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Research Article

Efficacy of Ami *Verticillium lecanii* Against Aphids and Whitefly in Cotton: A Comparative Study Under Laboratory and Field Conditions

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Abstract

Cotton (*Gossypium spp.*), a vital crop in India, faces significant yield losses due to pest infestations, especially from whiteflies (*Bemisia tabaci*) and aphids (*Myzus persicae*).The extensive use of chemical pesticides caused concerns about their environmental and human health consequences, leading to a variety of alternative pest-control approaches. Laboratory studies demonstrated that both filtrate and conidia formulations of V. lecanii were effective in causing mortality in both pests, with the filtrate formulation showing higher mortality rates. After 3 days, the filtrate caused 39.28% aphid mortality and 33.75% whitefly mortality, while conidia resulted in 47.39% and 8.75%, respectively. At 6 days, the filtrate treatment resulted in a mortality rate of 72.02% for aphids and 88.75% for whiteflies. Field trials also demonstrated the potential of *V. lecanii*, with 39.12% aphid mortality at 5 days after spray (DAS), increasing to 52.17% by 10 DAS. Whitefly mortality in the field was 46.11% at 5 DAS, reaching 74.11% by 10 DAS. The control group showed much lower mortality rates. The results indicate that *V. lecanii* is a promising biocontrol agent for managing cotton aphids and whiteflies, providing a long-term alternative to conventional pesticides.

Keywords: *Gossypium* spp.; Whiteflies; Aphids; Entomopathogenic; *Verticillium lecanii*

Abbreviation

ITTCC: Indian Type Culture Collection; PDA: Potato Dextrose Agar; RH: Relative Humidity; ANOVA: Analysis of Variance; DAS: Days After Spray; IPM: Integrated Pest Management

Introduction

Cotton (*Gossypium spp.*), often referred to as 'white gold' [1], holds significant economic importance in India, contributing substantially to the national economy through its role in ginning, fabric manufacturing, processing of textiles, and clothing production, among other sectors [2]. Major cotton-producing states include Gujarat, Telangana, Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra, Rajasthan, Haryana, and Punjab [3]. Cotton production in India is affected significantly by different insect pests, despite its extensive cultivation [4].

The primary factors impacting cotton yield are the cotton bollworm complex and the sucking pest complex [5]. Sucking pests such as aphids, mealybugs, whiteflies, thrips, black cotton bugs, and jassids are particularly damaging [6]. Among these pests are whiteflies (*Bemisia tabaci*), which are polyphagous and serve as effective vectors for agricultural viruses, and aphids, which affect greenhouse crops like peppers, tomatoes, and cucumbers, exhibit rapid population growth and are often seen in overlapping generations [7]. These pests can directly reduce cotton yields through sap extraction and indirectly through the transmission of fungal and viral diseases, additionally the several abiotic variables affect the density of aphid populations [8]. The use of chemical pesticides has been a traditional approach to managing these pests; however, it poses substantial risks to the environment, human health, and contributes to pest resistance [9].

In response to these challenges, biological control methods, which utilize natural predators, microorganisms, and microbial products, offer a promising alternative [10]. Among biological control agents, entomopathogens such as *Lecanicillium spp*., *Isaria fumosorosea*, *Beauveria bassiana*, and *Metarhizium anisopliae* have proven effective in pest management through mechanisms including nutritional deprivation, tissue breakdown, and toxin release [11]. Specifically, the commercial entomopathogenic fungus *Verticillium lecanii* has emerged as a highly effective biopesticide for controlling sucking pests at all developmental stages [12]. Available in both liquid and powdered formulations, *Verticillium lecanii* functions by adhering to the host's epicuticle, germinating, and initiating internal colonization, leading to the death of the insect [13]. This method provides an environmentally friendly alternative to chemical pesticides for sap-feeding pests such as mealybugs, scale insects, thrips, and whiteflies [14].

In another study, thirty-five strains of *Verticillium lecanii* were shown to be differently pathogenic against *Bemisia argentifolii* at different life stages. Hatching nymphs had the highest death rates (95–98%), while pupae and adults (34-53%) had decreased but still substantial mortality rates [15]. A similar study showed that *Verticillium lecanii* isolates achieved 100% mortality of *Myzus persicae* and *Aphis gossypii* within 4 days at high concentrations, while other isolates showed reduced effectiveness [16]. The current study demonstrates that commercial entomopathogenic fungus Ami *Verticillium lecanii* effectively inhibits whiteflies and aphids on cotton plants.

Material and Methods

Experimental site

The experiment was conducted from 2022 to 2023 at the Ami Experimental Farm in Ahmedabad, Gujarat.

Field crop for experiment

A research study was conducted between 2022 and 2023 on cotton plants to determine the effectiveness of the Ami strain of *Verticillium lecanii* in preventing whitefly and aphid pest infestations. *Verticillium lecanii*, a fungus known for its biological control functions against a variety of insect pests [17], was specially obtained from the Indian Type Culture Collection (ITCC). This collection plays a crucial role in preserving and enhancing microbial cultures, ensuring that the fungal strains utilized in agricultural research and applications are both pure and effective.

Population analysis of white flies and aphids

Borisade's method for rearing whitefly populations on potted cotton seedlings was used. 14 days after transplanting, adult whiteflies were released onto cotton plants. Netting (mesh size = 0.0322 × 0.0105) was used to cover the potted cotton plants to keep insects from moving among the pots. Egg laying was continuously observed while the plants were kept in a laboratory setup with 24 ± 2°C, 55 ± 3% humidity content, and 16:8 (Light) light periods.

Aphid collected from cotton plant and then moved to potted bean plants and covered with bug netting. These plants were preserved under laboratory settings with a 16:8 light period, 45–60% humidity content, and 24°C temperature. New plants were added to the garden every week to ensure the insects raised there had sufficient nutrition.

Fungus strain

The *Verticillium lecanii* fungal isolate was assessed against aphids and whiteflies. The isolate was kept in tubes at 4°C on Potato Dextrose Agar (PDA) slants. They were cultivated on PDA for 14 days at 25°C for assessment and were kept at 4°C.

Conidial suspension

Conidia from culture plates were harvested using a 0.02% Tween 80 solution on the 20 days of incubation, resulting in conidial suspensions of the isolate *Verticillium lecanii*. After thorough agitation, the resultant suspensions were filtered through sterile cheese cloths. Under a microscope, the concentration of conidia was measured using a hemocytometer and adjusted to 10^8 conidia per milliliter.

Filtrates of fungi

Each isolate's conidial suspension was added individually to 100 milliliters of Adamek's Liquid Medium, and the mixture was shaken at 150 rpm for three days. 250 milliliters of Adamek's Liquid Medium and 2.5 milliliters of primary culture were mixed, and the mixture was cultured for six days at 26°C in a shaking incubator with a 150 rpm setting to form a 1% secondary culture. After that, the culture was filtered, centrifuged for 30 minutes at 4°C at 10,000 rpm, and then filtered again using a 0.45 µm pore size Millipore filtration.

Filter-based biological assay

Each treatment involved two plants infested with 70 to 90 aphids per plant. The plants were sprayed with 3 ml of filtrate from each treatment. The control group received only medium as the spray. Four leaves from each treatment were excised and placed individually in Petri dishes, which were then stored at 24°C and 100% relative humidity with a photoperiod of 16:8 (Light). For whiteflies, a leaf dip method was employed, with 20 whiteflies per Petri dish. The concentration and incubation conditions were identical to those used for aphids. Mortality rates for fungus isolate was recorded daily.

Conidia-based biological assay

The pathogenicity of a single fungal strain against whiteflies and aphids was determined using controlled studies. An adult whitefly from each Petri plate was exposed to a conidial suspension of the fungal isolate in order to conduct the whitefly test. To sustain 100% relative humidity (RH) and promote conidial germination, these Petri plates were then put into a bigger Petri dish that was filled with 1% agar. Conidial suspension was applied to cabbage plants infected with 40–50 aphids per leaf for aphid testing. After treatment, the leaves were put on Petri plates with 1% agar to maintain 100% relative humidity and encourage conidial germination. Spore counts were made using fields of cover slips put on Petri plates, with the spore concentration being standardized to 150–200 spores per square millimeter. Over the course of six days, the mortality rates of aphids and whiteflies were measured at 24-hour intervals. Whiteflies and dead aphids were removed from the Petri plates and kept at 100% humidity content (RH) in the dark. After being stored for a few days, those who had fungus growth on their corpses were categorized as dead. For the aphid studies, untreated leaves were used as the control, while for the whitefly experiments, untreated Petri plates were used as the control. Every experiment was carried out three times. Based on a totally randomized design, a one-way analysis of variance (ANOVA) was used to analyze the data.

Field trial

The experiment was conducted in a randomized block design with three replications, utilizing plots measuring 4 m x 3.5 m with the cotton plants. The treatment group received applications of *Verticillium lecanii* at a dosage of 40 g per 15 liters of water, while the control group was treated with water. Sucking pests, specifically aphids, jassids, thrips, and whiteflies, were monitored on three leaves each from the upper, middle, and bottom sections of selected plants, with assessments made before treatment initiation and at 5 and 10 days post-treatment, and pre-treatment observations were recorded 24 hours before the first spray. The spray solution was produced by diluting the microbial mixture to the desired concentration. Post-treatment evaluations intended to measure the effectiveness of treatments by estimating the percentage reduction in pest populations using the following formula:

(Initial Population−Final Population)/Initial Population×100.

Results and Discussion

Effect of fungi on % mortality of Aphids in laboratory condition

Aphid mortality: Filtrate vs. Conidia (3 Days)

After three days, cotton plants were treated with *Verticillium lecanii* at a dose of 40 g per 15 liters of water, resulting in a 39.28% death rate of aphids with filtrate and 47.39% with conidia. In contrast, the control groups had much lower death rates (4.16% for filtrate and 7.95% for conidia) showed in Table 1. In another research, adult Green peach aphids had a corrected death rate of 35% ± 2.3 after three days at a concentration of 1×10^7 conidia ml^{-1} of *Verticillium lecanii* [18].

Treatment	% Mortality (Filtrate after 3 days)	% Mortality (Filtrate after 6 days)	% Mortality (Conidia after 3 days)	% Mortality (Conidia after 6 days)
V. lecanii Test	39.28	72.02	47.39	66.55
Control	4.16	10.41	7.95	8.33

Table 1: Effect of fungi on % mortality of aphids on cotton plant in laboratory condition.

Filtrate vs. conidia after 6 days

At 6 days of treatment with *Verticillium lecanii*, cotton plants showed a 72.02% aphid death rate with filtrate and a 66.55% mortality rate with conidia. In comparison, the control groups had substantially lower death rates: 10.41% for filtrate and 8.33% for conidia (Table 1). Other similar study indicate that, *V. lecanii* caused a notable 54.4% ± 2.3% death rate in *B. tabaci* after 6 days of treatment, whereas the control group had only 4.0% ± 0.04% [19].

Effect of fungi on % mortality of whitefly in laboratory condition

Whitefly mortality: Filtrate vs. conidia (3 Days)

Verticillium lecanii's effectiveness in reducing whitefly mortality was assessed on cotton plants. After 3 days, the filtrate treatment containing *V. lecanii* led to a death rate of 33.75%, which was considerably more than the 3.75% recorded in the control group. Similarly, the mortality rate for the conidia treatment was 8.75% as compared to 2.50% for the control group (Table 2). In different studies, adult whitefly populations on leaves showed a 23.18% reduction following treatment with *Verticillium lecanii* in comparison to the control. Moreover, reductions of 42.06% and 40.63% in adult whitefly populations were shown by entomopathogenic fungi, especially *Verticillium lecanii* [20].

Filtrate vs. Conidia After 6 Days

After 6 days, *Verticillium lecanii* filtrate treatment resulted in 88.75% whitefly mortality, compared to 8.75% in the control group. Whereas, the conidia treatment was more effective, resulting in a 27.50% death rate compared to an 8.75% mortality rate in the control group illustrated in Table 2. Other investigations found that *Verticillium lecanii* killed 80.9% of *Bemisia tabaci* six days after treatment [21].

Treatment	% Mortality (Filtrate after 3 days)	% Mortality (Filtrate after 6 days)	% Mortality (Conidia after 3 days)	% Mortality (Conidia after 6 days)
V. lecanii	33.75	88.75	8.75	27.50
Control	3.75	8.75	2.50	8.75

Table 2: Effect of fungi on % mortality of whitefly on cotton plant in laboratory condition.

Effect of formulations of fungi on percent mortality of whitefly and aphids

Table 3 shows that *Verticillium lecanii* filtrate had a mortality rate of 41.2% against whiteflies and 62.5% against aphids, whereas the conidia formulation had mortality rates of 19.1% for whiteflies and 54.1% for aphids. In comparison, the control groups had considerably lower mortality rates: 6.71% for whiteflies, 11.9% for aphids in the filtrate control, 4.21% for whiteflies, and 7.2% for aphids in the conidia control.

Table 3: % mortality of whitefly and aphids.

Treatment	Whitefly		Aphids	
	Filtrate	Conidia	Filtrate	Conidia
V. lecanii Test	41.2	19.1	62.5	54.1
Control	6.71	4.21	11.9	7.2

Effect of fungal formulation (*Ami V. lecanii*) on % mortality of Aphids and Whitefly in field trial

% mortality of Aphids at 5 Days after spray and 10 DAS in field condition

In the field examination on aphid mortality, the *Verticillium lecanii* treatment carried out more effectively than the control. At 5 days after spray (DAS), the *V. lecanii* treatment resulted in 39.12% mortality, which increased to 52.17% by 10 DAS. In comparison, the control group had much reduced mortality rates, with 7.4% at 5 DAS and 9.12% at 10 DAS shown in figure 1. Other research found that using *Verticillium lecanii* (1 x 10^8 cfu/ml, 4 g/l) substantially decreased sucking pest populations. Aphid numbers decreased by 59.28% 5 days after spraying (DAS) and 56.60% after 10 DAS. Similarly, whitefly populations were reduced by 57.09% at 5 DAS and 55.36% at 10 DAS, demonstrating that this treatment is effective in reducing both aphids and whiteflies [22].

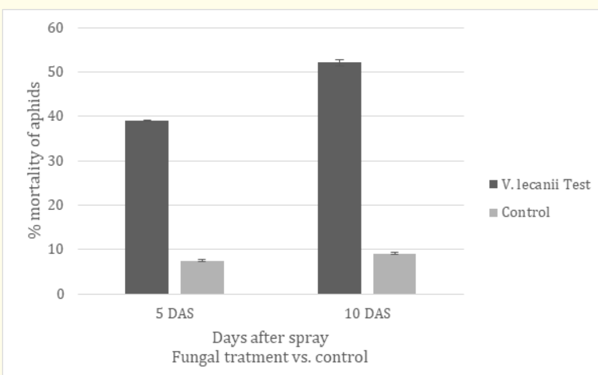

Figure 1: % mortality of Aphids at 5 Days after spray and 10 DAS.

% mortality of Whitefly at 5 DAS and 10 DAS in field condition

In figure 2. The *Verticillium lecanii (V. lecanii*) treatment showed a notable improvement in efficacy over time for whitefly mortality in the field analysis. The mortality rate after *V. lecanii* treatment was 46.11% at 5 days after spray (DAS) and 74.11% at 10 DAS. The mortality rate for the control group was notably lower, at 5.11% at 5 DAS and 10.34% at 10 DAS.

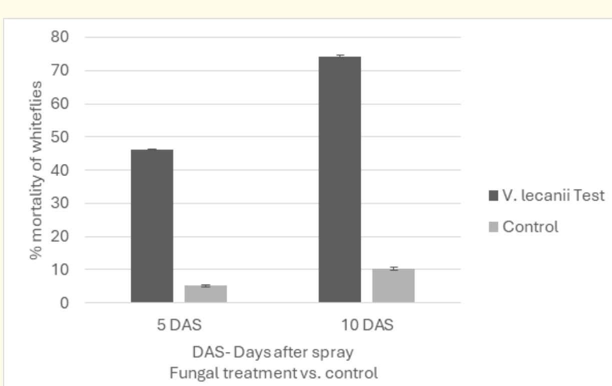

Figure 2: % mortality of Whitefly at 5 DAS and 10 DAS.

Conclusion

The results of this study show that Ami *Verticillium lecanii* is a highly effective biopesticide for controlling whiteflies (*Bemisia tabaci*) and aphids (*Myzus persicae*) in cotton farming. As an commercial entomopathogenic fungus, *V. lecanii* presents a viable substitute for chemical pesticides, reducing the risks to the environment and human health caused by with using traditional pest control methods. It also addresses the problem of insect resistance. By lowering reliance on chemical pesticides, *V. lecanii* can be used in integrated pest management (IPM) initiatives that promote more sustainable farming methods. The effectiveness of this biocontrol product as a component of a larger pest management strategy is increased by targeting several developmental phases of the insect lifecycle.

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