Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 8 Issue 2 February 2025

Research Article

Efficacy Study of the Commercial Biofungicide *Trichoderma viride* (Ami Trichostar) Against Fusarium wilt in *Arachis hypogaea* L

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DOI: 10.31080/ASMI.2025.08.1482

Received: December 11, 2024

Published: January 25, 2025

Abstract

The aim of this study is to investigate the effectiveness of Ami Trichostar, a biocontrol product that contains Trichoderma viride, in reducing the occurrence of Fusarium wilt in Arachis hypogaea L. (groundnut) plant. Using dual culture and agar well diffusion techniques, in vitro experiments were conducted to evaluate the antagonistic potential of Trichoderma viride against Fusarium oxysporum. Studies, including crop yield, germination percentage, survival rate, and the number of dried plants, were assessed through field experiments. Results determined that in both the agar well diffusion experiment and the dual culture assay, Trichoderma viride produced zones of inhibition against Fusarium oxysporum that measured 34.2 ± 6 mm and 34.3 ± 4 mm, respectively. Comparing Ami Trichostar treated plants to untreated ones, the application greatly increased germination percentage, survival rate, incidence of dried plants, and crop production. The application of Ami Trichostar as a biocontrol agent, administered in powder form at a rate of 1 kg/acre, significantly decreased Fusarium wilt incidence while boosting seed germination and other plant growth indicators.

Keywords: Ami Trichostar; Fusarium oxysporum; Groundnut; Plant Growth; Seed Germination

Introduction

Worldwide, *Arachis hypogaea* (groundnut) is grown in many tropical and subtropical climates as an important legume crop. Numerous plant species, including beans, cotton, groundnuts, cucumbers, tomatoes, watermelon, peppers, and muskmelon, are susceptible to vascular diseases caused by a pathogenic fungus *Fusarium* [1-3]. Through invading the xylem vessels, it infiltrates plant roots and causes wiltning [4]. Grain production is completely lost when this disease appears during the vegetative and reproductive periods of the crop [5]. The *Fusarium* wilt causes symptoms such as yellowing leaves, wilting plants, and root rot, which can lead to little or no crop production. With its variety of pathogenic forms, *Fusarium oxysporum* is the most harmful species in the genus when it comes to plant pathology. Recent studies have identified new cases of diseases caused by *Fusarium*, adding valuable information to agricultural research [6]. While chemical pesticides were initially used to control these diseases, their overuse has raised environmental concerns [7]. This has created a demand for alternative methods to manage these plant diseases.

An alternative to using synthetic pesticides is biological control, which is generally more popular with the public and causes less harm to the environment. This method aims to restore the natural balance of ecosystems, which is often disrupted in farming areas, by using microorganisms to regulate pest populations [8]. Biopesticides are biological agents or plant mixtures used to control populations of harmful organisms in environments [9]. The use of microbial antagonists for the purpose of biological control has garnered considerable interest as a very efficient method to handle a variety of plant diseases [10].

The fungus *Trichoderma* contains more than 100 species that have been identified. Their quick growth, adaptability in exploiting substrates, resistance to different pollutants, and ability to endure harsh environmental conditions are the reasons for their worldwide dissemination [11]. *Trichoderma* species are commonly found soil fungus that are widely distributed in the ecology around plant roots [12]. It is known that *Trichoderma* are effective antagonistic microorganisms that suppress *Fusarium* wilt [13]. It is well known that certain species of *Trichoderma* may obstruct fungi that are harmful to plants while at the same time promoting plant development and growth [14]. The present research aims to examine the effectiveness of *Trichoderma viride* in suppressing the wilt of *Fusarium* in *Arachis hypogaea* through the implementation of a biocontrol agent. A comparative field research was conducted to assess the performance of commercial strain of Ami Trichostar against a control group. Important agricultural characteristics, such as the percentage of germination, the percentage of survival, crop production, and the number of dried plants, are the focus of this research.

Materials and Methods

Sample site and collection

The research was accompanied in the year 2021-2022 at Ami experimental farm, Ahmedabad, Gujarat. The root specimen was obtained from the Khanpur, Dahegam and then carried to the lab for evaluation. Ami Trichostar, which includes *Trichoderma viridae*, is used as a biopesticide to control pests in fields. The study employed an experimental design with three replications and a completely randomized framework.

Isolation of plant pathogen

The pathogen was isolated from plant sections exhibiting the initial signs of wilting. First, debris was removed by rinsing the affected roots with tap water. The impacted root segments were then thoroughly rinsed three or four times with distilled water after being sterilized for one minute in a 0.1% aqueous solution of mercuric chloride. The pieces were placed between sterile blotting sheets to eliminate any remaining moisture. These segments were then inoculated into Potato Dextrose Agar (PDA) plates, and the plates were allowed to incubate at 25 ± 1°C for 24 hours.

Identification of pathogen

By comparing the pathogen's cultural and morphological features, which included growth habit and cultural and morphological traits, *Fusarium oxysporum*, the pathogen was identified.

Laboratory screening of biocontrol agent against the plant pathogen

Dual culture method

Using the dual culture plate method, the bioagent *Trichoderma viride*'s effectiveness against *Fusarium oxysporum* was evaluated. For inoculation, a 5 mm disc containing the antagonistic and test fungi was removed from the edge of cultures that were five days old. Seventy-two hours before the bioagent was infected, the test fungus was injected on to potato dextrose agar plates. The bioagents and pathogenic fungus were injected in opposition to one another, keeping a 5-mm gap from the petri plate's edge. Three duplicates of each treatment were carried out, along with a control group that did not inoculated with any bioagent. A regulated temperature of 25 ± 1°C was maintained during the incubation of all plates, and data was collected 96 hours after the bioagent was injected. The percentage of inhibition was measured in cases where a zone of inhibition was observed.

Agar well diffusion technique

This technique was implemented to evaluate the extract's antimicrobial properties. About 1.5 ml of each sample was uniformly distributed on Muller Hinton agar plates in glass petri dishes, containing a bacterial suspension at a concentration of roughly 1.5×10^8 cells/ml. Following a 15-minute waiting period, sterile cork borers were used to puncture each of the 6 mm-diameter wells into the culture medium. The stock solution was applied to each well with measures to keep the extract from overflowing. After that, the plates were put in an incubator and left upright for 24 hours at temperature of 37°C for bacterial cultures and 72 hours at temperature of 25°C to 28°C for fungal cultures. Concurrent maintenance of controls was done. The inhibitory zone's diameter was then calculated in millimetres. With regard to fungi, sterile plates containing Czapek Dox Agarwere spreaded with a spore suspension of the test organism made using sterile water. The culture mediums were then punctured using sterile cork borers to create wells that were each

6 mm in diameter and varied wells received *Trichoderma* extract. Then, for 3-4 days, these plates were incubated between temperature of 25 and 28 °C. The diameter of the inhibitory zone was measured in millimeters after incubation.

Seed treatment with biopesticides

The seeds were immersed in solutions containing different amounts of the bioformulation of the bioagent separately for an entire night. The seeds were planted in a well-prepared 28 × 46-meter block on the next day. Within a block, three rows were sowed for each treatment, with a 30 cm gap between rows. Beginning thirty days after seeding, observations were made on a daily basis and documented every 15 days. To prepare for further investigation, the number of plants was counted row by row. Furthermore, at 20-day intervals, the quantity of dead plants was recorded. The number of surviving plants were determined after five consecutive observations, and additional evaluations were carried out to evaluate the bioagent's effectiveness in reducing the occurrence of *Fusarium oxysporum* on groundnut.

In the field experiment, infected seeds were treated with a prescribed dose of bio-formulation WP at a rate of 1 kg per acre with three replications. One group was treated, while the other served as a control group that did not receive any treatment. Regarding every aspect of seed quality, such as germination rates, plant death at various stages, plant survival, and yield per plant, different presowing seed treatments generated distinct responses. Prior to seeding, the soil was inoculated with *Fusarium oxysporum* inoculum as part of the experimental protocol. Plant wilting was closely observed from the emergence of seedlings till crop maturity. As part of the study, wilt incidence, wilt control percentage, and percent increase in yield relative to control were also noted and examined. A vulnerable type of groundnut seedlings was treated with the antagonist *Trichoderma viride* and planted in a 28 x 46-meter wilt-infected patch at the Ami experimental farm in Ahmedabad, Gujarat. A randomized block design (RBD) was used to organize the experiment, with untreated seeds acting as the control group.

Result and Discussion

Laboratory screening of the biocontrol agent against the plant pathogen

Trichoderma viride was shown to be a highly potent bioagent in studies conducted in the lab to determine its antagonistic efficiency against *Fusarium oxysporum*. Using dual culture and agar well diffusion procedures, this biocontrol agent was evaluated *in vitro* against the plant pathogen. The antagonistic qualities of many *Trichoderma* species were assessed against a wide range of common plant diseases. Later studies concentrated on particular disease by using dual culture methods, which were developed by Morton and Stroube[19]. The results are given in a tabular style for easy interpretation and were assessed in terms of suppression of fungal growth in contrast to the control as displayed in Table 1.

Through the use of the dual culture method and the agar well diffusion method, *Trichoderma viride* efficiently inhibited *Fusarium oxysporum*, resulting in zone of inhibition readings of 34.3 ± 4 and 36.2 ± 6, respectively. The pathogenic fungus is important economically and is known for its ability to cause illness in an extensive range of plant species. Similar to this, earlier research showed that *Trichoderma viride* was antagonistic in contrast to *Fusarium oxysporum* f.sp. *lycopersici*, causing a significant 83.56% reduction in mycelial growth [15].

Effect of seed treatment with biopesticides

Throughout the field experimental, the percentage of germination, percentage of survival, number of dehydrated plants, and yield per plant were all evaluated from treated seeds. Every 15 days starting 30 days after seedling, there were measurements taken of the average yield and the number of plants that died. The findings of the experiment with seed treatments for the cultivation of groundnut showed significant differences between the Ami Trichostar-treated group and control group.

When compared to the control group, the treated group showed superiority and efficacy in raising germination rates. Similarly, treated groups excelled over the control in the influence of seed treatment on plant survival, which was remarkable. In addition, the treated group's average yield was maximized. Crucial markers of seed quality are yield per plant and the percentage of plants that survive as displayed in Figure 1. According to an analysis of the results, the treated group showed the highest germination (Figure 2) and yield per plant in Figure 3. Compared to the untreated groups, the treated groups clearly show a lower incidence of dried plants as shown in Figure 4. Similarly, Researchers also found that the biocontrol agent *Trichoderma* improves seed treatment, which increases the vigor and germination rates of lentil and chickpea crops [16,17].

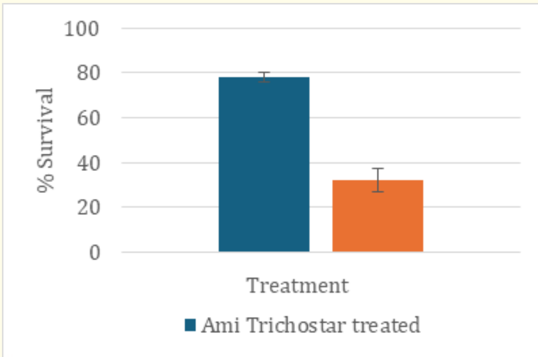

Figure 1: Effect of Ami Trichostar on % Survival.

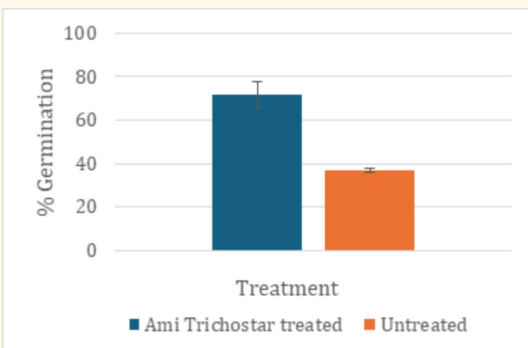

Figure 2: Effect of Ami Trichostar on % Germination.

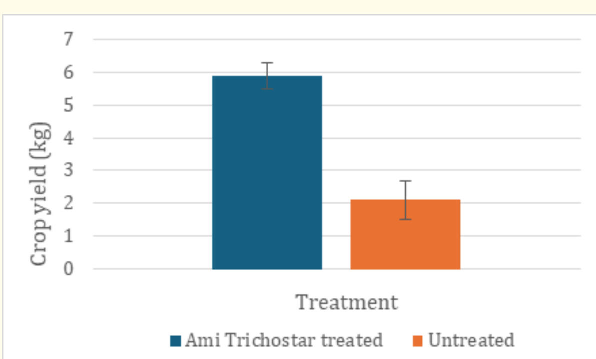

Figure 3: Effect of Ami Trichostar on crop yield.

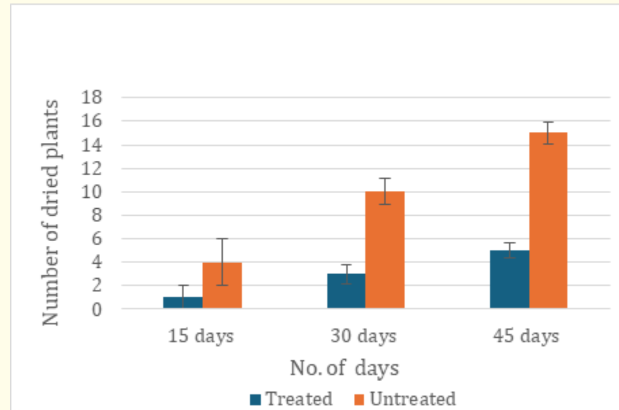

Figure 4: Number of dried plants at three different sample intervals.

Table 1: Effect of *T. viride* against *F. oxysporum.*

Treatment	Dual culture method (zone of inhibition in mm)	Agar well diffusion method (zone of inhibition in mm)
F. oxysporum against *T. viride*	34.3 ± 4	36.2 ± 6
Control	--	--

Based on these data, it can be concluded that treated plants are a better way to treat seeds in order to improve important metrics related to seed quality, which would lead to higher yields. All of the seed quality variables, including yield per plant, rates of plant survival, germination rates, and plant death at different growth stages, showed different responses in the treated and control groups.

Conclusion

Research conducted *in vivo* revealed that Ami Trichostar, a bioformulation consisting of *T. viride*, has a noteworthy suppressive effect on the mycelial growth of *Fusarium oxysporum*, a pathogenic fungus. In terms of improving seed quality and reducing the occurrence of wilt, it was shown to be the most effective seed treatment technique. These promising outcomes suggest potential applications in a variety of plant species, demonstrating an antagonistic action against many plant diseases.

Conflict of Interest

The authors declare no conflict of interest.

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