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Safe approach to the Biological Control of the Tomato Leafminer *Tuta absoluta* by entomopathogenic fungi *Beauveria bassiana* isolates from Gaza Strip

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Abstract
Tuta absoluta is the most important insect pests which pose a serious threat in tomato production in many countries. The effectiveness of entomopathogenic fungi (*Beauveria bassiana*) as safe prospects for the biological control of *tuta absoluta* in tomato crops 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 *T. absoluta* to *B. bassiana*. The development of safe approaches to control *tuta absoluta* would be facilitated through an information on this pest and data for the safe use of entomopathogenic fungi (*B. bassiana*) as efficient biological control programs. The current study will encourage us to find solutions more realistic and safer to humans and environment-friendly for pest management. Evaluation the activity of *B. bassiana* spores as a biological control agent was carried out in this study using liquid-semi solid fermentation techniques and t, the entomopathogenic *B. bassiana* fungus could be caused larvae mortality up to 95% compared to chemical treatment 88%.

Keywords: *Tuta absoluta*, entomopathogenic fungi, *Beauveria bassiana,* biological control

1. Introduction

Plant diseases have caused severe losses to human including starvation, direct economic loss and reduce the aesthetic values of landscape plants and home gardens (Maloy, 2005). There are many plant pathogens which have profound effects on mankind like *Phytophthora infestans* that caused late blight in potato (Ballvora, *et al.*, 2002) [1], nematoda and insects. Insect pests and plant pathogen destroy more than 40% of all food production each year (Pimentel, 2009) [17]. This loss occurs in spite of the use of nearly 3 million tons of pesticide per year plus the application of physical and biological methods (Pimentel, 2009) [17]. Tomato plant is one of the most important Solanaceous vegetable crops (Sabbour, 2014) [21]. Tomato crops are infected with many serious pests, recently the most distructive ones, *T. absoluta* (Sabbour, 2014) [21].

T. absoluta is a micro lepidopteran moth belonging to the Gelechiidae family and is considered the major pest that attacks tomato in many countries (Shalaby, *et al.*, 2013) [23]. It is a devastating pest of tomato originating from South America, After its initial detection in eastern Spain in 2006 (Shalaby, *et al.*, 2013) [23]. Damage that caused by this pest may be pose a serious threat in tomato production. Its damages are dangerous both in greenhouse and in field crops (Desneux, *et al.*, 2010) [4]. The yield and quality of tomato crops will be reduce by the direct feeding of *T. absoluta*, the wounds made by this insect will be facilitate entry secondary pathogens through the wound make by this pest (Kaoud, 2014) [12]. Synthetic chemical pesticides are still the main tool for pest eradication (Loni, *et al.*, 2011) [14]. Complex problems have been generated from the application of chemical pesticides in control of insect pests including ; safety risks for humans and wild and domestic animals; contamination of ground water; decrease in biodiversity and outbreaks of secondary pests normally held in check by natural enemies (Dolinski & Lacey, 2007) [5]. There is an increasing interest in evaluating the potential role of native natural enemies in reducing the pest populations (Loni, *et al.*, 2011) [14]. Cultural, biological and biotechnological methods for pest management become mandatory and the continued use of chemical insecticides could harm non-target organisms and the environment (Shalaby, *et al.,* 2013) [23]. Different approaches may be applied in an Integrated Pest Management (IPM) program for pest management including insecticides and biological control and the association of both (Dos, *et al.,* 2011) [6]. 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 (Haas-Costa, *et al.,* 2011).

B. bassiana is considered one of the most important entomopathogenic fungi used as biocontrol agents. There is great potential for the use of *B. bassiana* as biological control, due to it can be cost-effective to locally mass-produce (Qazzaz, *et al.,* 2015) [19].

Studies have been carried out under laboratory conditions to assess of resistance *T. absoluta* for various biocontrol agents. *B. bassiana* has been tested as biocontrol agent for pest management and has been successfully used in many countries. The ability and efficacy of *B. bassiana* to prevent crops damage in addition its infections are extremely rare events have encouraged studies to use it in biological control (Längle, 2006) [13]. There are no known reports of significant negative effects that can be attributed to the use it in biocontrol (Längle, 2006) [13]. Growing risk resulting from pesticide presence in our food and environment and Safety of *Beauveria bassiana* are encouraging to carried out this study that aim to use *Beauveria bassiana* spores as a biological control agent using liquid-semi solid fermentation techniques and low price crude materials.

2. Methodology

2.1 Materials

2.1.1 Chemicals and Reagents

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

Table 1: Chemicals, reagents and cultures mediums that were used in this work

#	Reagents & Cultures Media	Manufacture	Country
1	PDA media	HiMedia	India
2	SDA media	HiMedia	India
3	PDB media	HiMedia	India
4	Cholorophenicol tablets	HiMedia	India
5	Tween 20	Sigma-Aldrich	USA
6	Yeast Extract	HiMedia	India
7	Peptone	HiMedia	India
8	SDAY media	HiMedia	India
9	Dodine acetate	Toronto Research Chemicals	Canada
10	Crystal Violet	HiMedia	India
11	Methylene Blue	HiMedia	India

2.2 Protocols

2.2.1 Isolation of fungi

B. bassiana was isolated from dead larvae of *Tuta absoluta* found in the soil of one of the green house of infected tomato 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) [16].

2.2.2 Culture of *B. bassiana*

Selective medium is generally required for isolation of *B. bassiana* from soil. DOC2 medium for *B. bassiana*, autoclaved and poured into 15 cm Petri dishes (Shin *et al*., 2010). Soil sample (1g) from a corn field in Gaza strip was suspended in sterile distilled water (200 ml) containing Tween 80 as surfactant. Suspensions were applied at a concentration of 0.2 ml/plate and spread using a glass rod. Plates were incubated at 25 °C in the total darkness. For increasing of quantity of *B. bassiana* by using of Potato Dextrose Agar (PDA) medium. Incubation at 25 °C in the total darkness.

2.2.3 Spore suspension

Preparation of spore suspension from fungi in a liquid medium (PDB) media. Liquid medium (PDB) was used for production of spores required for experiments. Liquid mediums were autoclaved and inoculated with fungal spores propagated on PDA. Spores were harvested from 2 – 3 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 5 days at 25 ˚C. The suspensions were stirred and filtered through a single layer of linen to remove culture debris and mycelia. After this time the blast spore concentrations were determined using a haemocytometer and were calibrated to 2.5×10^7 spores/ml for *B. bassiana* respectively. (Gindin, *et al.,* 2006) [8].

2.2.4 Morphological Identification of Fungal Isolates

Cultures were examined periodically and identified when they sporulated. The cultures were separated into groups based on their morphological characteristics including growth pattern, colony texture, pigmentation, and growth rate of the colonies on PDA (Promputtha *et al.*, 2005) [18]. 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 (Olympus BX41 system microscope, Olympus America Inc, Melville, NY) for characteristics of their vegetative and reproductive structures such as hyphal color and structures, shape and size of conidia and conidiophores (Yu, 2010) [26].

2.2.5 Evaluation the influence of fungi against tomato leafminer

2.2.5.1 Divided the groups

Tomato plant was planted in the green house in 50 plots in each artificial infestation was made by spraying, three groups include (50 plots of tomato plants as control, 50 plots of tomato plants treated with chemical pesticides, 50 plots of tomato plants treated with our biological control agent (Biotutacide)). The plant with the bio-insecticide *B. bassiana* (Biotutacide) at the concentrations of 2.5×10^7 spores/ml. Control samples were sprayed by water only, and chemical pesticides samples treated by Chlorofenapyr 240g/l and Thiocyclam hydrogen oxalate 50%. The plants were examined every 24hr, the percentage of infestation was calculated until the end of the experiment. (Sabbour, 2014) [21].

2.2.5.2 The steps of treatment

We counted the larvae's of the *T. absoluta* 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 plants was done at the end of day before sunset.

2.2.5.3 The Counting
We count the number of larvae's on the all tree in the three groups after 24hr, 48hr, 72hr and 96hr.

2.2.6 Data Collection and Statistical analysis.
The effect of plant cultivar by *T. absoluta* on leaves was tested using one-way ANOVA. Significantly different means of treatments were determined using Duncan's multiple range test at $P = 0.05$. A t-test was also used to compare the number of larvae present on leaves between the three groups of tomato plants. All statistical analyses were performed using the software SPSS Statistics 17.0 (SPSS Inc. 2009).

3. Results
3.1 Isolation of *B. bassiana* from soil of Gaza strip
B. bassiana was isolated from dead larvae of *Tuta absoluta* found in the soil. After removing the surface layer of soil, collection of 100 gram has been done from the aforementioned areas in sterile bottles of polyethylene then proper storage in refrigerator were done and these samples shown in Figure 3.1

Fig 3.1: Soil samples that collected for *b. bassiana* isolation.

3.2 Cultural Characterization
The cultural characteristics of the suspected *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. this result presented in Figure 3.2

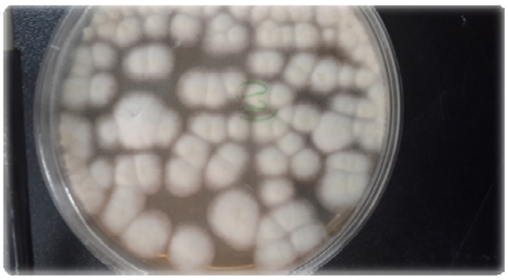

Fig 3.2: Culture of *b. bassiana* on DOC2 Selective Medium.

3.3 Microscopic Examination
Microscopic characters observation of *B. bassiana* were shape, size, color and thickness of hyphae, conidiophore, and conidium. Microscopic characters of *B. bassiana* was shown on figure 3.3.
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.

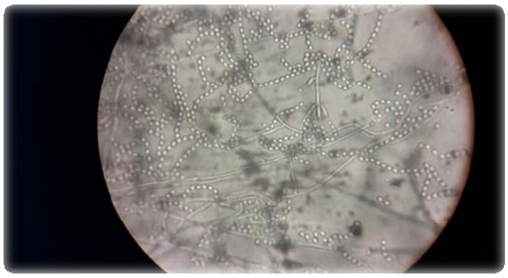

Fig 3.3: Microscopic examination for B. bassiana 1000X.

3.4 Enrichment on Selective medium and spore suspension
This selective media that contains dodine and used as effective media for enrichment of *b. bassiana*. Calibration for spores into $2.5X10^7$ spores/ml is the lethal concentration for *T. absoluta*. The enrichment of *b. bassiana* shown in Figure 3.5

Fig 3.5: Subculturing on Selective media containing on dodine.

3.5 Evaluation the influence of fungi against tomato leaf miner
After adjustment of *B. bassiana* at the concentrations $2.5 X 10^7$ spores/ml was tested against tomato leaf miner. Data in Table 2 show the effect of bioinsecticide *B. bassiana* and chemical treatment against the larvae of the tomato leaf miner moth *Tuta absoluta* and the results were as follows:

Table 2: Show the three groups of tomato plants compare with the time of treatment.

Treatment With	Before Treatment	After 24 hr. of treatment	After 48 hr. of treatment	After 72 hr. of treatment	After 96 hr. of treatment
Control	489	530	543	736	761
Chemical	496	266	218	93	65
Fungus	480	218	125	56	19

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

Table3: Show the P-Value for all groups.

Group	P- value
Control – Chemical	0.041
Control – Fungus	0.031
Chemical – Fungus	0.019

4. Discussion

Tomato leafminer *T. absoluta* is a major pest of tomato plants in South America (Goftishu, *et al.*, 2014) [9]. Its main host is tomato, but it also can feed, develop and reproduce on potato, eggplant and common bean (Bloem & Spaltenstein, 2011) [3]. Larvae cause mines that lead to cosmetic damage, leaves drying out or even early defoliation. Female adults also lead to cosmetic damage in plant by cause feeding marks where they feed (Kaoud, 2014) [12].The wounds made by larvae will be facilitate entry secondary pathogens through the feeding areas make by this pest (Dos, *et al.*, 2011; kaoud, 2014) [6, 12]. Infestation by this pest may be cause loss in tomato production and yield.

Growing risk resulting from pesticide presence in our food and environment have resulted in political and economic pressures to reduce chemical pesticide use, or at a minimum, emphasize the development and use of products that are less toxic and more environmentally safe (Rebek, *et al.*, 2012) [20].

B. bassiana is the most important entomopathogenic fungi currently that used as biological agents against a wide range of arthropod, mainly insect, pests, and the most common species developed as mycopesticides (Stafford & Allan, 2010) [22].

B. bassiana is a fungus that grows naturally in soils throughout the world and causes much infection in various insects by acting as a parasite. The spores of the fungus come into contact with the body of an insect host, they germinate and grow inside the body of insect eventually killing the insect. Then a white mold grows on the dead body and produces new spores (Hatem & Reda, 2012) [11].

A successful infection by *B. bassiana* is dependent primarily on the activity of different enzymes for degradation of proteins, chitin and lipids in the insect integuement (Feng, *et al.*, 1994) [7].

Many studies were conducted to determine the effects of *B. bassiana* against insect pests, This study completely agree with many others studies that the fungi like *B. bassiana* can be used without any doubt to control the tomato leafminer *T. absoluta*. Current studies were reached that entomopathogenic fungi like *B. bassiana* is have a high efficacy with no adverse effects on human health or the environment. (Haas-Costa, *et al.*, 2011; *Health Canada Pest Management Regulatory Agency*, 2014) [2].The ability of the pest for high reproductive capacity and very short generations, have increased risk of developing resistance, so you should submit this fungi as product for biological control of tomato leafminer, specially the cost of production and utilization of this product very low. (Kaoud, 2014) [12].

5. Conclusion

Obviously that biological control agent such as *B. bassiana* significantly differs from chemical pesticides in their properties. The properties of biological control should be taken into account when designing pesticide for control plant diseases with no adverse effects on human health and environment. Therefore, the use of the bio-pesticide is one of the best solutions and safe approach for plant disease management and this lead to reduce the costs of pest control, preserves human health and environment from pollution which caused by chemical pesticides usage. The use of *b. bassiana* pre-injury work to prevent disease and this product is a spores and play important role in Independent Automatic prevention. So in our country we are production of this fungus like *b. bassiana* as biological control agent called (Biotutacide) and it's currently used by farmers.

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