Eco. Env. & Cons. 30 (February Suppl. Issue) : 2024; pp. (S408-S411)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i02s.080

In vitro Efficacy of *Trichoderma viride* Against *Alternaria sesami*

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(Received 5 July, 2023; Accepted 12 September, 2023)

ABSTRACT

Sesame (*Sesamum indicum* L.) is an important oilseed crop cultivated in various parts of the world. *Alternaria sesami* is a devastating fungal pathogen that causes significant yield losses in sesame crops worldwide. The survey conducted revealed a maximum disease incidence of 55.00 percent at Namakkal. *Alternaria sesami* was isolated from the leaf blight-infected sesame leaves. *Trichoderma viride* recorded significant results against *Alternaria sesami in vitro*. T1R2 showed the highest percent inhibition over control (PIOC) (77.77%) on mycelial growth of *Alternaria sesami*. 72.22 and 67.77 percentages of inhibition were recorded in T1R1 and T1R3, respectively.

Key words: *Sesame, Leaf blight, Alternaria sesami, Trichoderma viride, Dual culture*

Introduction

Sesame

Sesame (*Sesamum indicum* L.) is an ancient and oleaginous crop that is grown worldwide, chiefly in the tropical and subtropical regions of Asia, Africa, and South America. It belongs to the family Pedaliaceae and is espoused in hot areas with sufficient rain. It serves as a substitute cash crop for smallholders, especially women, to generate income (Dossa *et al*., 2022).

Alternaria sesami is a major fungal pathogen that affects sesame crops, causing significant economic losses. Chemical fungicides have been widely used to combat this pathogen, but their harmful effects on the environment and human health necessitate the development of eco-friendly alternatives. *Trichoderma viride*, a filamentous fungus, has gained attention as a potential biocontrol agent due to its antagonistic properties against various plant pathogens. Hence, the present research work anticipates assessing the antagonistic activity of *Trichoderma viride* against *Alternaria sesami* under *in vitro* conditions.

Materials and Methods

Experiments related to work on **"*In vitro* Efficacy of *Trichoderma viride* Against *Alternaria sesami*"** have been carried out in the Plant Pathology Laboratory, Department of Crop Protection, PGP College of Agricultural Sciences, Namakkal, which is situated at 11.229545° latitude and 78.200957° longitude and an elevation of 218m above MSL. The details of the materials used and the methods espoused in the present research are described concisely hereunder.

Survey for the intensity of the disease

A roving survey was conducted to assess the severity of Alternaria leaf blight in sesame in Namakkal district in 2023. Alternaria leaf blight-infected samples were collected in and around the Namakkal district.

The percent disease index was calculated by the following formula (Mahapatra and Das, 2013).

$$\text{Percent Disease Index (PDI)} = \frac{\text{The Sum of individual ratings}}{\text{No. of leaves examined}} \times \frac{100}{\text{Maximum disease grade}}$$

Isolation of the *Alternaria sesami*

Sesame crops showing typical symptoms of Alternaria leaf blight were collected from crop fields around Namakkal in May 2023. A standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues were cut and surface sterilized for 30 seconds with a 70% ethanol solution, and such bits were transferred to Petri dishes containing sterile water three times, drained of the water, placed in sterilized tissue paper, and transferred into Petri dishes containing 15 ml of solidified potato dextrose agar medium and incubated at 28±1 °C for seven days. A pure culture of the fungus was obtained by the fungal disc method (Vinod Kumar, 2012). The purified isolates were preserved at 4°C and used during the study (Chandraprakash *et al.*, 2022).

Cultural and morphological characterization of *Alternaria sesami*

The pathogen was identified based on cultural and morphological characteristics seen in the Petri dishes. Cultural characters are mycelial growth, topography, and colour of mycelium, and morphological characters like septations, colour, the shape of mycelium, and conidia are noticed. For morphological characterization, spores of *Alternaria sesami* were taken from 7-day-old culture plates and mounted on a clean glass slide. Spores were thoroughly mixed with water to obtain a uniform spread, over which a cover slip was placed. The glass slide with pathogens was examined under an Almicro compound light microscope, and the pictures were captured with the help of a Strange View electronic eyepiece. The spore size has been measured with the help of a stage and an ocular micrometer.

***In vitro* assay of *Trichoderma viride* against *Alternaria sesami* (dual culture technique)**

Alternaria sesami was tested against the antagonist, *Trichoderma viride*. The *Trichoderma viride* was purchased from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, and stored at 4°C until used. *Trichoderma viride* was subcultured using Trichoderma selective medium (TSM) by the fungal disc method and later pure cultures were obtained. On the potato dextrose agar medium, 9mm of the fungal disc of the antagonist along with the test fungus were kept in the exact opposite direction. The plates were incubated for a week at 28±1 °C. The mycelial growth of antagonistic fungi and the growth of pathogens were also recorded separately. The observation of the interaction zone, or inhibition zone, was recorded. After the period of incubation, the growth of the mycelia of *Alternaria sesami* was recorded, and the percent inhibition of the mycelia over control was calculated by the following formula (Naik *et al.,* 2020).

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

Where,
C=Radial growth of the pathogen in control,
T= Radial growth of the pathogen in treatment.

Statistical analysis

The data were subjected to statistical analysis following the method of variance described by Gomez and Gomez (1984). The least significant difference (LSD) at the 5% level was calculated to determine significant differences between treatments.

Results

The present investigations have been conducted as per the objectives mentioned earlier, and the results of the experiments have been conducted on various aspects concerning a survey on Alternaria leaf blight, symptomatology, cultural studies on the growth of *Alternaria sesami*, and *in vitro* evaluation of a bio-control agent against *Alternaria sesami.* The results of the experiments are presented hereunder.

Survey on Alternaria leaf blight in sesame

Disease intensity during 2023

A survey was conducted to assess the severity of *Alternaria sesami* in sesame in the Namakkal district

in 2023. Alternaria leaf blight-infected samples were collected from the college campus, PGP College of Agricultural Sciences, Namakkal. The survey conducted revealed a maximum disease incidence of 55.00 percent at Namakkal (Plate 1).

Plate 1. Leaf blight of sesame caused by *Alternaria sesame*

Isolation and characterization of *Alternaria sesami*

Alternaria sesami was isolated from the leaf blight-infected sesame leaves. Light grey, fluffy mycelium later turns dark grayish white with concentric zones. On maturity, the entire culture has turned black colour (Plate 2). Muriform-shaped olivaceous brown conidia with several horizontal and 2-4 vertical septations are produced by *Alternaria sesami*. A long beak is present in the conidia (Plate 3). Conidia are present in chains**.** Mycelium is profusely branched, septate, and light brown**.** Germination of conidia was also observed under the microscope.

The size of the conidiospore was measured by stage and ocular meter. The length of conidia is between 33 and 59.4 µm. It has a beak length of about 13.2 to 16.5 µm and a width at the broadest part of the conidia between 13.2 and 16.5 µm.

In vitro*, screening of *Trichoderma viride* isolates against *Alternaria sesami

Trichoderma viride-treated dual culture plates showed the dominance of *Alternaria sesami* on mycelial growth. T1R2 recorded the highest inhibition with 2.00cm of mycelial growth of the pathogen, while the control plate without any treatment has shown full growth (9.00cm) of mycelium. T1R3 showed the lowest inhibition (2.9cm).

Trichoderma viride recorded significant results against *Alternaria sesami in vitro*. T1R2 showed the highest percent inhibition over control (PIOC)

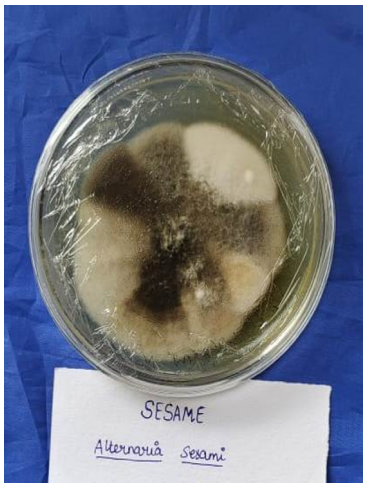

Plate 2. *Alternaria sesame*

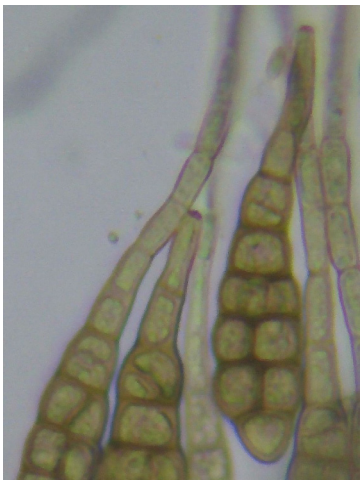

Plate 3. Muriform conidia produced by *Alternaria sesame*

Table 1. Effect of *Trichcderma viride* on the mycelial growth of *Alternaria sesami*

Treatment	Mycelial Growth of *Alternaria sesami** (cm)	Per Cent Inhibition Over Control*(PIOC) (%)
Trichoderma viride	2.45	72.58
Control	9.00	0.00
CD (P=0.05)	0.7228	8.0312

*Average of Three replications

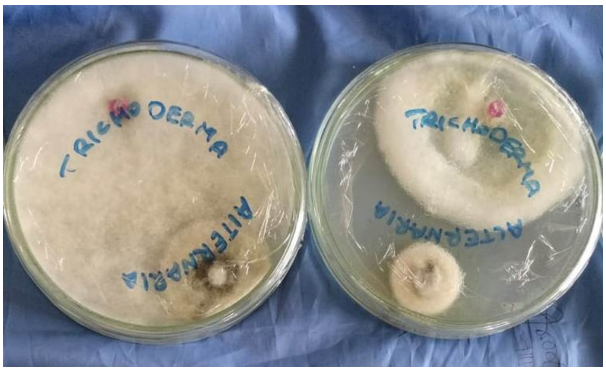

Plate 4. *In vitro* efficacy of *Trichoderma viride against Alternaria sesame*

(77.77%) on mycelial growth of *Alternaria sesami*. 72.22 and 67.77 percentages of inhibition were recorded in T1R1 and T1R3, respectively (Table 1 and Plate 4).

Discussion

Mandlik *et al.,* (2022), delved to find the efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Alternaria alternata*, the leaf spot-causing infectious agent on chilli. The results of this study showed that *Trichoderma viride* effectively inhibited the growth of *Alternaria alternata* with 7.10% PIOC under *in vitro* conditions.

This study aims to provide an original investigation on the use of *Trichoderma viride* as a biocontrol agent against *Alternaria sesami*. The research will shed light on the effectiveness of *Trichoderma viride* in suppressing the pathogen and offer valuable insights into the biocontrol mechanisms involved. This study will contribute to existing knowledge and serve as a foundation for future research and the implementation of *Trichoderma viride* as a sustainable approach for *Alternaria sesami* management.

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