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IN VITRO EFFICACY OF *TRICHODERMA VIRIDE* AGAINST *COLLETOTRICHUM CAPSICI* : THE CAUSATIVE AGENT OF TURMERIC LEAF SPOT

S. Chandraprakash[1]*, A. Deena[1], N. Kiruba[1], M.D. Kanishka[1], S. Suresh[1] and B. Muthuraja[2]

[1]Department of Crop Protection, P.G.P. College of Agricultural Sciences, Namakkal - 637 405, Tamil Nadu, India.

[2]Department of Social Sciences, P.G.P. College of Agricultural Sciences, Namakkal - 637 405, Tamil Nadu, India.

*Corresponding author E-mail : suriyaprakash235@gmail.com

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ABSTRACT

Turmeric (*Curcuma longa*), valued for its medicinal properties in India, faces a threat from *Colletotrichum capsici*, causing leaf spot and yield losses of 15% to 60%. *Colletotrichum capsici* was isolated from infected turmeric leaves, displaying characteristic features. *Trichoderma viride* effectively inhibited its growth *in vitro*. Treatment T1R3 showed the highest inhibition (72.22% PIOC) of mycelial growth, while T1R2 exhibited the lowest (2.85cm). This study underscores *Trichoderma viride*'s potential as a biocontrol agent against *Colletotrichum capsici*, offering insights for managing turmeric leaf spot and improving agricultural productivity.

Key words : Turmeric, Leaf spot, *Colletotrichum capsici*, *Trichoderma viride*, Dual culture.

Introduction

Turmeric (*Curcuma longa*) is a vital spice crop in India, known for its medicinal benefits, particularly its anti-inflammatory properties attributed to curcumin (Bhowmik *et al*., 2009). The productivity of turmeric in Tamil Nadu is notably reported as 3.80 MT/ ha (cured rhizome yield) (Indhumathi *et al*., 2023). India, the primary global producer of turmeric, faces challenges from pathogens like *Colletotrichum capsici*, causing leaf spot disease. This disease significantly reduces turmeric yields, with losses ranging from 15% to 60% (Binalata *et al*., 2017). To manage such diseases, biocontrol agents like *Trichoderma viride* have been employed. *T. viride* shows promising efficacy against *C. capsici* (Mamatha *et al*., 2006). This study aims to evaluate the effectiveness of *T. viride* against *C. capsici*, specifically targeting turmeric leaf spot, through *in vitro* experimentation. Such research endeavors are crucial for developing sustainable strategies to combat foliar diseases and enhance turmeric production.

Materials and Methods

Experiments on the "*In vitro* Efficacy of *Trichoderma viride* Against *Colletotrichum capsici*, the Causative Agent of Turmeric Leaf Spot" were conducted in the Plant Pathology Laboratory at PGP College of Agricultural Sciences in Namakkal. The laboratory is situated at 11.229545° latitude and 78.200957° longitude, with an elevation of 218 meters above Mean Sea Level (MSL).

Isolation of the *Colletotrichum capsici*

Leaf spot infected samples were collected from turmeric fields and the pathogen was isolated using a standard protocol. Leaf bits measuring 5mm were prepared from the junction of leaf spot-infected and healthy portions of turmeric leaves. These leaf bits were surface sterilized with 70% ethanol and rinsed twice with sterilized water. After removing excess water, the leaf bits were placed in potato dextrose agar medium under aseptic conditions. The culture was stored at 4°C (Binalata *et al*., 2017).

Cultural and morphological characterization of *C. capsici*

The pathogen was identified based on cultural and morphological characteristics observed in Petri dishes. Cultural traits and morphological features were noted. Spores of *C. capsici* were obtained from 7-day-old culture plates, mounted on a clean glass slide, mixed with water for uniform spreading, covered with a cover slip, and examined under an Almicro compound light microscope. Images were captured using a Strange View electronic eyepiece and the size of fruiting bodies and spores was measured using a stage and an ocular micrometer.

***In vitro* assay of *T. viride* against *C. capsici* (dual culture technique)**

The *T. viride* culture employed in this study was obtained from the commercial product sourced from Tamil Nadu Agricultural University, Coimbatore.

The efficacy of *T. viride* against *C. capsici* was evaluated using the dual culture technique. Seven-day-old *C. capsici* culture bits (6mm diameter) were placed at one end of a Petri dish containing potato dextrose agar medium, positioned 1cm away from the dish margin. Similarly, seven-day-old *T. viride* culture bits of the same size were placed at the opposite end of the same dish. Each combination of *C. capsici* and *T. viride* in a single dish was considered a treatment, with three replications per treatment. Control plates consisted solely of pathogen inoculation. After full pathogen coverage in control plates, radial growth was measured in both control and treatment plates to calculate percent inhibition over the control. The inhibition of radial growth of pathogen was calculated by the following formula (Bassi Santosh and Sobita Simon, 2020)

$$\text{Percent Inhibition Over Control (PIOC)} = \times \frac{(C-T)}{C} 100$$

Where,

C = Radial growth of the pathogen in control,

T = Radial growth of the pathogen in treatment.

Statistical analysis

The data were analyzed using the variance method outlined by Gomez and Gomez (1984). Although, the calculated p-value was greater than the chosen significance level of 0.05, indicating no statistical significance, meaningful differences were observed between the experimental and control groups. Thus, the null hypothesis was rejected. This suggests that the experimental conditions have a significant effect on the outcome variable compared to the control condition. Therefore, it can be concluded that the experimental interventions yield statistically meaningful improvements despite not meeting the conventional threshold for statistical significance.

Results and Discussion

The investigations align with the stated objectives and cover aspects including a survey on turmeric leaf spot, symptomatology, cultural studies on *Colletotrichum capsici* growth and *in vitro* evaluation of a bio-control agent against the pathogen.

Isolation and characterization of *Colletotrichum capsici*

C. capsici was isolated from leaf spot-infected turmeric leaves, appearing initially as small circular white colonies, which later matured into white to olivaceous grey, cottony or fluffy mycelium with concentric zones. The mycelium is branched, septate and olivaceous grey, with acervuli containing black-colored setae. Conidia were released from compactly present conidiophores in the acervuli, being hyaline, single-celled and sickle-shaped

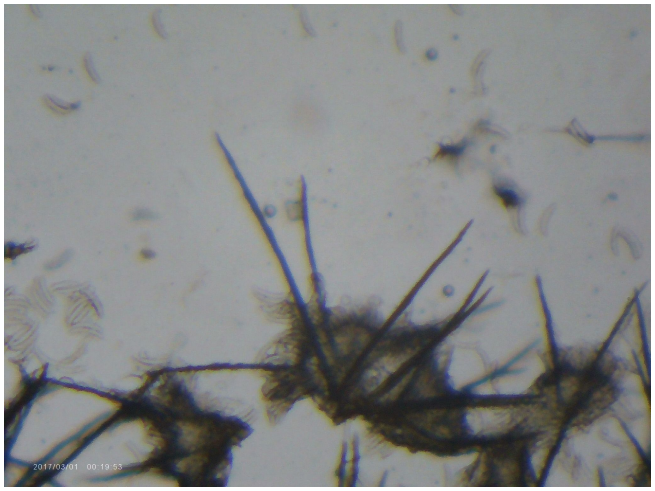

Fig. 1 : Acervuli produced by *C. capsici.*

Fig. 2 : Sickle shaped conidia produced by *C. capsici.*

Fig. 3 : *In vitro* efficacy of *T. viride* against *C. capsici*.

(Fig. 2). The size measurements were: acervuli diameter ranging from 303.3 to 454.95 µm, setae length ranging from 72.6 to 82.5 µm, and conidia size ranging from 19.8-23.1 × 2.6-3.3 µm (Fig. 3).

In vitro, screening of *Trichoderma viride* isolates against *Colletotrichum capsici*

T. viride-treated dual culture plates displayed the dominance of *C. capsici* in terms of mycelial growth. Among the treatments, T1R3 exhibited the highest inhibition with 2.50cm of mycelial growth of the pathogen, while the control plate, without any treatment, showed full mycelium growth (9.00cm). Conversely, T1R2 demonstrated the lowest inhibition at 2.85cm. *Trichoderma viride* showed significant efficacy against *Colletotrichum capsiciin vitro*, with T1R3 achieving the highest percent inhibition over control (PIOC) at 72.22% on mycelial growth. T1R1 and T1R2 recorded inhibitions of 70.00% and 68.33%, respectively (Table 1 and Fig. 3).

Similar results were reported by Mamatha *et al*. (2006) and Binalata *et al.* (2017), with pathogen inhibition of 68.17% and 66.67% respectively, against *T. viride* in turmeric. Nasreen Musheer and Shabbir Ashraf (2017) found that *T.viride* exhibited the highest inhibition (67.79%) against *Colletotrichum capsici*, consistent with the current study's results. This highlights *Trichoderma viride*'s consistent efficacy in combating turmeric leaf spot disease.

Table 1 : Effect of *T. viride* on the mycelial growth of *C. capsici*.

Treatment	Mycelial growth of *Colletotrichum capsici** (cm)	Per cent inhibition over control* (PIOC) (%)
Trichoderma viride	2.68	70.18
Control	9.00	0.00
CD (P = 0.05)	**0.2815**	**3.1282**

*Average of three replications.

This study aims to assess the efficacy of *Trichoderma viride* against *Colletotrichum capsici*, the pathogen responsible for turmeric leaf spot. While various chemical methods exist for controlling turmeric *Colletotrichum* leaf spot, this research focuses on developing environmentally friendly control measures using biocontrol agents. Considering the importance of next-generation health and sustainable agriculture, this research endeavors to pave the way for chemical-independent agricultural practices.

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