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A comparative study between two preparation methods of filtrate *Metarhizium anisopliae* in control *Aphis gossypii* glover

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Abstract
Aphis gossypii Glover causes various serious economic damages to many crops. Entomopathogenic fungi like *Metarhizium anisopliae* have been proven as biological control agents in controlling many pests. This study included testing the effectiveness of two methods for preparing *M. anisopliae* filtrate in the mortality of *A. gossypii* under laboratory conditions. Three replicates were used to test three concentrations of the two factors: the autoclave sterilized filtrate and the Millipore sterilized filtrate, in addition to the control treatment, distilled water. The results showed that the autoclave sterilized filtrate was superior to the Millipore sterilized filtrate for *M. anisopliae*, The percentage of mortality increased with increasing concentrations and increased in the treatment period, to be recorded at 100.00 and 83.00 % at the highest concentration of nymph mortality in the filtrate sterilized with autoclave and the filtrate sterilized with Millipore, respectively, compared with 70.00 and 53.30% at adults mortality at the same concentration after three days from treatment. The results of this study, which took place under laboratory conditions, supported the importance of using entomopathogenic fungi in several ways. However, their field testing and effectiveness must still be verified against other biological enemies.

Keywords: Aphis gossypii, filtrate, metarhizium anisopliae

Introduction
Aphis gossypii Glover (Hemiptera: Aphididae) spreads around the world, recording advanced economic importance in infecting many crops, and causing damage in quantity and quality. The damage to crops varies between direct, through feeding on them, by sucking plant sap, and by secreting honeydew, on which dust collects and mold grows [1]. The damage is indirect through its transmission of many viruses to plants which causes disturbances in the physiological processes of plants and thus affects plant growth [2].

It is known that the use of chemical pesticides harms humans, the environment, and non-target organisms such as bees and biological enemies, as well as the emergence of resistance among pests to them [3]. The efforts of researchers in searching for alternatives to pesticides that are safe and environmentally friendly have continued over recent years to find many alternatives, including the recent use of nanoparticles in pest control [4].

By returning to nature, the researchers have found one of the most important factors in controlling insect pests, which is entomopathogenic fungi which has shown the ability and effectiveness in controlling many pests belonging to many families and orders [5]. Entomopathogenic fungi have been used in various forms to control insect pests. For example, they have been used in the form of spore suspensions [6], While it was benefited in another way through the secondary metabolites they produce, which are enzymes and toxins, that play a major role in the mortality of insect pests such as secondary metabolites of *Metarhizium anisopliae* [7]. This study aims to test the effectiveness of the autoclave sterilized filtrate and the Millipore sterilized filtrate of *M. anisopliae* in the mortality of *A.gossypii*.

Materials and Methods
Study Area
This study was conducted for the period between October 2023 to March 2024 in the Plant Protection Laboratory, Field Crops Department, College of Agriculture - Al-Qasim Green University.

Rearing insects
In a plastic house planted with okra plants infested with insects, stages of the *A.gossypii* were transferred to the laboratory and raised for several generations for rearing on plants were grown in plastic pots (10 cm diameter) in cages (80 × 80 × 80cm) under the environmental condition at 26 ± 1 ºC, 70 ± 5% RH and L:D 16:8 h.

Fungi culture
The *M. anisopliae* was isolated from *Bombyx mori* larva and grew on potato dextrose agar (PDA) at 26± 1°C for 16 days to obtain the necessary quantity to make the filtrate. The fungal filtrate was prepared by placing three discs of the growing culture of the fungus in 200 ml of culture medium potato dextrose broth (PDB) which was placed in a sterile flask with 500 ml, where it was incubated for 21 days at 26°C [8]. After completing the period and obtaining the fungal filtrate, it was passed through filter paper to get rid of the mycelium, and then it was treated in two ways, the first by sterilizing it using an autoclave at temperature 121°C and pressure 1/2 time for 15 minutes, While the second method it was represented by the passage of the filtrate through Millipore sterile filter (Millipore0.45 µm syringe filter). The resulting filtrate in both methods was considered stock solution with and concentration of 100% and then diluted by sterile distilled water to prepare 50 and 25 % concentrations. The control treatment was sterile distilled water only.

Bioassay Test

10 nymphs and adults of the insect were counted and transferred to petri dishes with a diameter of 9 cm containing sterile blotting paper, then they were treated directly with an insulin syringe and with an amount of 1 ml of autoclave sterilized fungal filtrate, in three replicates, for all concentrations, including the control, represented by sterile distilled water, to prevent escape. Insects: The dishes were covered tightly and placed under laboratory conditions (26 ± 1 ºC, 70 ± 5% RH and L:D 16:8 h). The dishes were examined daily for 3 days and dead insects were removed. The same experiment was repeated for the fungal filtrate sterilized with Millipore.

Statistical Analysis

Using GenStat package 3 (3rh edition) The data obtained were analyzed in a completely randomized design with two factors. The percentage effects of the fungal filtrate on the mortality of nymphs and adults of *A.gossypii* were calculated and corrected by Abbott's formula [9]. At a 5% level of significance ($P \leq 0.05$) The treatment means were compared by the least significant difference (L.S.D).

Results and Discussion

Statistical analysis of the results of laboratory experiments conducted when spraying the nymph stage of A.gossypii by concentrations of *M. anisopliae* filtrate sterilized by autoclave and Millipore showed the effectiveness of the autoclave sterilized filtrate over the Millipore sterilized filtrate by increasing the cumulative mortality of *A.gossypii*. The results also showed increased mortality of the nymph stage with increasing filtrate concentrations and an increase in the period. Results of Table 1: A mortality rate was recorded at the highest concentration of 100% of the autoclave sterilized filtrate treatment 63.30, 80.00, 100.00 % on days 1, 2, and 3, respectively, compared with 13.30, 20.00, 23.30 % at the control treatment for the same periods. While, in the treatment of the sterile filtrate with Millipore, the cumulative mortality rate was recorded for days 1, 2 and 3 of the highest concentration 50.00, 63.30 and 83.00 % compared with 13.30, 20.00 and 23.30 % at the control treatment.

Table 1: Effect of different concentrations of M. anisopliae filtrate sterilized by autoclave and Millipore on nymph of A.gossypii at different time periods

Treatment	Concentration	Days			Mean
		1	2	3	
Autoclave	0	13.30	20.00	23.30	18.87
	25	30.00	40.00	53.30	41.10
	50	40.00	56.60	73.30	56.63
	100	63.30	80.00	100.00	81.10
Mean		36.65	49.15	62.47	
L.S.D (P ≤0.05)		Con.0.7 Day 0.6, Int. 1.2			
Millipore	0	13.30	20.00	23.30	18.87
	25	26.60	33.30	43.30	34.40
	50	36.30	46.60	60.00	47.63
	100	50.00	63.30	83.00	65.43
Mean		31.55	40.80	52.40	
L.S.D (P ≤0.05)		Con.0.9 Day 0.8, Int. 1.6			

The results of Table (2) A were similar in effect to the results of Table (1) with the difference in the stage of the treatment. The cumulative mortality rate for adult stage at the highest concentration of the autoclave sterilized filtrate was recorded 43.30, 53.30 and 70.00 % on 1, 2 and 3 days compared with 10.00, 10.00 and 20.00 in the control treatment. These results showed the cumulative mortality rate of highest concentration of the sterilized filtrate with Millipore recorded at 1,2,3 day, 36.60, 43.30 and 53.30 % respectively, compared with 10.00, 10.00 and 13.30 % at the control treatment.

Table 2: Effect of different concentrations of M. anisopliae filtrate sterilized by autoclave and Millipore on adult of A.gossypii at different time periods

Treatment	Concentration	Days			Mean
		1	2	3	
Autoclave	0	10.00	10.00	20.00	13.33
	25	23.30	30.00	36.60	29.97
	50	33.30	40.00	46.60	39.97
	100	43.30	53.30	70.00	55.53
Mean		27.48	33.33	43.30	
L.S.D (P ≤0.05)		Con.0.8 Day 0.9, Int. 1.6			
Millipore	0	10.00	10.00	13.30	11.10
	25	20.00	26.30	30.00	25.43
	50	30.00	36.60	40.00	35.53
	100	36.60	43.30	53.30	44.40
Mean		24.15	29.05	34.15	
L.S.D (P ≤0.05)		Con.0.7 Day 0.8, Int. 1.5			

Discussion

In our study, Statistical analysis showed significant differences between the two methods for preparing the fungal filtrate of *M. anisopliae*. The autoclave sterilization method showed higher efficiency than the Millipore sterilization method in controlling stages of *A.gossypii*, The reason may be that the heat and pressure used in autoclave sterilization may lead to the decomposition of the secondary metabolic compounds in the fungi into other compounds that are more toxic and effective against insects, or these crude secondary metabolic compounds, when decomposed, bond with other compounds and thus form compounds with a distinct chemical structure lead to High toxicity to insects. The effectiveness of the crude fungal filtrate in killing insects is due to them containing biological compounds such as destruxins A, B and E that are toxic to the insect's systems, especially the digestive system, which appears to have an effect on the insect's abstention from feeding [10]. The other reason for effectiveness of the crude fungal filtrate is that they contain many enzymes such as chitinase, lipase, and protease, which have a role in decomposing components of the insect's body wall [11]. The results are clear that nymph mortality was recorded more than adult mortality in A.gossypii because movement speed of the nymph In addition to possessing thin cuticle layers of nymph stages compared with the adult stage, which may allow the penetration of the fungal filtrate during the cuticle of the A.gossypii, as reported by [12]

Conclusion

This study investigated the use of two methods for preparing the fungal filtrate of *M. anisopliae* to determine their biological efficiency in the mortality of *A.gossypii*. Based on the study's results, it is possible to use the autoclave sterilization method, which showed higher efficiency than the Millipore sterilization method in controlling *A.gossypii* when comparing the two methods with the control

treatment. The results of this study indicate the possibility of preparing and utilizing the secondary metabolites of insect-pathogenic fungi in several possible and simple ways, and thus the results of the study are considered a new addition to the field of bioinsecticide production.

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