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Nymphicidal and adulticidal action of *Beauveria bassiana* isolates in whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) *in vitro*

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ABSTRACT

Whitefly *Bemisia tabaci* is one of the most dangerous and destructive pests for crops in fields and greenhouses. Entomopathogenic fungi have emerged as an effective management method compared to potential disorders created by chemical pesticides, including environmental pollution and development of resistance. Therefore, the bio- control agents (microbial pesticides) provide an alternative to chemical pesticides due to their cheapness and ease of handling with their safe use for farmers and more selective than chemical pesticides. The laboratory study objective was to evaluate the efficiency of the bio-control agent (*Beauveria bassiana*) in the control of the nymph and adult stages of *B. tabaci* on cucumber crop. Three concentrations of fungal filtrate of this fungus, 0.25, 0.50, 1.00% conidia mL^{-1} were used in this study. The results showed high virulent of two tested isolates against the nymph and adult stages of the *B. tabaci* that different significantly when compared to the control. The mortality rate was increased by elevating the concentration and by increasing in time period of the nymph and adult exposure to fungal filtrates. The 100% concentration for both two isolates (Bb100 and Bb90) was superior from the rest of the concentrations and exhibited the mortality rate in the nymph and adult stages of this pest as 55.55% and 55.55% respectively in nymphs stage, while 52.22% and 51.10% in adult stages. In addition, in the case of effects of time periods on mortality rate after treatment, the highest mortality rate of nymphs occurred after 9 days of treatment, amounting to 84.44% and 66.66% for Bb100 and Bb90 in nymph stage, while 68.88% and 65.55% in adult stage respectively. From the point view of interaction between concentrations and time periods, the highest mortality rate in nymphs was recorded at the concentration of 1.00% after 9 days of treatment amounting to 96.66%, 93.33% for Bb100 and Bb90 respectively and 83.33%, 86.66% in adults respectively. The lowest mortality rate in nymph was 6.66 after 3 days of treatment at the concentration of 0.25% for both isolates in nymph, while in adults were 13.33% and 10.0% for Bb100 and Bb90 respectively.

Keywords: Biological control, Entomopathogenic fungi, *Beauveria bassiana*, *Bemisia tabaci.*
Article type: Research Article.

INTRODUCTION

Whitefly *Bemisia tabaci* Gennadius (Hemiptera: Whitefly) is considered as one of the most economically important vegetable and ornamental crop pests in the world (Xu *et al.* 2012). Pests as polyphagous insects have a wide host range and can affect over 500 species and 74 plant families, resulting in direct and indirect damage to crops in both greenhouses and field (Ahmed *et al*. 2019; Anwar *et al*. 2019; Salehi *et al.* 2021; Alwan, SH 2022; Abbas & Al-Rahmanny 2022; Almuhsin Ahmed *et al.* 2022). White flies are characterized by lethal characteristics and capabilities on vegetable and field crops due to the economic damage they cause, represented by the absorption of plant juice by the nymphs. In addition to the fact that they secrete enzymes during their feeding that

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affect the physiological processes of the plant, they also secrete honeydew that covers the vegetative parts, flowers and fruits, and impede the processes of photosynthesis, respiration and transpiration through soil adhesion and dust (Osborn *et al.* 1990). Whiteflies have a characteristic life cycle of six stages: the egg, four immature stages (nymphal instars), and the adult stage (Perring *et al.* 2018). The main factors that significantly affect the life cycle of whiteflies are temperature, relative humidity, and host plants (Li *et al*. 2017). Adult *B. tabaci* is a fine insect (usually 1-3 mm long) that feeds heavily on the underside of its leaves to lay eggs (Choudhary *et al*. 2017). Pests can transfer a number of phytopathogenic viruses such as Ipomovirus, Carlavirus, Clinivirus, Tradvirus and Begomovirus. They can slow down the rate of photosynthesis in plants through excretion of honeydew during feeding (Cuthbertson 2013; Gao *et al.* 2017). Over 111 different plant viruses are transferred by *B. tabaci* (Fortes *et al.* 2016). Chemical pesticides have been widely used to control these pests. However, it has been found that such control can cause undesired consequences such as the rapid development of resistance to numerous pesticides, including organophosphates, pyrethroids and carbamate. Although pesticides have a negative impact on agriculture, they play an important role in controlling pests and increasing yields. Therefore, it is necessary to develop a mechanism within the environmental infrastructure to balance the use of pesticides with the integrated management of pests (Anjum & Wright 2016). Many researchers tend to find other options for controlling the number of pests without causing environmental pollution such as biological control. Biological pest control agents such as *Aspergillus niger* and *Beauveria bassiana* are known for their ability to control many pests due to their ease of separation and rapid growth. Attempts were made to use entomopathogenic fungi as biological agents to combat them on some of its families, such as the cucumber, tomato crops, where the fungi spread widely in different environments (Zhang *et al.* 2018). Entomopathogenic fungi have emerged as an effective management method compared to potential disorders created by chemical pesticides, including environmental pollution and development of resistance. Many entomopathogenic fungi are used as biocontrol agents. These fungi usually infect them hosted through a special pathogenic mechanism including successful adhesion, germination, differentiation and direct penetration of fungal hyphae in the body wall of the insect. Successful infection usually depends on adhesion of conidia, penetration within the pest tissues, multiplication and invasion of other tissues as well as the depletion of the insect's materials rapidly, since these fungi secrete some enzymes (toxins) such as lipase, protease, chitinase, etc. which hydrolyzes the epidermis of insects. In addition to its ability to penetrate that work, it disrupts the work of some tissues internally or affects the growth of the insect and finally causes pest death (Ahmed *et al.* 2019; Sanaa *et al.* 2020). *B. bassiana* is considered as one of important entomopathogenic fungi due to its possession of the analysed enzymes, since it produces a set of toxins, including Beauvericin, Beauverolides, Bascyanolide, Tenerin and Bascyanin. These toxins play a role in killing the host by lysing tissues, breaking down cells, thereby formation the germ tube and budding out of the host body, as well as the emergence of fungal hyphae on the outer surface of the pest and restoring the fungal life cycle (Maan 2017; Geroh *et al.* 2014; Serkan 2017). The purpose of this paper was to highlight and discover the biological control mechanisms of entomopathogenic fungus, i.e., *B. bassiana* against some pest stages (nymphs and adults) on cucumber crop.

MATERIALS AND METHODS

Location of the laboratory study

This study is performed in the laboratories of Department of Biology, Collage of Science and College of Education for Pure Sciences, Wasit University, Iraq in cooperation with Medical Technical Institute, Kut, Middle Technical University.

Source of pest (*Bemisia tabaci*)

The *Bemisia tabaci* population was obtained from infected cucumber leaves with *B. tabaci* in covered houses in Medical Technical Institute, Kut in May 2019.

Preparation of host plant pots and breeding cages

To ensure the availability of host cucumber for different studies related to biology of the *B. tabaci*, cucumber (Italy variety) was reared in plastic pots (13 cm diameter, 10 cm height) in a climate-controlled room (25 ± 1 ºC, 60–70% RH, 16:8 h (L:D). After maturity (three weeks old), and infecting the cucumber leaves, they were brought from the plastic house at the Medical Technical Institute, Kut for one year to obtain a permanent colony (sensitive strain without using pesticide), the same culture was used for mass multiplication of pest and employed in

subsequent experiments. Cages for breeding were also made of wood with dimensions of 40 × 40 × 40 cm. Their all sides were made of special cloth. The cage base was made of wood and equipped with a tight door to insert anvils, insects and feeding materials according to Siddhapara (2015) and Sabrine *et al*. (2015).

Fungal isolates

In this study, two local isolates were stored in the Agricultural Research Directorate, Ministry of Science and Technology. It was previously isolated from Iraqi gardens and farms soil using the technique of insect bait traps and employing larvae of *Galleria mellonellaa* which were appeard to be highly virulent on various insect kinds. It was stored after purification using the singular conidia technique, and deposited within the GenBank database with accession numbers (B100, B90) respectively.

Culture media used in the study

1. Potato Dextrose Agar (PDA)

This medium was used to develop fungus, *Beauveria bassiana*, according to instruction by the manufacturer. Two methods were used to prepare this medium (Al-Zubaidi *et al*. 2010).

A-First method: We prepared it ourselves

Composition of Potato Dextrose Agar (PDA)

Ingredients	g L^{-1}
Patato infusion	200
Dextrose	20
Agar	15
Distilled water	1 L

Note: 200 g of potato infusion is equivalent to 4.0 g of potato extract.

B-Second method: Preparing from commercial medium powder, attended as instructed by the manufacturer

Ingredients	
Commercial PDA Powder (20 g dextrose, 15 g agar, and 4 g potato starch)	39 g L^{-1}
Distilled water	1 Litre

2- Potato dextrose broth (PDB)

The medium was prepare by boiling 200 g potato peeled and cutting into small pieces with 500 mL distilled water for 20 min in a glass beaker. We filtered the cooked potatoes with a clean cloth, then took the filter and added 20 g dextrose to it, thereafter completed the volume to 1 L by adding distilled water. The filtrate was distributed in glass beakers with a capacity of 250 mL at a rate of 150 mL per beaker. The media were sterilized by autoclave at a temperature of 121 °C and a pressure of 15 pounds for a period of 20 min. Then we used the medium to prepare the fungus filtrate (Al-Zubaidi *et al*. 2010).

Laboratory Experiments

Evaluation of the effectiveness of biological control factor (*B. bassiana*) against adults and nymphs of the pest.

Activation of fungal isolates

Potato dextrose agar (PDA) medium was used to activate the isolates of the fungus employed in this study. It was prepared by dissolving 39 g commercial medium powder per litre of distilled water. Tetracycline (antibiotic) was added to the medium at 250 mg L^{-1}, then poured into petri dishes and kept at 4 °C until use. The fungal isolate of *B. bassiana* were activated by taking 0.5 cm of stored fungal culture and placing them in petri dishes containing PDA medium followed by incubating at 25 °C for a week. The isolates were preserved by transporting disc from pure colonies of 0.5 cm diameter, cut with sterile cork borer by sterile needle to 15 mm sterile glass tubes containing slanted PDA medium, then incubated for a week under 25 ± 2 °C and stored under refrigerated conditions till further use (Kanika *et al*. 2015; Serkan & Nurcan 2017).

Preparation of fungus filtrates

The liquid nutritional medium, potato dextrose broth (PDB) was prepared and distributed in beakers with a capacity of 250 mL and an amount of 150 mL / beaker. Then the Chloramphenicol, an antibiotic, was added to it at an amount of 250 mg mL^{-1}. Each flask was inoculated with three tablets of 5 mg in diameter each with a cork borer from the edge of the fungal colony purified in culture media (PDA) and extracted at the age of 7 days for *B. bassiana*. The beakers were incubated at a temperature of 25 ± 2 ºC, taking into account that the beakers were shaken every 3-4 days to distribute the fungal growth. After 28 days, the inoculum was filtered using filter paper and a vacuum device, then re-filtered using the fine filter. Afterward, different concentrations (0.25, 0.50 and 1.00%) of the fungal filtrate for the fungus (*B. bassiana*) were prepared, then used in the subsequent experiments (Singh and Prakash 2010; Al-Zubaidi *et al*. 2010).

Laboratory experiments

Leaf disk method

Before using the leaves for different experiments, the healthy thin green leaves of cucumber selected from potted plants were thoroughly washed with tap water, dried and examined under the microscope to remove or kill any insect or mite stages found on them. Cucumber leaves were cut by circular cutter into discs and these discs kept upside down on wet filter paper (7 cm × 5 cm) overlaying a wet cotton swab in 9-cm diameter petri dish to ensure the leaf remained hydrated. The cotton swabs were kept saturated with water from time to time. The development of two-spotted spider mite was studied at 27 ± 2 °C temperature maintained in biological oxygen demand (BOD) incubator. The old leaf-discs were replaced periodically (every week) with fresh ones so as to ensure their good quality. After spraying, petri dishes remained exposed for a short period of time (30 min), allowing to dry the surface of the leaf disc. It was then covered and kept under controlled environments. In general, a pest that cannot walk a space equal to the length of its body is considered dead (Manal & Hany 2019; Flore *et al.* 2019; Farman *et al.* 2019).

Bioassay

The aim of bio tests was to assess the efficiency of bio-control agent (*B. bassiana*) in the physiology of this pest which is generally associated with determining the toxicity of compound or resistance to it *in vitro*. The pesticidal efficacy of *B. bassiana* were evaluated against nymphs and adults of *B. tabaci in vitro* as per leaf disc bioassay method (Ullah & Lim 2015).

Evaluating the toxic efficacy of fungal filtrates of *Beauveria bassiana* in the *Bemisia tabaci* stages (nymphs and adults)

We placed 10 moving individuals from each of the stages (nymphs and adults) separately by transferring them from infected cucumber leaves using camel hair brush on ventral surface of healthy cucumber leaves placed in 9-cm petri dish surrounded by tangle foot substance. We then treated it with fungal filtrates 2 mL per replicate at different concentrations (0.25, 0.50 and 1.00 %), while the control was sprayed with distilled water only. Hand-held sprayer size 2.5 mL was used for spraying. The petri dishes were labelled according to fungal filtrates and their replicates. Three replicates of treatment and one control were also applied for comparison. The dishes were placed in incubator with a temperature of 25 ± 2 ºC and humidity 65 ± 5%. Afterward, the death of individuals were calculated after 3, 6 and 9 days of spraying as well as the mortality rate (%) and corrected values according to the Orell & Schnider equation (Al- Jubouri *et al.* 2000; Haider & Wajih 2016).

$$\frac{\text{No. of living individual in control} - \text{No. of living individual in treatment}}{\text{No. of living individual in control}} \times 100 = \text{Mortality rate (\%)}$$

Statistical Analysis

All experiments were designed according to the Completely Randomized Design (CRD) and results were analysed using SPSS version 20 program which includes Duncan's Multiple Range Test (DMRT) to compare rates in all coefficients and determine the significant differences at the probability level 0.05.

RESULTS AND DISCUSSION

Nymphicidal action of the *B. bassiana* isolates on *B. tabaci* nymphs

According to the bioassay results listed in the Table 1, we found the high effectiveness of two tested isolates of *B. bassiana* against the nymphs stage of the *B. tabaci* which was different significantly compared to the control, exhibiting that *B. bassiana* was high pathogenic to *B. tabaci*, which is in agreement with the results obtained by Ghulam *et al*. (2018). The mortality rate was increased by the elevated concentration and also by time duration of nymphs exposure to fungal filtrates, which was compatible with Somoza-Vargas *et al*. (2018). It was also observed from the results of isolates (Bb100 and Bb90) in nymphs (Table 1) that there is a significant effect of the concentrations of the fungal filtrate of *B. bassiana* (0.25, 0.50 and 1.00%) conidia mL^{-1} in the *B. tabaci* nymphs, when compared to control. The concentration of 1.00% conidia mL^{-1} was superior to the rest of the concentrations in the nymph mortality rate exhibiting the highest mortality rate (55.55%) for both isolates (Bb100 and Bb90). In the case of the effects of time periods on mortality rate after treatment, the highest mortality rate of nymphs' occurred after 9 days, amounting to 84.44% and 66.66% respectively. It was significantly different from mortality rates after 3 and 6 days of treatment. In the case of their effects on the nymph stage, these two treatments were also differed significantly from each other. from the point view of the interaction between the concentration and time period, it became clear from the same table that the highest mortality rates in nymph was recorded at the concentration of 1.00% after 9 days of treatment amounting to 96.66% and 93.33% for isolates (Bb100 and Bb90) respectively, and that the lowest mortality rate in nymph was 6.66% for both isolates after 3 days of treatment at the concentration of 0.25%.

Table 1. Nymphicidal action of the B. *bassiana* isolates on *B. tabaci* nymphs.

isolates	Fungal filtrate concentration conidia mL-1	Mortality rate (%) post treatment			mean concentrations
		3th day	6th day	9th day	
Bb100	0.25	6.66	30.0	70.0	35.55[a]
	0.50	16.66	43.33	86.66	48.88[a]
	1.00	20.0	50.0	96.66	55.55[a]
	Control	0.0	0.0	0.0	0.0
	mean time period	14.44[c]	41.11[b]	84.44[a]	0
Bb90	0.25	6.66	23.33	43.33	24.44[a]
	0.50	13.33	30.0	63.33	35.55[a]
	1.00	23.33	50.0	93.33	55.55[a]
	control	0.0	0.0	0.0	0.0
	mean time period	14.44[a]	34.44[ab]	66.66[b]	0

Note: *Similar letters in same column indicate that there is no significant difference at p = 0.05; *Each number represents an average of three replicate.

Adultiicidal action of the *B. bassiana* isolates on *B. tabaci* adults

According to the data presented in Table 2, the different concentrations of *B. bassiana* fungal filtrates exhibited good toxicity against *B. tabaci* adults and different significantly from the control group. The effect of bio-pesticide increased by the elevated concentration and also time duration of exposure, where the mortality rates of adults caused by fungal infection generally increased over time and also raised by the elevated concentration. It is noted from the effect results of isolates (Bb100 and Bb90) in adults (Table 2) that there is a significant effect of 1.00, 0.25, 0.50% conidia mL concentrations compared to control, and that the concentration 1.00% exhibited a tangible superiority in the mortality rates over the rest of the concentrations. Where it gave mortality rates amounted to 52.22% and 51.10% for Bb100 and Bb90 respectively. As for the effect of the periods after treatment in mortality rates, Table 2 also indicated that the highest mortality rate was 68.88% and 65.55% for the two isolates after 9 days of treatment respectively. As for the effect of the interaction between the concentration and the time period after treatment in the mortality rate of adults, the highest mortality rates were 83.33% and 86.66% for the two isolates (Bb100 and Bb90) at the concentration of 1.00% after 9 days of treatment respectively, and the lowest mortality rate was 13.33%, 10.0% for the two isolates at the concentration of 0.25% after 3 days of treatment respectively. The variance between the isolates of the same species might be attributed to the genetic variance, and this variance was recorded among the isolates of *Metarhizium anisopliae* and *B. bassiana* in many studies (Garcia *et al*. 1984; De La Rosa *et al*. 2002). Our results also showed that the mortality of nymphs initiated after 72 h of fungal application and increased by the upraised exposure time in agreement with the results obtained by

Ahmed *et al.* (2018). The mortality began to appear since the fourth day of the treatment, however, elevated over time, which supported by the previous studies on other pests (Negash *et al.* 2014; Tehri *et al.* 2014; Serkan 2017; Yeşilayer 2018; Elhakim *et al.* 2020).

Table 2. Adulticidal action of the *B. bassiana* isolates on *B. tabaci* adults.

Isolates	Fungal filtrate concentration Conidia mL-1	Mortality rate (%) post treatment			Mean concentrations
		3th day	6th day	9th day	
Bb100	0.25	13.33	30.0	53.33	32.22 [a]
	0.50	16.66	40.0	70.0	a 42.22
	1.00	23.33	50.0	83.33	52.22[a]
	Control	0.0	0.0	0.0	0.0
	mean time period	17.77 c	40.0[b]	a68.88[a]	0
Bb90	0.25	10.0	26.66	46.66	27.77[a]
	0.50	16.66	40.0	63.33	39.99[a]
	1.00	20.0	46.66	86.66	51.10[a]
	control	0.0	0.0	0.0	0.0
	mean time period	15.55[b]	37.77[b]	65.55[a]	0

Note: Similar letters in same column indicate that there is no significant difference at p = 0.05; *Each number represents an average of three replicate.

It was found that the adulticidal and nymphicidal activities of *B. bassiana* against *B. tabaci* were time-dependent, i.e. the toxicity increased by the time, where the time duration (9 days) exhibited maximum mortality rate compared to other time durations. This results are compatible with studies carried out by Serkan (2017), Yesilayer (2018), Ahmad *et al.* (2018) who found that the effect of *B. bassiana* against other pests was increased by the upraised concentrations of conidial suspension. Other studies revealed that effect of *B. bassiana* against pests differ according to the concentration of conidial suspension and time duration (Ortucu & Iskender 2017; Ahmad *et al.* 2018; Yesilayer 2018). A study was performed to evaluate the effects of HPI-019/14 strain of *B. bassiana* on white fly of *B. tabaci* nymphs exhibiting that the effect of the fungus in the laboratory (*in vitro*) was higher than its effect in the field (in vivo) and that the effects upraised by the elevated concentration (Somoza-Vargas *et al.* 2018). Accordingly, it was clear from our results that the fungal filtrates of *B. bassiana* had an effect on the biological performance criteria of the *B. tabaci*, due to its possession of the analysed enzymes, since *B. bassiana* produces a set of toxins, including Beauvericin, Beauverolides, Bascyanolide, Tenerin and Bascyanin. These toxins play a role in killing the host by lysing tissues, breaking down cells, thereby formation the germ tube and budding out of the host body, hence leading to the emergence of fungal hyphae on the outer surface of the pest and restoring the fungal life cycle (Maan 2017; Geroh *et al.* 2014; Serkan 2017). Pathogenicity is the most important indicator when measuring the effectiveness of pathogenic fungi against pests. It is adopted in laboratory biological tests, and fungal isolates are selected as successful biological control agents as a result of its high pathogenicity, specialization, ease of quantitative production, and adaptability to environmental conditions (Reay *et al*. 2008; Ptlamul & Parasertan 2012). We observed from our results that the nymph stage is more sensitive to the effect of *B. bassiana* than the adults due to the lack of completeness of its defensive means. The increase in the mortality rates may be due to the type of mycotoxins and decomposing enzymes secreted by these fungi, which affect the vital activities of the pest bodies, or may work to disrupt the work of some physiological processes within the pest, affecting the growth and development of the pest through tissue destruction or starvation of the pest by depleting nutrients then kill it. Our results are in line with previous work by Mascarin *et al.* (2013) who demonstrated that whitefly adults showed less sensitivity to *B. bassiana*. Several studies confirmed the effectiveness of *B. bassiana* in the control of *B. tabaci*. It achieved good results, and the process of sifting the fungal isolates to determine the characteristics of their virulence. It is of vital importance to the success of strategies in controlling the whitefly and other insects and also pests (Faria & Wraight 2001; Lacy *et al*. 2008; Cabanillas & Jones 2009). In another study, the virulence of five isolates of *B. bassiana* and *Isaria fumosorosea*

and four isolates of *Lecanicillium muscarium* was determined on whiteflies on bean leaves under laboratory conditions. The results showed that the greatest effectiveness was for the fungi *B. bassiana* and *I. fumosorosea*, with a mortality rate of 71-86% during 8 days (Mascarin *et al.* 2013).

CONCLUSION

All the concentrations of the fungal filtrate of *B. bassiana* used in present study exhibited varying pathogenicity in nymphs and adults of *B. tabaci*. It is natural that the mortality rates increase by the upraised concentration of the fungal filtrate and by elevating the time duration, where the highest mortality rates were recorded at the concentration of 1.00% after 9 days of treatment. This confirms the effectiveness of *B. beauveria* as a good biological control agent that can be used with other control agents within the integrated pest management program to suppress this pest in open fields or covered houses.

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