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LABORATORY EVALUATION OF THE ADULTICIDAL AND NYMPHICIDAL ACTIVITY OF *Aspergillus niger* In *Bemisia tabaci* (HEMIPTERA: ALEYRODIDAE)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Whitefly, *Bemisia tabaci* (*Gennadius*), is a widespread polyphagous insect pest and a dangerous vector for many viruses that cause plant diseases. Farmers urgently need safe pesticides to protect their crops and plants, making biopesticide a good alternative to chemicals. Biopesticides are part of an integrated pest management program and offer a safer, more natural alternative to chemical pesticides. Since biocides were introduced, several pest management products have been released, some of which dominate the market. The current laboratory study tested the biological agent *Aspergillus niger* at three concentrations (0.25, 0.50, and 1.00 con/ml) to manage and reduce *Bemisia tabaci* population density in greenhouses and field crops. Laboratory tests showed that the two isolates were highly virulent against *Bemisia tabaci* nymphs and adults. Mortality was significantly different from controls in relation to isolate concentrations and time. Culture filtrate concentration and duration affected nymph and adult mortality. The 100% concentrations of both An1 and An2 were superior to the remaining concentrations, giving mortality in nymphs of *B.tabaci* 48.88 and 45.55 for isolates (An1 and An2) respectively, 42.21 and 36.66 for isolates (An1 and An2) respectively in adults stage. Effect of duration on post treatment mortality, highest mortality in nymphs and adults occurred 9 days after treatment, 74.44 , 56.66% in nymph and 53.33, 45.55 in adults for isolates (An1and An2) respectively. Concerning the interaction between concentration and duration, as is clear from Tables (1) and (2), the mortality was highest at 1.00% concentration. After 9 days of treatment, the isolates (An1, An2) achieved mortality 86.66, 80.0 in nymphs and 73.33%, 63.33% in adults, respectively. These findings demonstrate *A. niger's* biopesticide potential.

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1. INTRODUCTION

The *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is one of the important pests spread in most parts of the world, and this insect is more common in tropical and subtropical regions and less common in temperate regions and affects a wide range of plant families, the most vulnerable plants to infection with this insect are tomatoes, cucumbers, okra, and beans eggplant, cotton [1,2] along with ornamentals [3]. This pest can attack more than 600 species of plants in both open field and greenhouse environments [3]. Whiteflies are distinguished by their lethal characteristics and their ability to cause economic damage to most vegetable and field crops, as evidenced by the nymphs' consumption of plant sap. In addition to its effect on the physiological processes of the plant through the secretion of enzymes during its feeding, it also has an effect on the plant's defense mechanisms. It inhibits photosynthesis, respiration, and transpiration by secreting honeydew, which coats plant parts, including fruits and flowers, and helps soil and dust adhere to them [4,5]. It causes direct damage by sucking the sap, but mostly indirectly by transmitting large numbers of plant viruses (Begomovirus, Ipomovirus, Crinivirus, Tradovirus) leads to heavy losses in the crop yield [6,3]. The nature of the damage caused by this pest to plants is represented by attacking the bark juice, absorbing the juice and excreting toxic substances, which negatively affects the plant, which is represented by reducing the process of photosynthesis, yellowing of the plant and falling leaves, and the honeydew on the affected plant parts becomes a catalyst for the growth of some molds and their consequences [7]. It has negative effects and contributes to the transmission of many pathogenic viruses to plants, include Bean Golden Mosaic Virus(BGMV), Tomato Mottle Virus (TMOV), Tomato yellow leaf curl virus (TYLCV) and the large losses these viruses cause in quantity and quality of production [2,8]. Also, this insect is one of the important insect pests in Iraq and causes great economic losses to many important economic crops, also mentioned that it infects the cucumber crop and causes great losses in production in addition to the poor quality [9]. Previously, reliance on chemical pesticides to pests control was mainly in different parts of the world, which led to the emergence of many complex problems, including the emergence of resistance to chemical pesticides and toxic effects in field crops and the cumulative effect of pesticides on the health of humans, animals and the environment in general, as well as other effects of pesticides such as the elimination of natural biological enemies in addition to production costs [10,11]. These factors led to the search for safe alternatives compared to chemical pesticides, perhaps the most prominent of them is biological control that depends on the use of pathogenic diseases against insects such as microorganisms, including fungi [12,11]. A biopesticide is a natural substance derived from living organisms, such as microorganisms, plants, minerals, nematodes, Bacteria, viruses, and fungi, that reduces the population of pests. Biopesticide targets more specific pests and contribute in reduce risk than chemical pesticides in other organisms such as human, birds and mammals [13,14]. Where the interest in fungi began as control agents through the use of spores or secondary metabolites [15] some fungi have achieved outstanding success in management various types of insects are either pests or vectors of disease [14]. Use of biocides provides suitable and excellent ways to improve crop production, so it must be increased their use and popularity over the next few years [16]. Microbial pesticides generally work to control pests by the presence of certain toxic metabolites that lead to pest's diseases can also inhibit or confuse some important physiological processes led reduce reproductive processes and reduce the pest population [17]. *Aspergillus niger* is the most famous fungus, used in biotechnology to produce a wide variety of products such as organic acids, biofertilizers, proteins and enzymes, and also has a role in insect diseases [14]. *A. niger* insecticidal properties have been previously reported alongside other insects such as: Spodoptera littura , Dysdercus koenigii , Culex sp. Aedes sp., etc [18,19]. The majority of Aspergillus species are parasitic, especially A.niger that infects bee colonies, and is considered a “vital” factor against larvae of Chrysomya chloropyga [20,14].

2. MATERIALS AND METHODS

2.1 Pest Source (*Bemisia tabaci*)

The different phases of B. tabaci was collected from the infected cucumber plant with the pest inside the covered houses in May of 2019 at the Kut Medical Technical Institute

2.2 Preparation of Containerized Host Plants and Breeding Cages

In this study, grew cucumber plants (Italy variety) in a controlled environment (251Co, 60-70 RH, 16:8 h (L: D), for host plant availability assurance in order to obtain a permanent colony (sensitive strain without used pesticide) for a year, infected cucumber leaves

were brought from the covered houses. The same culture has been used for mass multiplication of pest and used in subsequent experiments; the breeding cages were made of wood and measured 40 * 40 * 40 cm. It had special fabric on all sides and a wooden base with a tight-fitting door for inserting insects, anvils and other research implements [21,22].

2.3 The Study's Cultural Media

2.3.1 Potato Dextrose Agar (PDA)

Developed fungus (*Aspergillus niger*) based on the recommendations of the manufacturer.

This medium was prepared using two techniques 22.

A-First method: We had prepared it on our own.

Structure of Potato Dextrose Agar (PDA)

No	Ingredient	Gms/L
1	Patato infusion	200gm
2	Dextrose	20gm
3	Agar	15gm
4	Distilled water	1 liter

The equivalent amount of potato extract in 200 gm of potato infusion is 4.0 gm.

B-Second method: Using commercial medium powder for preparation in accordance with the manufacturer's instructions

No	Ingredients	Gms/L
1	Commercial PDA Powder (20 gm dextrose, 15 gm agar, and 4 gm potato starch)	39gm
2	Distilled water	1 Liter

2.3.2 Potato Dextrose Broth (PDB)

In a glass beaker, 200 grams of peeled and diced potato were boiled for 20 minutes in 500 milliliters of distilled water. The boiled potatoes were strained through a clean cloth before being combined with 20 grams of dextrose and one liter of distilled water. In order to test the efficacy of the filtrate, it was poured into 250 ml glass beakers at a rate of 150 ml per beaker. Twenty minutes at 121°C and 15 pounds of pressure were used to sterilize the media in the autoclave. This medium was utilized for the preparation of the fungus cultures [23].

Laboratory Trials: Examination of *A. niger*'s (a biological control agent) effectiveness in combating the pest's adult and nymph stages

2.4 Activation of Isolated Fungi

To cultivate the fungus isolates, Potato Dextrose Agar (PDA) medium was made by dissolving 39 g of Commercial Medium Powder per liter of distilled water. The medium was supplemented with 250 mg per liter of tetracycline (antibiotic), then poured into petri dishes and stored at 4°C. The fungal isolate of *A. niger* was activated prior to use by placing 0.5 cm of stored fungal culture in petri dishes containing PDA medium. Incubate for one week at 25°C. The fungal isolates were preserved by transferring discs from pure colonies of 0.5 cm in diameter, cut with a sterile cork borer and a sterile needle, to 15 mm sterile glass tubes containing slanted PDA medium and incubating them for a week at 25°C before storing them in the refrigerator until further use [24,25].

2.5 Fungus Filtrate Preparation

Beakers of 250 ml capacity were filled with 150 ml of the liquid nutritional medium Patato Dextrose Broth (PDB). After that, 250 milligrams of the antibiotic Chloramphenicol per milliliter was added. After 7 days in PDA culture media, an *Aspergillus niger* colony was cleaned up and split into three 5 mg tablets, which were then placed in separate flasks and inoculated with the fungus. The beakers were stored at a constant 25 2 C in an incubator. The fungal growth was dispersed by shaking the beakers every 3–4 days. The inoculum was filtered twice; once with filter paper and a vacuum, and once with the fine filter, after 28 days. The next set of tests utilized filtrate from the fungus *A. niger* at various concentrations (0, 25, 50, 1, 00) [26,23].

2.6 Laboratory Experiments

2.6.1 Techniques used in laboratory

Method of leaf disk:

The leaves were washed with running water, dried, and examined under a microscope to identify and remove any insect or mite stages before they were used in the experiments. Using a circular cutter, cucumber leaves were sliced into discs, which were then placed upside down on a filter paper pad (7 cm x 5 cm) atop a moist cotton swab in a 9 cm diameter petridish to maintain moisture. The cotton swabs were dipped in water at regular intervals. *Bemisia tabaci*'s growth was monitored at 27±2°C degrees Celsius in a biological oxygen demand incubator (B.O.D.) The quality of the leaf-discs was maintained by replacing worn out ones on a regular basis (once a week). In order to dry the leaf disc surface after spraying, Petri

dishes are left out in the open for 30 minutes. Then, it's sealed up and kept in temperature and humidity controlled warehouses. Insects are presumed dead if they are unable to travel a distance proportional to their own length [27-29].

2.7 Bio Test

In order to determine the toxicity or resistance of a compound in a controlled environment, bio tests are conducted to evaluate the effectiveness of the bio-control agent (*Aspergillus niger*) in the physiology of this pest. The pesticidal efficacy of *A. niger* against *B. tabaci* nymphs and adults was tested in the lab using the leaf disc bioassay method [30].

2.8 Toxic Efficacy of *Aspergillus niger* Fungal Filtrates on *Bemisia tabaci* Phases

The camel hair brush was used to move about 10 active individuals (nymphs and adults) from infected cucumber leaves to the underside of healthy cucumber leaves. They were then sprayed with either distilled water or one of three different concentrations of fungal filtrate while in a 9cm petri dish surrounded by tangle foot substance. The spraying was done using a 2.5 ml hand-held sprayer. Fungi filtrates and their carbon copies were used to label the petri dishes. More specifically, we compared three treatment replicates to a single control. The dishes were put into an incubator set to 25±2 degrees Celsius and 65% relative humidity. Percentage of mortality and corrected values were determined after 3, 6, and 9 days of spraying with the help of the Orell & Schnider equation [31,32].

$$\text{Percentage of mortality \%} = \frac{\text{death rate in treatment} - \text{death rate in control}}{100 - \text{death rate in control}} * 100$$

2.9 Statistical Analysis

Lab experiments were conducted in accordance with Completely Randomized Design (C.R.D.) as single-factor experiments, and data were analyzed using variance analysis, with averages compared using the least significant difference (R.L.S.D.) and at a Probable value (0.01) [33].

3. RESULTS AND DISCUSSION

3.1 Lethal Effects of Culture Filtrates of *A. niger* against nymphs of *B. tabaci*

The results of the toxicity tests shown in Table (1) show that both *A. niger* isolates tested were highly effective against the nymphs stage of the *Bemisia tabaci* and significantly different from the control. Our findings are consistent with those of a previous study [19]. The mortality rate of nymphs exposed to fungal filtrates increased with increasing concentration and exposure time, which is consistent with previous findings Essien [20].

Table (1) shows that the concentrations of *A. niger* fungal filtrate (0.25, 0.50, 1.00 conidia/ml) had a significant effect on the mortality rate of B. tabaci nymphs when compared to the control**,** the concentration of 1.00% conidia/ml was superior to the other concentrations. The highest mortality rate was 48.88% 45.55% for both An1 and An2 isolates respectively. In addition to the period effect on mortality rate after treatment, the two treatments had significantly different effects on nymphs after 3 and 6 days of treatment, with the highest mortality rate of nymphs occurring after 9 days of treatment at 74.44%, 56.66% for isolates (An1 and An2). The highest nymph mortality rates were found at a concentration of 1.00% after 9 days of treatment (86.66% and 80.0% for isolates (An1 and An2), respectively; the lowest nymph mortality rates were found at a concentration of 0.25% after 3 days of treatment (6.66 and 3.33% for both isolates (An1 and An2)).

3.2 Lethal Effects of Culture Filtrates of *A. niger* against Adults of *B. tabaci*

The data in Table (2) indicate that various concentrations of *A. niger* filtrate are significantly more toxic than the control against adult B. tabaci. The biopesticide's effectiveness increased as concentration and duration increased. In this region, adult mortality tends to increase with time and with increasing concentrations.

Table (2) shows that the mortality rates for the two adult isolates (An1 and An2) are 42.21 and 36.66 percent, respectively, at a conidia/ml concentration of 1.00, 0.25%, and 0.50%, all compared to a control concentration of 0%. Looking at Table (2), we can see that nine days after treatment, both An1 and An2 isolates had their highest mortality rates (53.33 and 45.55 percent, respectively). Adult mortality was found to be lowest at a concentration of 0.25% after 3 days of treatment and highest at a concentration of 1.00% after 9 days of treatment, according to the results of the interaction between the concentration and the post-treatment period.

Many studies have documented genetic differences between isolates of *Beauveria bassiana* and *Metarhizium anisopliae* and between isolates of *A. niger* of the same species [34,35].

Table 1. Lethal effects of culture filtrates of *A. niger* against nymphs of *B. tabaci*

Mean	Mortality rate% post treatment			Fungal filtrate concentration conidia/ml	Isolates
Concentrations	9 th	6 th	3 th		
31.1	60	26.66	6.66	0.25	An1
42.21	76.66	36.66	13.33	0.5	
48.88	86.66	43.33	16.66	1	
0	0	0	0	Control	
0	74.44	35.55	12.21	average period	
17.77	33.33	16.66	3.33	0.25	An2
31.1	56.66	26.66	10	0.5	
45.55	80	40	16.66	1	
0	0	0	0	control	
0	56.66	27.77	9.99	average period	

*R.L.S.D 0.01 for time = 0.4808, for con = 0.4808, for (con*time) = 0.8328). An1 (An2 (R.L.S.D 0.01 for time = 0.781 , for con = 0.781 , for (con*time) = 1.353) .*

Table 2. Lethal effects of culture filtrates of *A. niger* against adults of B. tabaci

Mean	Mortality rate%			Fungal filtrate concentration	Isolates
Concentrations	Post treatment			Conidia/ml	
	9 th	6 th	3 th		
19.99	33.33	20	66.6	0.25	An1
33.33	53.33	33.33	13.33	0.5	
42.21	73.33	36.66	16.66	1	
0	0	0	0	Control	
0	53.33	29.99	12.21	average period	
16.66	33.33	13.33	3.33	0.25	
25.55	46.66	23.33	6.66	0.5	
36.66	63.33	33.33	13.33	1	An2
0	0	0	0	control	
0	45.55	23.33	7.77	average period	

*.An1 (R.L.S.D 0.01 for time = 0.577 , for con = 0.577 , for (con*time) = 0.999 An2 (R.L.S.D 0.01 for time = 0.4192 , for con = 0.4192 , for (con*time) = 0.7260)*

Our findings revealed that nymphal mortality began 72 hours after fungal application and increased with exposure time. Similar observations were made by other researchers [36,19,29].

Mortality began to manifest on day four after treatment and grew over time. In contrast to fast-acting neurotoxic chemical pesticides, fungal pathogens do not cause immediate mortality or other immediate effects in insects. It may take longer to penetrate the cuticle barrier and develop within the hemocoel. Also our current study is consistent with past research on pests [37,38,25,39,40].

We found that whitefly stages mortality depended on time, with the period (9 days) having the highest mortality compared to other periods. This result is consistent with a study conducted by [25,39,36,41].

The results observed that the efficacy of *Aspergillus niger* against pests varied with the concentration and duration of the fungal filtrate, our results come compatible with study results Kumari [19]. Also our study agree with other studies conducted to estimate pathogensity of other entomopathogenic fungi against whitefly [42,36,39].

Therefore, it was showed from the results that the fungal filtrate of *Aspergillus niger* affects the biological performance criteria of the pest because this fungus is an opportunistic, creatine-loving Perfect et al. [43,14], and restorative fungi, as it possesses many analyzed enzymes. where *A. niger* produces a set of enzymes, including glucoamylase, Lysozymei cellulose, amylases, extracellular lipasesi , glucose oxidasei citric acid xylanases [44-46,14]. *A. niger* also produce the following essential enzymes protease [47,14], chitinase [48,14,49,14]. *A. niger* has the ability to secrete many secondary metabolites that the fungus uses as virulence factors like as Oxalic acid & [14]. Like other entomopathogenic fungi such as beauveria bassiana ect., A.niger has the same pathogenicity against harmful pests as a result of possessing these enzymes and secondry metabolites

enable the fungus to it plays a key role in host demise by dissolving tissues and destroying cells. Thus, many branched hyphae are formed, and fungal hyphae emerge from the body of the pest, causing the fungus' life cycle to begin again [25].

Several species of Aspergillus leporis, including A. flavus, A. ochraceus, A. nomius, A. tubingensis, and A. sulphureus, have been reported to possess insecticidal activity against other pests, including Helicoverpa zea and Culex sp. and Anopheles sp [50-52].

Indicators of pathogenic fungi's efficacy against pests center on their pathogenicity. Beneficial fungal isolates are chosen as biological control agents after its use in laboratory biological tests. It is highly pathogenic, highly specialized, easily reproducible in large numbers, and highly tolerant of different environments [53,54].

The adult stage is less sensitive to the effects of *A. niger* than the nymph stage, the hypersensitivity of the nymphs due to imperfect defenses, and the increased mortality of nymphs may be due to the types of degradative enzymes and mycotoxins secreted by *A. niger* that affect the pest's vital functions or that may interfere with the way some of its physiological processes work, or by starving pest [55] confirmed that the adult whiteflies are not as much of susceptible to *B. bassiana*.

Selection of fungal isolates for virulence characteristics is critical to the success of control strategies for whitefly and other pest and insect species, and numerous studies have confirmed *A. niger*'s efficacy in preventing B. tabaci [20,14].

4. CONCLUSION

Aspergillus niger has toxicity properties and highly virulent against whitefly *Bemisia tabaci* stages , All fungal filtrate concentrations of A.niger used in the current study showed varying percentage mortality in nymphs and adults of the pest.The greatest mortality was seen after 9 days of treatment with a concentration of 1.00% of fungal filtrate, and this mortality rose with both increasing fungal filtrate concentration and length of time pest stages were exposed to these filtrates. As a result, *A. niger* has become a highly effective biological control agent against this pest in green houses or open fields as part of an integrated pest management strategy.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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