Isolating and identifying the fungi associated with the black bean aphid Aphis fabae Scop (Homoptera: Aphididae) on the bean plant and the possibility of using them to control the pest.

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Abstract

This study aimed to isolate and diagnose fungi associated with the black bean aphid and test their pathogenicity in the different stages of the insect. Five types of fungi were isolated: (Aspergillus niger, Alternaria alternata, Beauveria bassiana, Cladosporium oxysporum, Penicillium brevicompactum), and the most frequently appearing fungus was A. niger, where the fungus recorded the highest occurrence rate of (40.74%), while the fungus recorded A. alternata had the lowest occurrence rate (11.11%). As for pathogenicity, the fungi A. niger and P. brevicompactum recorded the highest pathogenicity against the black bean insect in the laboratory compared to the rest of the isolated fungi. Laboratory results showed an increase in the mortality rate of A. fabae with increasing concentrations and time periods of the different treatments, where the A. niger fungal filtrate outperformed the P. brevicompactum fungal filtrate with significant differences. The results showed that the A. niger fungus filtrate was superior to the P. brevicompactum filtrate in terms of the rate of aphid mortality, as the highest percentage of mortality reached 100.0% at the concentration (1 x 102) for the time period of 72 hours, while the fungus P. brevicompactum achieved the highest percentage of mortality, reaching 83.3%. At a concentration of (1x102) for a period of 72 hours. The mortality rate for the comparison treatment was non-existent in all time periods, and this shows that the longer the time period after the insect was exposed to the effective fungal leachate, the greater the mortality rate .

Keywords: Legumes, Aspergillus niger, Penicillium brevicompactum, Aphis fabae .

Introduction

Legumes are an important crop for food production worldwide and a good source of protein for both humans and animals [1]. Leguminous crops are grown all over the world, and among legumes, one of the oldest crops cultivated globally is faba (Vicia faba). It is a vegetable that can be eaten raw, cooked, dried, or canned, and is a common breakfast dish [2]. Peas are an important crop in environmental, nutritional and economic terms [3]. Ripe bean seeds contain a high percentage of dietary fiber (25%), proteins (26.1%), and carbohydrates (58.3%) in terms of nutrition [4]. It is also considered a good source of nutritional minerals and elements such as potassium, phosphorus, iron, zinc, and vitamins such as vitamin B9 (folic acid) and vitamin K [5] and [3]. The bean plant is always affected by many insect pests, which reduces its production. These pests include the

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black bean aphid (Aphis fable Scop.), Liriomyza trifolii (Becker), Agrotis ipsilon, Bruchus rufimanus Boh, and the black bean aphid is a major insect pest [6] and [7]. Wide family range, more than 37 plants. Among the most important factors that reduce crop production and feeding by aphids are direct damage (penetration of the bark leads to the loss of nitrogen compounds and sugars) and indirectly viral diseases (introduction of pathogenic viruses to the plant) transmitted by vectors, and their damage includes in the symptoms of severe infestation are chlorosis, necrosis, wilting, stunting, abortion of flowers and fruits, deformation, falling or curling of leaves, and yellowing [6] and [8]. In addition to the secretion of honeydew that covers the affected parts, which leads to the accumulation of dirt and dust, which may cause the respiratory stomata in the leaf to close, affecting the photosynthesis process taking place in the plant, which causes the growth of fungi, which leads to many physiological damages to the plant [8] and [9]. Despite the importance of the above-mentioned pests, the low cost of purchasing and using insecticides, coupled with their broad-spectrum effectiveness, has reduced the incentive to expend significant efforts in developing new control strategies [10]. As a result, aphid control relied mainly on the use of insecticides [11]. This type of control has generated increasing concern due to the harmful effects on human health and other non-target organisms, especially beneficial insects and natural enemies [12] and [13]. In addition to environmental pollution and the development of resistance among aphid colonies [14], [15], and [16]. The interest in finding alternative pest control methods is of great importance [17] and [18]. Biological pesticides have appeared, which are natural preparations that contain strains of some living organisms, such as bacteria, fungi, and viruses, which are characterized by their ability to infect and eliminate insect pests. Among the most important of these organisms are insect-pathogenic fungi, which constitute a safe alternative to the environment and can be used on pests that have formed resistant strains. Compared to traditional chemical pesticides, its family range is wide, and its strains have the ability to penetrate the insect's cuticle with an enzymatic mechanism that is compatible with the host [19] and [20]. Entomopathogenic Fungi (EF) stands out among microbial control agents not only because of its mode of infection by direct penetration through the epidermis and ease of mass production, but also because of its lifestyles that interact with plants, which places it at the forefront of crop protection and production tools [21] and [22]. Entomopathogenic fungi have proven their ability to eliminate many insect pests, such as the olive fruit fly, the fruit fly, and other pests [23] and [24]. It was widely used within integrated pest management programs in many regions of the world, with fungal pathogens being introduced by 49.3% within 136 biological control programs [25]. Given the importance of pathogenic fungi in controlling pests and the lack of research related to them in Iraq, it is important to search for pathogenic fungi present in our local environment, and then evaluate their effectiveness against pests. This study aims to: 1. Using biological control methods to control A. fabae. 2. Isolating and diagnosing the fungi associated with the insect A. fabae and testing their pathogenicity on the nymphs of the insect .

Materials and methods

Collecting and raising black bean insects :

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A number of bean plants infected with the aforementioned insect were collected in plastic boxes containing small holes for the purpose of ventilation from one of the orchards in the Al-Husseiniyah area in Karbala on February 24, 2024. The infected plant parts were taken and placed at the top of the growing tops of the healthy bean plants previously planted in pots measuring 18 x 18 cm. Inside Al-Musayyib Technical College, a square plastic house measuring (1.5 x 1.5 meters) of wood and nylon was built, with the door of the house left open (to avoid global warming and plant mortality) in both the college and the house. Bean plants were grown in pots of the same size inside the plastic house for the purpose of breeding and multiplying aphids in anticipation of changes in climate conditions, especially strong rains. I left these insects to grow and reproduce until they were used in subsequent experiments, taking care to water the plants every 3 days to keep the plants in good condition, in addition to the survival of the insect, for the purpose of conducting subsequent experiments on them. When conducting laboratory experiments, I collected the aphids that were raised in the college using a soft brush and placed the nymphs in a plastic box with a diameter of 11 cm and a height of 6 cm, perforated with small holes for the purpose of ventilation .

Diagnosis of the black bean aphid :

The insect was diagnosed with the help of Dr. Haider Badri Ali (University of Baghdad/College of Science/Department of Life Sciences) and Dr. Ahmed Saeed Muhammad al-Hattab (Al-Qasim Green University/College of Agriculture) as Aphis fabae Scopoli, 1763 (Hemiptera, Aphididae .(

Isolation of fungi associated with an insect from beans: Media used in the experiment :

I prepared these media according to the method [33],[34] and adapted from [26] as follows:

Medium Potato Dextrose Agar (P.D.A :(

Prepare by dissolving 20 gm in a liter flask containing 500 ml of distilled water according to the manufacturer's instructions, then sterilize it with an autoclave at a temperature of 121°C and a pressure of 15 pounds for 15 minutes. After the end of the sterilization period, leave the flask to cool slightly, then add the antibiotic Chloramphenicol to it. Then pour the medium into petri dishes and store in the refrigerator until use. (Used for cultivation and propagation of fungi .(

Preparing the culture medium Potato Dextrose Broth (P.D.B :(

To prepare one liter of this medium, 200 g of peeled potatoes were taken, cut into small pieces, and boiled in a pot containing 500 ml of distilled water for 15 minutes. It was filtered through a piece of gauze, then the filtrate was placed in a one-liter beaker, 20 g of dextrose was added to it, and the volume was completed. With up to a liter of distilled water, sterilize the medium with an autoclave at a temperature of 121°C and a pressure of 15 pounds for 15 minutes, after which the antibiotic chloromphenicol was added to it at an amount of 250 mg/liter. (Used to prepare fungal filtrate .(

Isolating the fungi associated with the black bean insect, purifying them, diagnosing them, and calculating the percentage of their appearance :

A group of aphids were found on bean plants grown in the field of the College of Agriculture. They were washed well with sterile distilled water, then surface sterilized with 10% sodium hypochlorate (NaClO) from the commercial solution for two minutes, then transferred to sterile distilled water for two

minutes, then transferred to Whatman No type 1 filter paper to remove water from them. 4 insects in the shape of a plus sign were placed in each sterile Petri dish with a diameter of 9 cm containing the P.D.A nutrient medium, and the dishes were incubated at a temperature of 25±2°C for 7 days. The growing fungi were isolated and purified on solid media by taking a piece of the medium containing the fungal growth using a cork piercer from the edge of the growing fungal colony to a Petri dish containing a solid culture medium prepared in advance. Then the dish was left in the incubator at a temperature of 25±2°C for a week to obtain On a pure fungal colony and continuing the process of activating these fungi every 10-14 days. After purifying it and ensuring that one fungus grew in each dish .

Diagnosis of fungi

it was first diagnosed by Dr.prof. Ahed Abdul Ali Hadi, Department of Bioresistance Technologies, Al-Furat Al-Awsat University/Al-Musayyib Technical College .

The direct wet mounts or smears are prepared and for culture the specimen is inoculated on culture media. Almost all the specimens are processed for direct microscopic examination. This provides the presumptive diagnosis for the physician and also aid in the selection of appropriate culture media. KOH/calcoflurol mounts .Lactophenol cotton blue (LPCB) mounts.

The percentage of its appearance was calculated according to the following equation :

$$\text{Percentage of impression} = \frac{\text{The number of times the fungus appears}}{\text{Total number of samples}} \times 100$$

Preparation of fungal filtrate:

Bring the previously prepared liquid nutrient medium (Potato Dextrose Broth (P.D.B)) and then distribute it into 500 ml glass beakers at a rate of 250 ml/beaker. The beakers containing the liquid medium were then sterilized in an autoclave device at a temperature of 121 °C and 15 pound/inch2 atmospheric pressure for 15 minutes, and after leaving it for a period of time to cool, each flask was inoculated with several discs with a diameter of 0.5 cm of fungal colonies at 9 days old. Then the flasks were incubated in the incubator at a temperature of 25±2 C for 31 days, taking into account shaking the flasks every 3-4 days. For the purpose of distributing the fungal growth, it was then filtered using Whatman No.1 filter paper using a Buchner funnel with the help of a vacuum pump device. The filtrate was used in subsequent experiments. Laboratory work was carried out in the graduate laboratory of the Department of Bioresistance Technologies at Al-Musayyib Technical College/Al-Furat Al-Awsat University.

Preparing concentrations of filtrate of two fungi (Aspergillus niger and Penicillium brevicompactum) for laboratory experiments :

The following concentrations were prepared (1 × 104, 1 × 103, 1 × 102) for the purpose of determining the effectiveness and efficiency of the isolated fungal filtrate on the insect A. fabae, where 8 medical test tubes with a

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capacity of 12 ml were taken and 10 ml of distilled water was added using a medical syringe. Then take 1 ml of the filtrate and add it to the first test tube so that its concentration is (1 x 101). From this dilution, 1 ml is taken and added to the second test tube so that its concentration becomes (1 x 102), and so on with the rest of the concentrations, where 1 ml is taken from each concentration to prepare the next concentration. The control treatment was with distilled water only .

Laboratory evaluation of the toxic efficacy of the filtrates of two fungi (Aspergillus niger and Penicillium brevicompactum) on nymphs of A. fabae:

I carried out a factorial laboratory experiment with a completely randomized design (C.R.D.). I prepared bean leaves and placed 10 nymphs on them with three replicates for each treatment in plastic boxes with a diameter of 9 cm. Then the leaf blade was covered with a piece of cotton and moistened to obtain permanent moisture. The replicates were sprayed with three sprays for each replicate of The fungal filtrate was prepared as in paragraphs 3–15 using a sterile sprayer with a capacity of 10 ml and a distance of 15 cm from the paper. Each filtrate was used in concentrations (1 x 104,1 x 103,1 x 102). As for the control treatment, it was sprayed with only sterile distilled water. I left the dishes at a laboratory temperature of 25±2°C, calculated the percentage of destruction after 24, 48, and 72 hours of spraying, then corrected the values and converted them angularly according to the Orell and Schneider equation mentioned before [27], as follows:

$$\text{Percentage of mortality} = \frac{\text{Mortality rate in treatment -- mortality rate in comparison}}{\text{-- 100mortality rate in comparison}} \times 100$$

statistical analysis :

The data obtained were analyzed statistically according to Completely Randomized Design (C.R.D.) factororial experiments, which were completely randomized randomized for laboratory experiments, and the least significant difference (L.S.D) test was used using the GenStat Release 2009 program to ensure the significance of the differences between the different parameters at a probability level of 0.05. The percentage loss was corrected according to the Abbott equation [28]. The corrected percentage of destruction was converted to angular values for inclusion in statistical analysis [29 .[

$$\text{Corrected mortality percentage} = \frac{\text{\% of mortality in the treatment} - \text{\% of mortality in the comparison}}{\text{\% - 100 of mortality in comparison}} \times 100\%$$

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Results and discussion

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Isolating the fungi associated with the black bean insect, purifying them, diagnosing them, and calculating the percentage of their appearance:

Five types of fungi shown in Table No. 1 were isolated from A. fabae, which were grown in 27 dishes. They are (Aspergillus niger, Alternaria alternata, Beauveria bassiana, Cladosporium oxysporum, Penicillium brevicompactum), where the fungus A. niger recorded the highest occurrence rate of (40.74%), while the fungus A. alternata recorded the lowest occurrence rate of (11.11%). Then came the fungus B. bassiana with an appearance rate of (25.92%), and the fungus C. oxysporum with an appearance rate of (14.81%), while the fungus P. brevicompactum had an appearance rate of (33.33%). These results were consistent with other studies that indicated the isolation of many fungi from a bean insect, some of which were pathogenic for the insect. [30] and [31] isolated two types of fungi, A. niger and A. alternata from a black bean aphid. [32] also isolated several fungal genera from mosquitoes (Cx. Quinquefasciatus) namely Aspergillus, Fusarium, Penicillium, and Trichoderma. The results of the current research are consistent with the findings of [33], where 36 species of fungi were isolated from aphid species, including Aspergillus spp. Alternaria spp. and Cladosporium spp. ,As for [34], they isolated a group of fungi from the black bean aphid, namely A. alternata, A. chlamydospore, A. niger, B. bassiana, C. oxysporum, P. chrysogenum, P. compactum, and Ulocladium atrum.

Table No. (1) Types of fungi isolated and their occurrence rate

Fungi	Number of times the fungus appears	Percentage of impressions
A. niger	11	40.74
A. alternata	3	11.11
B. bassiana	7	25.92
C. oxysporum	4	14.81
P. brevicompactum	9	33.33

The effect of the filtrates of the two fungi (Aspergillus niger and Penicillium brevicompactum) on the nymphs of A. fabae in the laboratory:

The results shown in Tables 2 and 3 indicated that the filtrate of the fungi A. niger and P. brevicompactum had an effect on the nymphs of black beans and gave high mortality rates, which were significantly different from the control treatment. The fungus A. niger was superior to the fungus P. brevicompactum. In the mortality rate, the highest mortality rate reached 100.0% in the concentration (1x102) for the time periods of 72, 48, and 24 hours,

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followed by the two concentrations (1x103 and 1x104) with a mortality rate of 100.0% and 73.7%, respectively, after 72 hours, while the fungus P brevicompactum had the highest mortality rate of 83.3% at the concentration (1x102) for the time period of 72 hours, followed by the two concentrations (1x103 and 1x104) with a mortality rate of 56.7% and 33.3%, respectively, for the same time period. While the lowest percentage of mortality was recorded for the 24-hour period for the filtrate of the fungus A. niger, which amounted to 76.7% and 46.7%, respectively, for the two concentrations (1x103 and 1x104), for the fungus P. brevicompactum, the lowest percentage of mortality was recorded during the 24-hour period for all concentrations (1x104, 1x103, 1x102), which amounted to 50.0%, 33.3%, and 13.3%, respectively. The mortality rate for the comparison treatment was non-existent in all time periods, and this shows that the longer the time period after the insect was exposed to the influential fungal leachate, the greater the mortality rate .

The ability of these filters to affect the nymphs is due to their containing toxins that lead to the .[mortality or destruction of some cells, as they reach the inside of the pest's body either through the respiratory stomata or through surface contact with the pest's body. Mortality rates increase with an increase in the concentration of the toxins and the duration of the insect's exposure to them [35] and [36]. [37] indicated in a study that the appearance of significant differences in mortality rates is also due to the sensitivity of the treated insect stage and the time period of its exposure to the fungal ooze, as the nymph stage is more sensitive than the adult stage due to its incomplete means of defense. [38] also mentioned that increased concentrations of mycotoxins lead to an increase in the mortality rate after these toxins enter through the mouth and respiratory pores and come into contact the insect's body wall. Or through the fungus penetrating the insect's body wall, as it was found that the areas that can be most penetrated are the areas where the rings articulate, and thus the insect's defensive ability decreases, thus increasing its susceptibility to infection with the disease [39

Table No. (2): Results of the filtrate of A. niger

Concentrations	Mortality percentage/hour			Concentration rate
	24	48	72	
Comparison	0.0	0.0	0.0	0.0
$1x10^2$	100.0	100.0	100.0	100.0
$1x10^3$	76.7	93.3	100.0	90.0
$1x10^4$	46.7	70.0	73.7	63.3
Average time period	55.8	65.8	68.3	

L.S.D ($P \leq 0.05$) For periods = 5.62 For concentrations = 6.49 For interference =11.23

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Table No. (3) Results of P. brevicompactum filtrate

Concentrations	Mortality percentage/hour			Concentration rate
	24	48	72	
Comparison	0.0	0.0	0.0	0.0
$1x10^2$	50.0	66.7	83.3	66.7
$1x10^3$	33.3	43.3	56.7	44.4
$1x10^4$	13.3	23.3	33.3	23.3
Average time period	24.2	33.3	43.3	

L.S.D ($P \leq 0.05$) For periods = 5.25 For concentrations = 6.07 For interference =10.51

The results presented by [31] showed that they are consistent with the results of Table No. (2), where two types of fungi, Aspergillus niger and Alternaria alternata, were isolated from a bean insect. The cause of the suspended fungus A. niger had the highest mortality rate of 80% and the lowest mortality rate of 40%. It was followed by the A. alternata with a mortality rate of 75% and the lowest mortality rate of 35%. As for the leachate, the highest mortality rate reached 90% when using a fungal filtrate. A. niger had the lowest mortality rate of 50%, followed by A. alternata with a mortality rate of 80% and the lowest mortality rate of 45%. [40] found the highest percentage of mortality recorded in the fungal filtrate A. niger, amounting to 90%, after 4 days of treatment. This is consistent with the results of the current study, while [41] recorded that the fungal filtrate A. niger achieved a 90% mortality rate after 6 days of treatment when used to control house fly adults. [42] isolated 21 fungi of the genus Penicillium in search of insecticides and tested the pathogenicity and efficiency of the toxins contained in them using filtrates and suspensions of these fungi on the milkweed bug (Oncopeltus fasciatus Dallas), especially on juvenile hormones and antifungal activities. The filtrates were the most active, especially against insects, and approximately 25% of them showed high insect toxicity (100% lethality rate at 100 µg/cm2). Strong anti-JH activity of P. brevicompactum filtrate was detected in vivo for O. fasciatus Dallas, especially fourth instar nymphs. The results presented by the researcher [43] showed that they agree with the results of Table No. (3), where the fungus Penicillium oxalicum was recorded as a pathogen of the sugarcane insect Ceratovacuna lanigera, and the fungus grown on P.D.B medium achieved a 60% mortality rate after seven days. Treatment of the milkweed insect O. fasciatus Dallas. The study conducted by [44] showed that the life expectancy of the green peach insect M. persicae treated with the fungi Aspergillus and Penicillium is less than the life expectancy of insects not treated with the fungi, and the

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reason was attributed to the toxic secretions of the two fungi mentioned. The results presented by [45]. Showed the isolation of 26 fungal strains of aphids and tomato leafworms as local species, and of the red palm weevil and peach fruit fly as invasive insects. The fungal strains were tested for their ability to attack insects in beans and wheat. The mortality rate of aphids due to this fungus has been estimated at 90%-100% .

Conclusions :

A field survey of some nurseries and fields in Karbala and Babil governorates showed the presence of the black bean aphid, where the infestation rate varied according to weather conditions. Different types of insect pests were found, and the infestation rate varied on the bean plant. It was found that aphid colonies increase with lower temperatures and increased humidity. and the Aspergillus niger fungus filtrate was superior to the Penicillium brevicompactum filtrate in controlling the black bean aphid in the laboratory. possibility of manufacturing a bioinsecticide from the fungi A. niger and P. brevicompactum instead of chemical pesticides .

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