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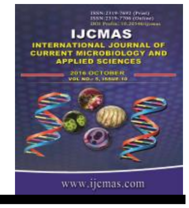

Original Research Article

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In vitro, Alternative Methods to the Biological Control of the *Aphids* by Entomopathogenic Fungi *Beauveria bassiana* Isolates from Gaza Strip

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A B S T R A C T

Aphid's species is one of the most important insect pests which threaten most of crops in Gaza strip such as Mentha, Citrus, Rosa, Almond, Pomegranate, Fruits and most species of flowers. It has a rapid reproduction which cause a serious damage. *Aphids'* species are one of the main targets of *Beauvaria bassiana* which is used as safe prospects for the biological control of this pest. This study was carried out to find safer and environment solutions for pest's management by using entomopathogenic fungi (EPF) (*B. Bassiana*). Spores of *B. bassiana* which is used as biological control agent in this study using Potato Dextrose Agar (PDA) media and liquid fermentation techniques. *B. Bassiana* fungus killed all adult aphids by 95%-97% compare to chemical treatment 50%.

Introduction

Sustainable vegetable production it depends mainly on yield and quality, if not properly managed, pests and diseases can dramatically reduce crop yield, quality and subsequent returns. Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world (Pal and Gardener, 2006). Disease control is an essential component of crop management for increase yield potential. A low disease loss in your fields in the recent past does not ensure disease losses will remain low (Tobacco disease management (2015). Every year gardeners confront many insect pests feasting on fruits and vegetables (Jackman, 1998). Plants have many pests, such as aphids and mites. Aphids are important herbivores of both wild and cultivated plants that feed on the phloem of vascular plants (Züst and Agrawal, 2016). Most vegetables crops attached by this pest and also it can stunt and distort the growth of plants and cause wilting and bud drop, resulting in poor flowering and fruit set.

Synthetic pesticides have played a central role for control plant pathogen (Chandler *et al.*, 2011; El-Wakeil, 2013). In the recent years, the negative effects on health and the environment as a result of the indiscriminate use of pesticides have led the EU to the prohibition of many synthetic pesticides (D'Addabbo *et al.*, 2014). Consequently, some pest management researchers have focused their efforts on developing alternative methods to synthetic chemicals for controlling pest's plant pathogen (Pal *et al.*, 2006). A lot of studies encourage us to find solutions more realistic and safer to humans and environment-friendly for pest management. The development of resistance by many important insect pests led to increase chemical insecticides price used for pest control in addition the concern about the environment protection have encouraged studies to use of biological control (Laird *et al.*, 1990; Slinninger *et al*., 2003; Haas-Costa *et al.*, 2011). Use of entomopathogenic fungi as biological control agents for insect species has increased the global attention during the last few decades (Latifian *et al.*, 2014). *B. bassiana* is considered one of the most important entomopathogenic fungi that used as biocontrol agents. *B. bassiana* is used as an efficient bio-control agent for controlling several tea pests e.g. termites, thrips, whiteflies, aphids (Bani *et al.*, 2014). The present study aims to use Entomopathogenic fungi *B. bassiana* that have wide host range against plant pathogens as fungal bio-pesticide on aphids.

Materials and Methods

Chemicals and reagents

Chemicals, cultures medium and reagents used in this study are shown in Table 1.

Methodology

Isolation of fungi

B. bassiana was isolated from dead of *aphids* found in the soil of one of the green house of infected citrus fields in Gaza strip. Soil sample was also collected from Gaza strip. The sample was placed into plastic bags and stored at 4–8 °C (NouriAiin *et al.*, 2014).

Culture of *B. bassiana*

Selective medium and PDA Medium are generally required for isolation of *B. bassiana* from soil. DOC2 medium for *B. bassiana*, autoclaved and poured into Petri dishes (Shin *et al.*, 2010). Soil sample (1g) from each isolate was suspended in sterile distilled water (200 ml) containing Tween 80 as surfactant. Suspensions were applied on PDA and selection medium using streaking method. Plates were incubated at 25 °C in the total darkness.

Table.1 Chemicals and reagents used in this study

Reagents & Culture Media
PDA Media
PDB Media
Chloramphenicol tablets
Tween 20
Yeast Extract
Peptone
Crystal Violet
Methylene Blue
Agar

Spore Suspension

Potato Dextrose Broth (PDB) medium was prepared for the preparation of the spore suspension from fungi. PDB was autoclaved and inoculated with fungal spores propagated on PDA. Spores were harvested from 1 week old surface cultures by scraping and used to inoculate the liquid medium in flasks. The flasks were held on a shaker (110 rpm) for 7 days at room temperature. The suspensions were stirred and filtered through a sterile Gauze to remove culture debris and mycelia. Spore concentration was adjusted using a haemocytometer and were calibrated to 2.5×10^7 spores/ml for *B. bassiana* (Gindin *et al.,* 2006; El kichaoui *et al.*, 2016).

Morphological Identification of Fungal Isolates

Cultures were examined periodically and identified when they sporulated. The cultures were identified based on their morphological characteristics including growth pattern, colony texture, pigmentation, and growth rate of the colonies on PDA (Promputtha *et al.,* 2005). When fungal colonies sporulated on PDA, small plaques from the edge and the center of each growing colony were transferred onto glass slides, and then were examined using a compound light microscope for characteristics of their vegetative and reproductive structures such as hyphal color and structures, shape and size of conidia and conidiophores (Yu, 2010).

In vitro, Evaluation the influence of fungi against aphids

Divided the group

Three groups of *aphids* were divided into (control, chemical and biological control *B. bassiana*). Each groups contain on 500 insect, air condition and nutrients at room temperature 25^0C (Rashki & shirvani, 2013).

The insects with the bio-insecticide *B. bassiana* at the concentrations of 2.5 x 10^7 spores/ml. Control samples were sprayed by water only, and chemical pesticides samples treated by Chlorofenapyr 240g/l and Thiocyclam hydrogen oxalate 50%. The insects were examined every 24hr, the percentage of infestation was calculated until the end of the experiment (Sabbour, 2014).

Treatment in field

We counted the *aphids* found in the plant areas for the three groups; we used the chemical pesticide on the second group for one time, and used our biological control agent *B. bassiana* of the third group for one time. Treatment of insects was done at the end of day before sunset.

The counting

We count the number of larvae's on the all tree in the three groups after 24hr, 48hr and 72hr.

Data Collection and Statistical analysis

The effect of *B. bassiana* on aphids was tested using T-test. Statistical analyses were performed using the software SPSS Statistics 17.0 (SPSS Inc., 2009).

Results and Discussion

Isolation of *B .bassiana* from the dead of *Aphids*

B. bassiana was isolated from dead of aphids found in the soil, after removing the surface layer of soil and these samples shown in figure 1.

Cultural Characteristics

The cultural characteristics of *B. bassiana* isolates were examined. Generally, in culture, *B. bassiana* grows as a white mould. It produces many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells shown in Figure 2 & 3.

Microscopic Examination

Microscopic characters observation of *B. bassiana* was shape, size, color and thickness of hyphae, conidiophore, and conidium. Microscopic characters of *B. bassiana* was shown on figure 2. Microscopic observation result show that hyphae size about 1-2 µm which grouped on conidiogene cells with 3-6 µm in size. Hyphae then branched and formed conidiogene cells with bottle like form, small neck, and branch long were up to more than 20 µm and 1 µm wide. Fertile hyphae was found on branch, circular and normally thicken or swollen. While mycelium which is hyphae aggregate of *B. bassiana* was white and insulated shown in Figure 4.

Evaluation of influence of fungi against *Aphids*

After adjustment of *B. bassiana* of concentration 2.5 X 10^7 spores/ml. Aphids on infected plants were treated by isolated fungi in vitro. Data in table 2 show the effect of bio-insecticide *B. bassiana* and chemical treatment against the Aphids for 3 days and based on collected data in table 2.the results were as follows:

The results in the current study reported that *B. bassiana* have exhibited satisfactory efficacy against *Aphids* compared to chemical treatment. This study recorded that; the entomopathogenic *B. bassiana* fungus could be caused larvae mortality up to 95-97%, and the *P-value* as shown in table3.

Aphid has been a significant pest which threatens most of crops in Gaza strip such as Mentha, Citrus, Rosa and *Ocimum basilicum*. Despite advances in integrated pest management, and frequent use of insecticides, the industry is still plagued by the insect. There is a need to shift emphasis on biological control agents and softer chemicals (Ochieng & Nderitu, 2011). Due to these problems, there was need to find alternative methods with different modes of action that would be effective, user and environment friendly.

Table.2 Shows the 3 groups of Aphids compare with the time of treatment.

Treatment	Before Treatment	After 24hr Treatment	After 48hr Treatment	After 72hr Treatment
Control	500	530	543	800
chemical	500	450	300	250
Fungus (*B. bassiana*)	500	350	150	25

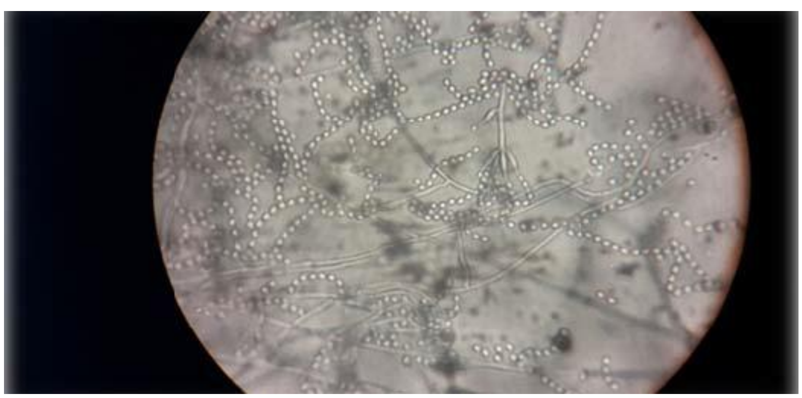
Table.3 Show the P-Value for all groups.

Group	*P-value*
Control – Chemical	0.045
Control – Fungus	0.037
Fungus- Chemical	0.016

Fig.1 Soil samples that collected for *B. bassiana* isolation.

Fig.2&3 Culture of B. bassiana on DOC2 Selective Medium and Culture of *B. bassiana* on PDA Medium

Fig.4 Microscopic examination for *B. bassiana* 1000X.



The effectiveness of entomopathogenic fungi *B. bassiana* as safe prospects for the biological control of aphid and the safe use of this fungus as a biological control where no negative effects on the surrounding environment and on the farmers and consumers health promote many studies to estimate the susceptibility of aphid to *B. bassiana*. The results indicate that the sample 1 & 8 from *B. bassiana* isolates recorded the most effective isolates with aphid mortality up to 97%. So the results in our study suggests that opportunities exist for revisiting aphid pest problems and the potential of *B. bassiana* as new tools to support a new biological control program targeting this insect.

In 2013, Rashki & Shirvani Recent study has results which clarified the high significantly influenced of the Entomopathogenic fungi. *B.bassiana* strain DEBI008, to use integrated pest management (IPM) programs as an efficient biological control against the Aphid pest.

Based on recent study for using *B.bassiana* for controlling of Aphids and the maximum mortality observed by the highest concentration of 1 X 10^8 spores/ml for *B. bassiana* and this insect pathogenic. Fungus can be used as potential biocontrol agent for the management of Aphids (Akmal *et al.*, 2013).

In conclusion, the results from this study were encouraging to find new environmentally friendly product, the advantages of using of this product are to reduce the costs of pest control. Additionally it preserves human health and environment from pollution, which caused by chemical pesticides usage. Also it minimizes the formation of insecticides resistance in some pest.

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