PDA medium. A total of 10 fungi were obtained from rhizosphere soil and root endosphere.

Based on colony and morphological characters, mycoflora were identified. Ten *Trichoderma* isolates designated from CT1 to CT10 were purified. Ramesh and Korikanthimath [14] isolated biocontrol agents like *Trichoderma viride*, *T. harzianum* from rhizosphere of various crops and tested their efficacy against the *Macrophomina phaseolina* causing root rot of groundnut.

In vitro evaluation of efficacy of antagonistic mycoflora (*Trichoderma* spp.) against *R. bataticola* in dual culture technique.

All the native antagonists i.e *Trichoderma* isolates showed significant reduction in mycelial growth of *R. bataticola* when compared to control. The data pertaining to per cent inhibition of mycelial growth of *R. bataticola* due to *Trichoderma* spp. are presented in Table 1. Among 10 *Trichoderma* isolates, *Trichoderma* isolate-7 (CT7) showed maximum inhibition of growth of *Rhizoctonia bataticola* (83.33%) followed by *Trichoderma* isolate-4 (81.11%) and *Trichoderma* isolate-10 (74.44%) which were on par with each other (Plate 19). The least inhibition was shown by *Trichoderma* isolate-9 (45.56%). These results were in agreement with Bandyopadhyay [3] who reported that strain of *Trichoderma* inhibited the growth of *Rhizoctonia bataticola* by 51.1 per cent under *in vitro* conditions. Paul [13] reported that among 11 *Trichoderma* isolates tested, *Trichoderma harzianum* showed higher inhibition of the growth of *Rhizoctonia solani* by 77 per cent under *in vitro* conditions. Kaushal (2008) reported that *T. harzianum* was effective in inhibiting the mycelial growth of *R. bataticola* the causal organism of chickpea dry root rot.

Pan [11] reported the antagonistic potential of *Trichoderma* isolates through production of volatile and non-volatile substances against *Macrophomina phaseolina*. Choudhary [4] tested four bioagents viz, *Trichoderma viride*, *Trichoderma harzianum*, *Aspergillus versicolor* and *Bacillus firmus* for the control of dry root rot in mungbean. In dual cultures, *Trichoderma viride*, *Trichoderma harzianum* and *Aspergillus versicolor* were effective in inhibiting the growth of *Macrophomina phaseolina* to an extent of 61 to 65%.

Table 1. In vitro evaluation of efficacy of antagonistic *Trichoderma* isolates against *Rhizoctonia bataticola* in dual culture technique

S.No.	Antagonist	Linear growth of <i>R. bataticola</i> (Cm)*	Per cent inhibition of mycelial growth of <i>Rhizoctonia bataticola</i>
1.	<i>Trichoderma</i> isolate-1 (CT1)	2.6	71.11 (57.50)
2.	<i>Trichoderma</i> isolate-2 (CT2)	3.6	60.00 (50.77)
3.	<i>Trichoderma</i> isolate-3 (CT3)	3.8	57.77 (49.52)
4.	<i>Trichoderma</i> isolate-4 (CT4)	1.7	81.11 (64.28)
5.	<i>Trichoderma</i> isolate-5 (CT5)	4.5	50.00 (45.04)
6.	<i>Trichoderma</i> isolate-6 (CT6)	4.7	47.78 (43.72)
7.	<i>Trichoderma</i> isolate-7 (CT7)	1.5	83.33 (65.96)
8.	<i>Trichoderma</i> isolate-8 (CT8)	4.0	55.55 (48.24)
9.	<i>Trichoderma</i> isolate-9 (CT9)	4.9	45.56 (42.44)
10	<i>Trichoderma</i> isolate-10 (CT10)	2.3	74.44 (59.64)
11	Control	9.0	-
	S. Em ±		2.42
	C.D (0.05)		7.21

* Mean of three replications

Figures in parenthesis are angular transformed values

In vitro evaluation of the compatibility of potential fungal antagonists with different fungicides.

The highly potential fungal antagonist *Trichoderma isolate-7* (CT7) was selected for fungicidal compatibility studies since it has shown maximum inhibition of *Rhizoctonia bataticola* growth in dual culture studies when compared to all other antagonists. Poisoned food technique was used to evaluate the compatibility of *Trichoderma isolate-7* (CT7) with different fungicides and the results are presented in Table 2. The results revealed that the *Trichoderma isolate-7* (CT7) was more compatible with validamycin (72.22%), followed by copper oxychloride (66.66%). The *Trichoderma isolate-7* (CT7) showed complete incompatibility with the fungicides hexaconazole and tebuconazole, whereas the fungicide captan has shown 22.22 per cent compatibility. The present results were in agreement with Tiwari and Singh [15] who reported that the mycelial growth of *T. harzianum* was completely inhibited by carbendizam and hexaconazole @ 1500 ppm, whereas copper oxychloride and mancozeb @ 1500 ppm has shown 90 and 41 per cent inhibition respectively.

Table 2. In vitro evaluation of compatibility of the potential fungal antagonist *Trichoderma isolate-7* (CT7) with different fungicides in poisoned food technique

S. No.	Fungicides	Linear growth of <i>Trichoderma isolate-7</i> (CT7) (Cm)*	Per cent compatibility
1	Copper oxychloride (0.25%)	6.0	66.66(54.77)
2	Captan (0.25%)	2.0	22.22(28.04)
3	Hexaconazole (0.2%)	0	0.00(0.00)
4	Tebuconazole (0.1%)	0	0.00(0.00)
5	Validamycin (0.1%)	6.5	72.22(58.24)
6	Control	9.0	-
	S.Em ±		1.61
	CD (0.05)		5.26

* Mean of three replications

Figures in parenthesis are angular transformed values

Naseema Beevi [9] tested the *in vitro* compatibility of *T. harzianum* with mancozeb, carbendazim and copper oxychloride and found that carbendazim at 0.1 per cent completely inhibited the mycelial growth while mancozeb and copper oxychloride showed compatibility with the antagonist at 0.2 and 0.1 per cent respectively. Parab [12] studied the sensitivity of *T. harzianum* to different fungicides and the results indicated that all systemic fungicides were found completely inhibiting growth of *T. harzianum* whereas zineb, copper hydroxide, mancozeb and copper oxychloride were found safe for the growth of *T. harzianum*. Gaur and Sharma [5] tested the *in vitro* compatibility of *Trichoderma harzianum* (TG-1) against different fungicides. Metalaxyl, fosetyl-Al, mancozeb, cymoxanil 8% + Mancozeb 64% mixture and copper oxychloride fungicides were found compatible with tolerance limits (ED₅₀) of >1000 µg/ml.

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