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Research Article

Evaluation of the Effectiveness of Commercial Biofungicide (Tricho Hstar) in *Controlling fusarium* Wilt Disease in *Cuminum cyminum L*

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Abstract

This study aims to assess efficacy of Ami Tricho Hstar, which includes *Trichoderma harzianum*, as a biocontrol agent for reducing Fusarium wilt in *Cuminum cyminum L*. Utilizing dual culture and agar well diffusion methods, *Trichoderma harzianum* was evaluated *in vitro* against *Fusarium oxysporum*. Such methods were used for their efficacy in evaluating fungal inhibition, providing accurate quantification of inhibitory effects and direct interactions between the biocontrol agent and the pathogen.Under field circumstances, parameters including the percentage of germination, the percentage of survival, the number of dried plants, and the crop yield were evaluated. The results demonstrated that in the dual culture and agar well diffusion experiments, *Trichoderma harzianum* showed zones of inhibition against *Fusarium oxysporum* that measured 41.1 mm ± 2 mm and 43.1 mm ± 3 mm, respectively. When compared to untreated plants, the application of Ami Tricho Hstar greatly improved metrics like the percentage of germination, the percentage of survival, the number of dried plants, and crop production. The present research introduced the application of Ami Tricho Hstar in a powder formulation at a rate of 40g per 15 litres of water resulted in considerable reduction in the prevalence of *Fusarium* wilt and notable improvements in seed germination and other plant development indicators. This study emphasizes its potential as a reliable biocontrol agent in agriculture.

Keywords: Antifungal Activity; Environmentally Friendly Control Methods; Integrated Pest Management; Sustainable Agriculture

Introduction

As the global human population continues to expand, the primary challenge confronting agriculture is the need to enhance crop production while minimizing the environmental footprint, thereby mitigating the detrimental effects of agricultural activities on the ecosystem [1]. Sustainable agricultural strategies and practices offer viable solutions for cultivating food and other agricultural commodities in an environmentally responsible manner, thereby minimizing ecological degradation and ensuring the long-term availability of food and well-being for future generations [2]. In agriculture, plant pathogens and pests reduce the global annual crop yield by an estimated 30 to 50%, which is a loss that must be combatted to ensure food security for an ever-increasing human population [3].

A common agricultural plant disease caused by *Fusarium* may infect a wide variety of tropical crops. Most species of *Fusarium* are soil-dwelling organisms that serve as pathogens as well as saprophytes. *Fusarium* is a pathogen that affects plant tissues both above and below ground, frequently acting as the main or secondary cause of illness [4]. *Fusarium oxysporum f. sp. cumini* causes cumin wilt, a severe disease that severely limits global cumin production. This disease causes significant yield losses, which can approach 100% in extensively infected areas under ideal climatic circumstances [5]. Wilt has caused a serious danger to the growth of cumin in Gujarat and Rajasthan due to its high incidence and broad devastation. Since asexual fungal spores, such as chlamydospores, may persist in soil for up to six years even in the absence of a suitable host, *Fusarium* wilt poses a significant barrier to its eradication [6]. The pathogen secretes pectinolytic and cellulolytic enzymes, which together have a deleterious effect on the host plant, in addition to producing toxins that cause vascular browning and wilting [7].

This continuous threat to crop production needs alternative methods of management that not only address immediate pathogen-related difficulties but contribute to the long-term viability of agricultural practices [8]. Chemical fungicides, although capable of providing significant resistance to *Fusarium* wilt, are often prohibitively expensive and pose significant risks to human health and the environment [9]. Excessive use of chemical treatments leads to severe problems, including pesticide resistance [10]. For example, fungicide-resistant Fusarium strains, which damage crops, are becoming more difficult to control. Moreover, these pollutants contaminate the environment, harming beneficial organisms such as insects and microorganisms while destroying biodiversity. This shows the need for using sustainable pest control methods to manage these issues effectively. Consequently, there is an imperative need for the development of less costly and more environmentally benign methods of controlling *Fusarium* wilt [11].

An excellent, cost-effective, and ecologically beneficial substitute for synthetic fungicides in the management of plant diseases is the use of antagonistic microorganisms [12]. Pesticides derived from living organisms, including bacteria, fungus, and plants, are known as biopesticides. They are an environmentally benign and non-toxic method of controlling and protecting crops from harmful infections that damage plants [13]. These biocontrol agents help treat plant diseases, promote soil fertility, improve plant growth, and minimize the development of harmful pathogens over time.

Trichoderma is a well-known cultivable fungus in the family *Hypocreaceae* that is found in many different ecological settings. This fungus is known to be a microbial biocontrol agent that can take the place of chemical fungicides and fight against the several fungi that cause root rot, foliar diseases, and soilborne illnesses [14]. It is the most widely used and studied fungal biocontrol agent among a wide range of bacteria and fungi because of its exceptional biocontrol ability against a number of the devastating and most resistant soil-borne fungal pathogenic genera, such as *Rhizoctonia*, *Sclerotinia*, *Pythium*, and *Phytophthora* [15].

In addition to disease control, *Trichoderma harzianum* has many environmental and economic benefits over chemical fungicides. One notable benefit is that it promotes sustainable agriculture by increasing soil microbial diversity [16]. Unlike chemical fungicides that reduce microbial populations and disrupt the soil ecosystem, *Trichoderma* harzianum not only controls harmful fungi but also fosters beneficial microbes in the soil, leading to a healthier and more resilient agricultural system. This contributes to long-term soil fertility and supports the overall health of the ecosystem. Furthermore, *Trichoderma* species can reduce the need for chemical fertilizers by facilitating nutrient cycling, enhancing phosphorus availability, and promoting nitrogen fixation [17].

From an economic perspective, *Trichoderma harzianum* provides a more affordable and accessible option for small-scale farmers [18]. In many regions, chemical fungicides are often out of reach due to high costs, limiting their use and effectiveness in smallholder farms. By contrast, *Trichoderma* harzianum can be applied more cost-effectively and has a low production cost, making it more viable for resource-limited farmers. Additionally, the use of biocontrol agents like *Trichoderma* reduces the dependency on external chemical inputs, resulting in overall lower production costs. Importantly, these biocontrol agents do not pose the same risks to workers' health as synthetic chemicals, providing a safer working environment [19]. Studies have shown that agricultural fields that utilizing biocontrol agents based on Trichoderma obtain an improved gain on their expenditures because they get higher results and spend less on chemical treatments [20].

When *Trichoderma* species invade the root system, they cause profound modifications to metabolic pathways and increase defensive mechanisms. During host-pathogen contact, the genes encoding the extracellular protease and oligopeptide transporter in *Trichoderma* species are expressed, triggering the host plant's defensive mechanism through the pathways of ethylene and jasmonic acid signaling [21].

The objective of this research is to examine the effectiveness of *Trichoderma harzianum* in reducing *Fusarium* wilt in *Cuminum cyminum* L. (cumin) as a biocontrol agent. To compare the effectiveness of a commercial strain of Ami Tricho Hstar to a control group, a comparative field experiment was carried out. The main focus of this study is on important agricultural indicators such as crop yield, germination rate, survival rate, and incidence of dried plants.

Materials and Methods

Research site

The experiment was carried out at the Ami Experimental Farm in Ahmedabad, Gujarat, during 2021 to 2022. Root samples were collected from Ucharpi, Mahesana, and then sent to the lab for examination. The biopesticide used in the fields for pest control was Ami Tricho Hstar, which contains *Trichoderma harzianum*. The experimental setting used a totally randomized design with three replications.

Isolation of pathogen

Plant portions exhibiting early signs of wilting were the source of the pathogen's isolation. At first, debris was removed by running tap water over the impacted roots. The affected root segments were then sterilized for one minute in a 0.1% aqueous solution of mercuric chloride, followed by three or four full rinses with distilled water. The remaining moisture was subsequently removed by placing these pieces between sterile blotting sheets. The Potato Dextrose Agar plates were inoculated with these segments, and then the plates were left to incubate at 25 ± 1°C for 24 hours.

Identification of pathogen

The pathogen was identified as *Fusarium oxysporum* by a comparative investigation of cultural and morphological features, including growth pattern and cultural and morphological properties.

Laboratory screening of bioagent against plant pathogen

Dual culture method

A dual culture plate approach was used to evaluate *Trichoderma harzianum*'s effectiveness as a biocontrol agent against *Fusarium oxysporum*. A 5 mm disc was excised from the periphery of a five-day-old culture, thereby revealing the antagonistic and test fungi for inoculation. The test fungus was inoculated onto petri plates containing potato dextrose agar medium 72 hours prior to the inoculation of the biocontrol agent. Subsequently, the biocontrol agent and the test fungus were inoculated in opposite positions on the petri plates, maintaining a 5 mm distance from the edge of the plate. Each treatment was replicated three times, with a control group for comparison. All treatments were incubated at a temperature of 25 ± 1°C, and data analysis was carried out 96 hours after the biocontrol agent was inoculated. Zone of inhibition were observed, and the inhibition percentage was measured.

Agar well diffusion technique

The agar well diffusion technique was employed to assess the antimicrobial properties of the extract. Each glass petri dish, having an 88 mm inner diameter, contained a microbial solution at a concentration of approximately 1.5 × 108 cells/ml, and 1.5 ml of each sample was evenly distributed onto Müller-Hinton agar plates. Following a 15-minute incubation period, sterile cork borers were utilized to create 6 mm-diameter wells in the culture medium. Subsequently, the extract was added to each well, taking care to avoid overflow. The plates were then placed in an incubator and maintained upright for 24 hours at 37°C for bacterial cultures and 72 hours at a temperature range of 25-28°C for fungal cultures. Control plates were concurrently maintained. A millimeter measurement was subsequently taken to determine the zone of inhibition.

A spore suspension of the test organism was prepared using sterile water and added to sterile plates containing Czapek Dox Agar for fungal cultures. Six millimeter-diameter cork borers were employed to create wells in the culture medium, and distinct wells were treated with *Trichoderma* extract. Following this, the plates were incubated for a period of three to four days at a temperature range of 25°C to 28°C. Subsequently, the diameter of the inhibitory zone was calculated in millimeters.

Seed treatment with biopesticides

The seeds were immersed in a solution containing the bioagent for an overnight period. The subsequent day, the seeds were planted in a 28 × 46-meter rectangular plot, prepared in advance. Each treatment was planted in three rows within each block, with a spacing of 30 cm between rows. Daily observations were initiated 30 days after planting, with recordings made every 15 days. Plant counts were conducted row by row to facilitate further investigation. Additionally, the number of deceased plants was noted every 15 days. After five consecutive observations, the number of surviving plants was determined. Subsequent evaluations were conducted to assess the efficacy of the bioagent in reducing the incidence of *Fusarium oxysporum* in cumin.

In the field trial, a predetermined dosage of bio-formulation WP was applied to infected seedlings at a rate of 40 g/15 liters of water, with three replications. The first group underwent treatment, while the second group remained untreated. Varying pre-sowing seed treatments resulted in varying reactions in terms of germination rates, plant survival, number of dried plants and yield per plant over the seed quality spectrum. Prior to sowing, *Fusarium oxysporum* inoculum was added to the soil as part of the experimental methodology. The wilting of plants was meticulously monitored and recorded from germination to crop maturity. At the Ami Experimental Farm in Ahmedabad, Gujarat, vulnerable cumin seedlings were treated with the antagonist *Trichoderma harzianum* and planted in a 28 × 46-meter wilt-infected area. The experiment was structured using a randomized block design, with untreated seeds serving as the control group.

Statistical analysis

The data collected from the experiment were analyzed statistically using Analysis of Variance (ANOVA).

Results and Discussion

Laboratory screening of bioagent against the plant pathogen

Trichoderma harzianum was tested in laboratory experiments to evaluate its antagonistic capabilities against *Fusarium oxysporum*, demonstrating the biocontrol agent's remarkable efficacy. The mycelial diameter of *Fusarium oxysporum* was substantially inhibited by the presence of *Trichoderma harzianum*, which was characterized by the development of an inhibition zone surrounding the pathogen. Similar results were observed in the recent study on *Trichoderma spp*. against *Fusarium oxysporum f. sp. cepae* displayed a significant zone of inhibition through dual culture method [22]. The results were tabulated and evaluated in terms of the suppression of fungal growth compared to the control group as displayed in Table 1.

Trichoderma harzianum effectively inhibited *Fusarium oxysporum* using the dual culture method and the agar well diffusion method, producing readings of 41.1 ± 2 and 43.1 ± 3, respectively, for the zone of inhibition. Similarly, the dual culture assay revealed that all *Trichoderma* species exhibited substantial antimycotic activity against the radial growth of *Fusarium oxysporum* under *in vitro* conditions [23]. Previous research on *Trichoderma viride* and *Trichoderma harzianum* has demonstrated significant inhibitory effects on the growth of various *Fusarium* pathogens under *in vitro* conditions [24].

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

Treatment	Dual culture method (zone of inhibition in mm)	Agar well diffusion method (zone of inhibition mm)
F. oxysporum against *T. harzianum*	41.1 ± 2	43.1 ± 3
Control	--	--

Effect of biopesticides on seed treatment

The results of the field trial revealed significant differences between the Ami Tricho Hstar treated group and the control group regarding seed treatments for cumin cultivation. Field trials demonstrated that *Trichoderma harzianum* not only exhibited antifungal properties by inhibiting *Fusarium oxysporum* growth, but also exhibited beneficial effects on plant growth. Furthermore, *T. harzianum* treatment resulted in a reduction in the incidence of dried plants.

The treated group exhibited a superior performance compared to the control group in terms of germination rates and plant survival. As displayed in Figure 1 and Figure 2. The treated group also demonstrated a notable average yield per plant (Figure 3). The yield per plant and the percentage of surviving plants are important ways to measure the quality of seeds, because they provide insight into the potential growth and success of the seeds. The results indicated that the treated groups experienced a substantially reduced incidence of dried plants compared to the untreated groups as shown in Figure 4. Previous studies have revealed that *Trichoderma harzianum* possesses the ability to effectively suppress *Fusarium oxysporum* infection in tomato plants, while also promoting growth and yield traits, thereby indicating its potential as a biological control agent [25].

The comparative analysis of inhibition zones indicates Ami Tricho Hstar's more substantial antifungal activity and larger zones of fungus growth suppression compared to other treatments. This shows that Ami Tricho Hstar can protect plants against fungal diseases, hence improving seed quality and crop productiv-

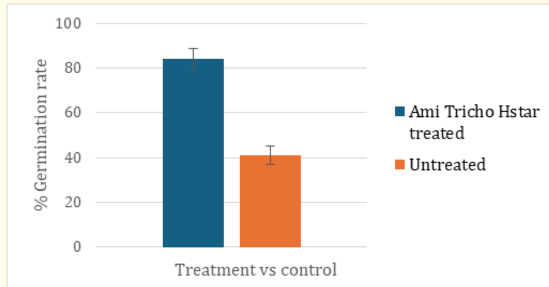

Figure 1: Effect of Ami Tricho Hstar on % Germination of cumin.

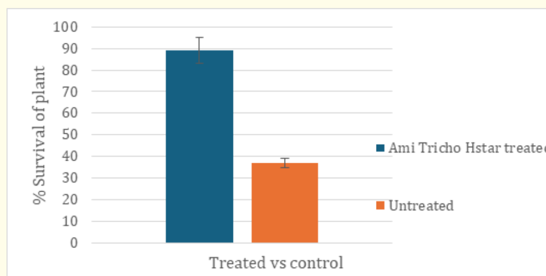

Figure 2: Effect of Ami Tricho Hstar on % Survival of plant.

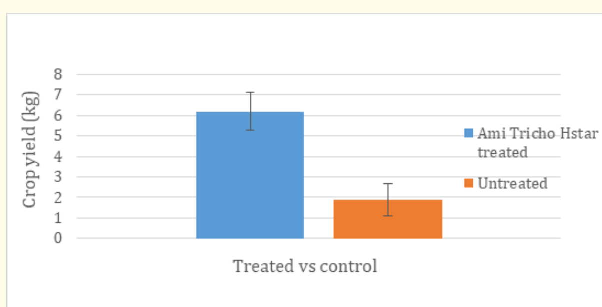

Figure 3: Effect of Ami Tricho Hstar on crop yield.

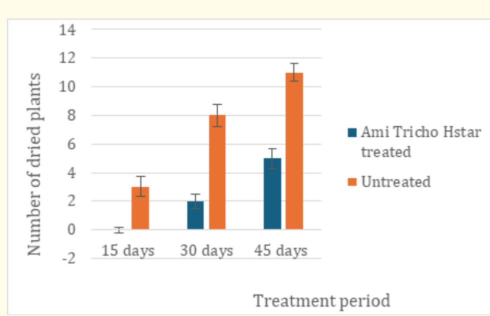

Figure 4: Number of dried plants at three different intervals of sample.

ity. Its application decreases the need for chemical fungicides, enabling more sustainable agricultural methods through integrated pest management (IPM). Furthermore, the treatment's biocontrol properties control targeted pathogens while maintaining soil health and beneficial bacteria.

Conclusion

The result of the study shows that Ami Tricho Hstar, containing *Trichoderma harzianum*, effectively controls Fusarium wilt in *Cuminum cyminum* (cumin) by inhibiting the growth of *Fusarium oxysporum*, as demonstrated in both laboratory and field trials. The treatment substantially improved seed germination, plant survival, and crop yield compared to untreated plants. These results demonstrate its potential as a sustainable, cost-effective alternative to chemical fungicides. Furthermore, the biocontrol agent provides an environmentally friendly solution, reducing the reliance on harmful chemicals, and promoting sustainable agriculture. Its affordability and effectiveness make it an excellent option for small-scale farmers and an important tool for integrated pest management (IPM), contributing to food security and environmental sustainability.

Conflict of Interest

The authors declare no conflict of interest.

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