Molecular Identification and Biological Control of Tomato Leafminer, *Tuta absoluta* Using Plant Extracts and Microbial Bio-Agents

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Abstract

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This study was conducted to evaluate the efficacy of some biopesticides (i.e. aqueous plant extracts and entomopathogenic microorganisms) against the tomato leafminer, *Tuta absoluta* Meyrick, as either an alternative or as integrated with chemical pesticides. The results obtained revealed that the aqueous plant extracts from *Eucalyptus camaldulensis* showed highest mortality (57.78%) against the larval stage, followed by *Cupressus sempervirens* (46.67%) seven days after treatment. Highest mortality was obtained by using 50,000 or 25,000 ppm extracts with no significant difference between the two concentrations. However, the 12.500 ppm concentration produced the lowest mortality rate. On the other hand, the larvae were more susceptible to the tested entomopathogenic microorganism. Nevertheless, the susceptibility and resistance varied in response to the pathogen species utilized in the test. The results obtained indicated that both *Beauveria bassiana* and *Bacillus thuringiensis* achieved maximum mortality rate (92.5 and 85.83 %, respectively) seven days after treatment, and the mortality rate for all bio-agents gradually increased with increasing concentration. The LC_{50} value of the above mentioned bioagents was 682340.58 and 995679.42 conidia/ml, respectively. Both positive and negative controls (Indoxacarb 15% and sterilized distilled water) displayed the highest and lowest mortality rate throughout the study. The identification of the bio-agents species was carried out via a DNA-based test. Various specific primers were designed for species identification with a product size ranging between 164 and 560 bp for further use in future agricultural research.

Keywords: LC_{50}, exposure time, DNA extraction, mortality.

Introduction

The tomato crop (*Lycopersicon esculentum* Mill.) of the Solanaceae family is a nutritious and economically rewarding vegetable farmed all over the world for fresh market and processing. (Jassam, 2017; Iftikhar *et al*., 2021). However, tomato production in Iraq, in general, and especially in the Kurdistan region, is constrained by a variety of factors such as the abundance of insect pests and the scarcity of effective pest management alternatives.

The tomato leafminer (TLM), *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), is a micro lepidopteron nocturnal moth that originated from South America mainly first described in Peru in 1917, where it is regarded as a significant pest threatening tomato crop production, and was found later in the Mediterranean basin (EPPO, 2006; Guenaoui, 2008). However, the leafminer was initially observed in Iraq in 2010, and after that, the pest has been spreading quickly, destroying tomato growing areas either in the open fields or in greenhouses in the whole country (Abdul Razzak *et al*., 2010; Assaf *et al*., 2015).

Tomato leafminer larvae feeds on all tomato above-ground parts which might result in up to 100% crop loss (Desneux *et al*., 2010). Even though the continuous use of synthetic chemical insecticides has been reported to result in the build-up of resistant biotype populations of *T. absoluta*, primarily due to the overall endophytic behavior of the larval instars, which reduced the ability to control these insects. In spite the demand to produce healthy crop free from chemical substances, the use of conventional pesticides is still the primary tool for pest management (Haddi *et al.*, 2012; Khidr, 2018; Lietti *et al*., 2005; Silva *et al*., 2011; Yalçin *et al*., 2015). On the other hand, several natural enemies (predators and parasitoids) were examined against the TLM with promising results (Al-Jboory *et al.*, 2012; Zappala *et al.*, 2013). However, there have been few attempts based on the use of biopesticides for the management of TLM either in the form of plant extracts (eucalyptus and cypress) (Pinto *et al*., 2020; Trabuco de Evert *et al*., 2015), since these species of plants can display insecticidal activities on various pests (Kanat & Alma, 2004, Agha *et al*., 2017) or in the form of entomopathogenic microorganism such as *Beauveria bassiana* (Balsamo) and *Bacillus thuringiensis* (Berliner) (Al-Eisa *et al.*, 2017; Elkichaouim *et al.*, 2016; Saranraj & Jayaprakash, 2018).

To promote environment friendly approaches for the control of tomato leafminer, this study aimed to explore the toxic impact of aqueous plant extracts from cypress (*Cupressus sempervirens*), eucalyptus (*Eucalyptus camaldulensis* Dehnh), and barabin (*Portulaca oleracea)* and the commercial formulations of microorganism *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metsch), *Verticillium lecanii* (Zimmerman), *Paecilomyces ilacinus* (Thoms.) and *Bacillus thuringiensis* (Berliner) as effective biocontrol agents against the most destructive pest of tomato. The mortality of larval stage and susceptibility of tomato leafminers to various concentrations of biopesticides were tested based on LC_{50} values and designing specific primers for species identification which will facilitate future research in this area.

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Materials and Methods

Insect cultures

This study was conducted in 2021 in laboratories of Agricultural Engineering Science College, Salahaddin University, Iraq and Central Forest Nursery, Erbil, Iraq. *Tuta absoluta* larvae were collected from mined tomato plants of Golden variety cultivated in a greenhouse at a temperature of 23±4˚C and relative humidity of 60±10%. The plants were artificially infested with the insect to have a continuous culture, and fresh leaflets were collected daily.

Design and preparation of primers

The Primer 3 Website (Rozen & Skaletsky, 2000) was used to design species-specific primer pairs for tomato leafminers (Table 1). The volume indicated in the preceding material was completed by adding molecular biology grade water to prepare a stock solution of 100 pmol/µl. Following vortexing and spinning, the tubes were kept on ice for 30 minutes to generate a 10x primer stock; 1 µl of the stock was combined with 9 µl SDW for the forward and reverse primers, which were placed in a -20ºC freezer until used for DNA extraction.

DNA extraction

A sample size of 15 larvae was used for the test and then separated into three pooled samples consisting of five insects from three Erbil locations [Choman (Eastern) Y= 36.3521 X=44.5906, Shamamik (Southern) Y= 36.0107 X= 43.5423, Kawrgosik (Western) =36.1757 X= 43.4740] were collected. Samples were immersed in liquid nitrogen and then crushed into a fine powder using a pre-cooled sterilized mortar and pestle. Genomic DNA was isolated from the insects in the three locations, and samples were extracted by using Beta Bayern tissue DNA preparation kit (Beta Bayern GmbH, 90453 Bayern, Germany) and eluted/resuspended into 25 µl of Tris-EDTA buffer in the final step, then stored in the freezer at -20ºC until use.

Polymerase chain reaction (PCR)

DNA was amplified using PCR machine (Bioresearch PTC-200 Gradient thermocycler.) in 25µl reaction mixture composed of 12.5 µl of PCR master mix (Ampilqon A/S Stenhuggervej 22, Denmark), 8.5 µl of DNase and RNase free water (Bioneer), 2 µl of DNA template (20 ng/µl) and 1 µl of both forward and reverse primers (10 pmol). The reaction mixture for the negative control included all above-mentioned components except DNA template. PCR profiles were 5 min at 95˚C (initial denaturation), followed by 35 cycles of 40 sec at 95˚C (denaturation), 40 sec 58˚C (annealing) and 72˚C for 1 min (extension) and the last step was set at 72˚C for 5 min (final extension).

Agarose gel electrophoresis

The 2 % agarose gel (Molecular Grad, Bioline) was prepared in 1x TBE (Tris- Borate- EDTA) buffer, and was placed in the electrophoresis chamber. Samples to be loaded on the gel were mixed with 6x gel loading blue buffer (Promega) in a 1:1 ratio (one part sample to one part loading buffer). Consequently, 5 µl of DNA extracted from each sample was mixed with 5 µl of loading buffer. In addition, the appropriate size marker (3k bp DNA ladder; New England Biolabs, Ipswich, MA, USA), was loaded in the first lane. In response to the PCR products, a similar procedure has been conducted. The gel ran at 100 volts for approximately 1 hour. After electrophoresis, results were visualized and photographed under UV light (UV Transilluminator Biostep-UST-20M-8K).

Preparation of aqueous extracts

Fresh leaves of Mediterranean cypress, *Cupressus sempervirens* (C.s), eucalyptus, *Eucalyptus camaldulensis* (E.c) and barabin, *Portulaca oleracea* (P.o) were collected from Erbil Central Forest Nursery in the Hawler region, washed carefully with water, air dried in the shade, and ground into a fine powder using an electric herb grinder (Germany). To prepare a 10% stock of the aqueous plant extracts, 100 g dry powder from each plant was mixed with 1000 ml distilled water and then placed in the shaker overnight and then filtered with muslin cloth and stored in the refrigerator (Abdulhay, 2012). Serial dilutions (5, 2.5 and 1.25%) of the stock solution were prepared which correspond to 50,000, 25,000 and 12,500 ppm.

Bioactivity of the extracts

A sample size of 330 individuals was used for the experiment. The toxicity of the three aforementioned aqueous extracts was tested on the mature larval stage (3rd and 4th instars). A Petri dish contains fresh tomato leaves and lined inside with filter paper to maintain leaves freshness and adequate required moisture. Replicates, each consisting of 10 insects, were sprayed with 50,000, 25,000 and 12,500 ppm using a hand sprayer, and then covered with a muslin cloth. All treatments were kept at room temperature of 25±2 ˚C. A synthetic chemical insecticide, Indoxacarb 15% (0.3 ml/L) at 150 ppm concentration and sterilized distilled water were used as positive (PC) and negative control (NC) and kept under the same conditions. Larval mortality was recorded daily for seven consecutive days after treatment. Insects were considered dead when they changed in color, became sluggish and were not able to move back to the ventral position after being placed on their back.

Table 1. Primer pairs used to confirm the identity of tomato leafminer, *Tuta absoluta.*

Name	(Ps)bp	Forward primer 5′-3′	Length	Reverse primer 5′-3′	Length	Reference
T abs1	560	TTAGCCGGAATACCTCGTCG	20	AAACGAAGAGAGGGGAGAGC	20	This study
T abs2	401	CGAGCCTATTTCACTTCAGCA	21	CCGGCTAATCCTAAGAAATGTTG	23	This study
T abs3	221	GGAATTTGAGCAGGAATAGTAGG	23	CGTGGGAAGGCTATATCAGG	20	This study
T ab4	218	CGATGTCTACGACGTTTTCG	20	TGTCGATGTTCAAATGTGTCC	21	This study
T abs5	168	AGCCCCTGATATAGCCTTCC	20	TCTACTGAACTACCTCCATGAGC	23	This study
T abs6	164	CGAGGTATTCCGGCTAATCC	20	GGAGCTGTATTTGCTATTTTAGGG	24	This study

Preparation of microorganism formulation

The commercial entomopathogenic fungi formulation (EPF) (Rajan Labs, India) containing 1.87×10^8 conidia per gram powder and entomopathogenic bacteria (EPB) 1.87×10^8 CFU were used and compared with the chemical insecticide Indoxacarb 15% SC (The Scientific fertilizers CO. India). Both biopesticides EPF and EPB were grown on potato dextrose agar (PDA) and nutrient agar (NA) media, respectively, and incubated at 25±2°C for seven days to confirm their viability (Youssef, 2015). The fungal microorganisms were *Beauveria bassiana* (B.b), *Metarhizium anisopliae* (M.a) and *Verticillium lecanii*, and the bacterial species was *Bacillus thuringiensis* (B.t). Stock solution (0.748×10^7 spore/ml) of each EPF was prepared by mixing 1 g of the commercial powder with 25 ml distilled water containing 0.1% of Tween-80. The stock solution was diluted to obtain concentrations of 0.748×10^6, 0.748×10^5 and 0.748×10^4 spore/ml. Indoxacarb 15% (0.3 ml/L) and sterilized distilled water (0 spore/ml) were used as positive and negative controls, respectively.

Assessment of larvicidal effect under laboratory conditions

A total sample size of 540 mature larval stages of *T. absoluta* treated with the tested microorganism was carried out by the spray-bioassay method. Three replicates, each with ten (either 3rd or 4th) instars, were gently placed on filter papers inside a Petri dish that contained a fresh tomato leaf for feeding. Three filter paper discs were utilized to absorb excess suspension and preserve the freshness of tomato leaves while they were exposed to different concentrations (Migiro *et al.*, 2010). Furthermore, the treatments were sprayed with water whenever required to prevent the leaflet's desiccation and maintain sufficient moisture for the microorganism. Larval mortality was recorded one week after treatment with the microorganism suspension. To allow fungal overgrowth on the corpses of the dead larvae, they were kept in the Petri plates for an additional few days, so the cause of death could be further confirmed.

Statistical analysis

Analysis of variance was used to check the significance of differences between treatments. The data was analyzed using multi-factor analysis of variance (ANOVA) in Statgraphics Centurion XV followed by Fischer's least significant difference (LSD) at P=0.05. Abbott's algorithm (Abbott, 1925) was used to adjust mortality data to determine lethal concentration that causes 50% mortality (LC50) values. Mortality data were subjected to the maximum likelihood program of Probit analysis using SPSS software version 20. The efficacy of several bio-insecticides was evaluated as covariables while the larval mortality and exposure time as response and explanatory variables, respectively.

Results

Insect identification by PCR

The six primer pairs used had optimal annealing temperatures close to 58°C, allowing simultaneous amplification in the same thermoblock, and the specificity of the primers for *T. absoluta* was 100 % via the NCBI-BLAST after the gel electrophoresis results were visualized.

The primer pairs used amplified partial genes were designed for tomato leafminer (TLM) identification have yielded band sizes in the range 164-560 bp, and the PCR products were electrophoresed and visualized on 2% agarose gel using UV light (Figure 1).

Efficacy of plant extracts for the control of tomato leafminer

The laboratory test conducted using several aqueous plant extracts exhibited a significant effect on the mortality of TLM larval stage ($F_{(4,314)}$ =2478.48, P <0.001). Eucalyptus extract was found to be the most toxic of the three plant extracts, resulting in a 31.59% mortality rate, followed by Cypress (27.46 %). However, all types of biopesticides were significantly different from the chemical pesticide, which attained the highest mortality rate of 81.90% (Figure 2A). Larval mortality was also impacted by plant extract concentration ($F_{4,314}$ =116.07, P <0.001). The highest dose 50.000 ppm, with a 37.14 % mortality rate, was not significantly different from the effect of 25,000 ppm which gave a 35.71 % mortality rate, and the concentration of 12.500 ppm attained the lowest mortality rate (Figure 2-B). Mortality rate was also significantly affected by the exposure time of the larvae to the aqueous plant extracts ($F_{6,314}$ = 456.88., P <0.001, Figure 2-C). Thus, the highest mortality rate was observed 7 days after treatment and the lowest was following the first day of exposure.

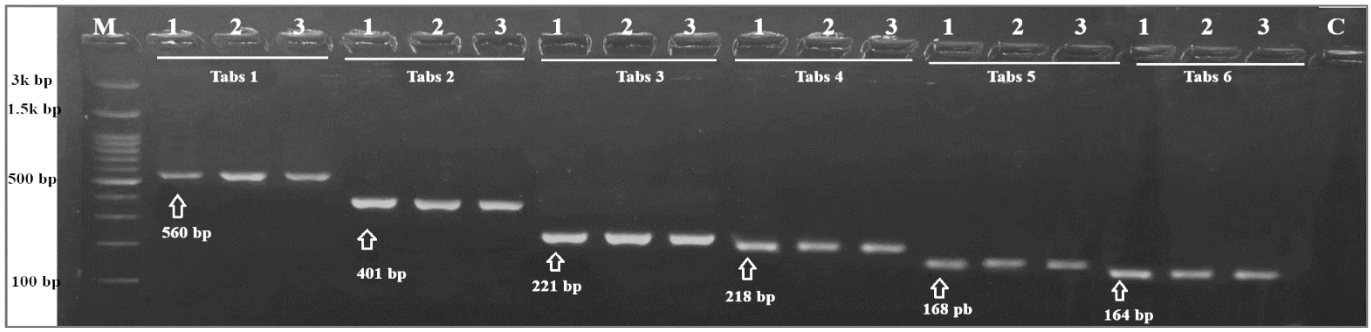

Figure 1. PCR amplification of partial genes from pooled larval samples collected from three directions of Erbil. The first lane includes molecular markers ladder (0.1-3k bp) and the second to nineteenth lanes are amplicons with size between 164 bp and 560 bp. The last lane C includes a negative control (primer pairs name correspond to those in Table 1).

In addition, insect survival was significantly influenced by both the treatment type and the time larvae were exposed to the aqueous plant extracts ($F_{24.314}$ =36.07., P <0.001). The highest mortality rate (57.77 %, Figure 3-A) was recorded for the Eucalyptus extract seven days after treatment, which was significantly different from the negative and positive controls (10 and 100 %), respectively.

Likewise, *T. absoluta* mortality rate was influenced by the interaction between the concentration and treatment type $F_{8.314}$ =18.72., P <0.001, Figure 3-B) as well as between the concentration and exposure time ($F_{12.314}$ =7.34., P <0.001). Thus, maximum mortality rate was obtained via the use of highest concentration with maximum exposure time (Figure, 3-C).

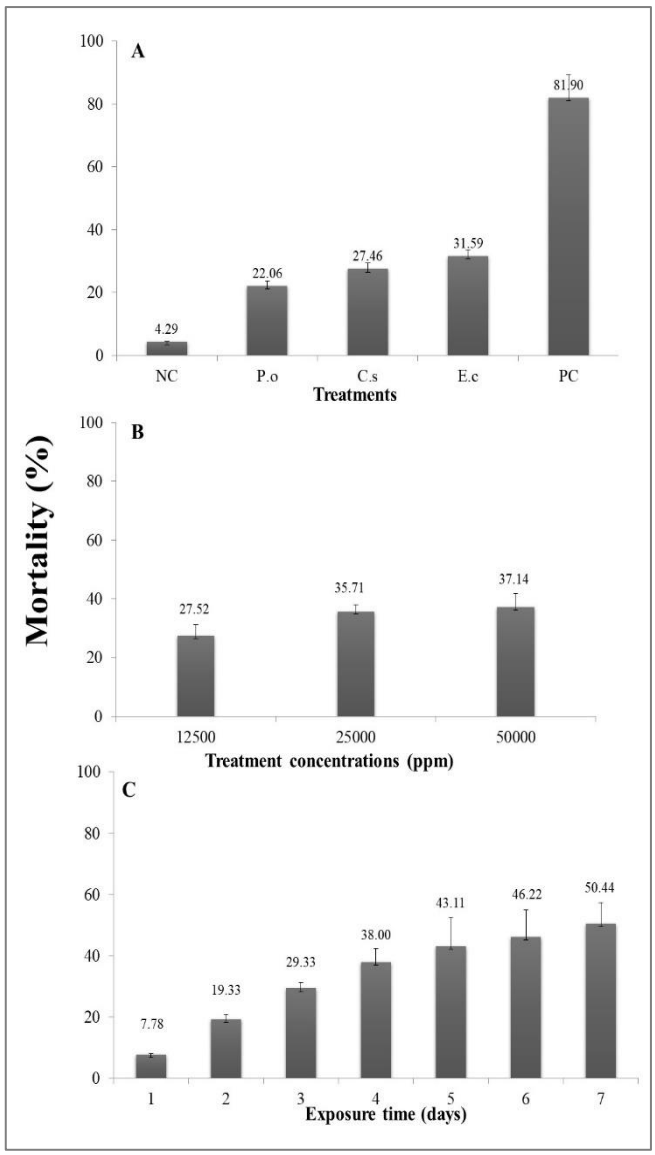

Figure 2. Mortality rate of *T. absoluta* larvae inside Petri dish based on (A) treatment type, (B) extract concentrations and (C) exposure time.

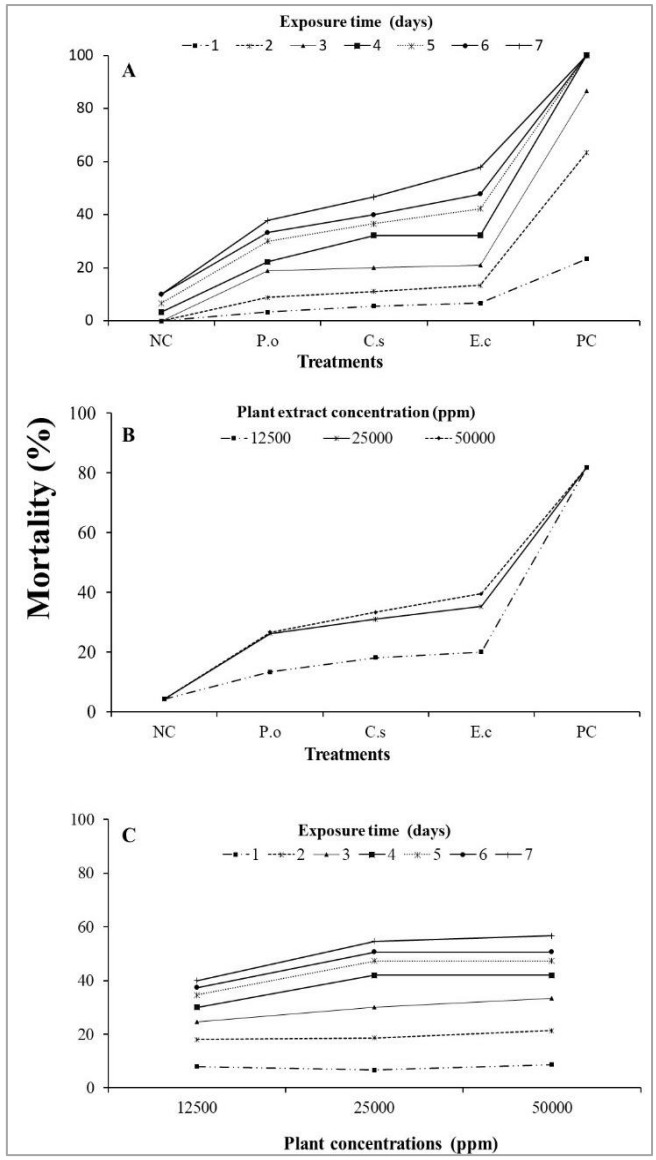

Figure 3. Mortality rate of *T. absoluta* in relation to interaction term between (A) treatment types and exposure times (B) treatment types and plant extract concentrations (C) plant extract concentration and exposure times

Insect species examined based on LC_{50} value

The LC_{50} values of different aqueous plant extracts examined on *T. absoluta* revealed variation in accordance to not only the kind of plant but also a constant decrease in the value observed whenever the larvae were exposed for a more extended time to the biopesticides. For instance, the LC_{50} values of the treated larvae with Eucalyptus extract for 1 and 7 days were (185626.31 & 52897.371) ppm, respectively. On the other hand, the larvae were more tolerant to barabin extract, which had the least insecticidal efficacy in comparison with the other biopesticides over the same period and consequently required higher doses to induce 50 percent mortality (Table 2).

Table 2. The LC_{50} values (ppm) using various aqueous plant extracts on tomato leafminer larvae following different exposure times.

Time (days)	LC_{50} (ppm)	Average
Eucalyptus camaldulensis		
1	185626.37	111332.7
2	155466.62	
3	131455.19	
4	102738.07	
5	81441.93	
6	69703.08	
7	52897.37	
Cupressus sempervirens		
1	214461.64	131248.4
2	181307.58	
3	147745.89	
4	111614.93	
5	98726.60	
6	89092.19	
7	75789.97	
Portulaca oleracea		
1	259429.07	428356.4
2	2096929.66	
3	163360.17	
4	147476.72	
5	121723.16	
6	110081.68	
7	99494.24	

Efficacy of entomopathogenic microorganism for the control of *Tuta absoluta*

The use of different entomopathogenic microorganisms under controlled environmental conditions produced a significant impact on the mortality rate of *T. absoluta larvae* ($F_{5.503)}$ =2095.08., P <0.001). Tomato leafminers treated with *Beauveria bassiana* achieved the highest mortality rate (56.79%), followed by the use of *Bacillus thuringiensis* (54.28%), without a significant difference between the two treatments, as compared to the positive and negative controls (Indoxacarb and sterilized distilled water (84.29 and 3.33% mortality), respectively (Figure 4-A).

Furthermore, the TLM susceptibility varied with the bio-agent concentration used ($F_{3.503}$ =43.52, P <0.001). The concentrations of 0.748×10^7 and 0.748×10^4 spore/ml produced the highest and lowest larval mortality rate of 49.84 and 42.86 %, respectively, regardless of the bio-agent used (Figure 4-B). The survivorship of the insect survival was significantly influenced by whether the larvae were subjected to the treatment for a short period (24-48 hours) or an extended period (144-168 hours) ($F_{6.503}$ =1496.07, P< 0.001, Figure 4-C).

The toxicity of various entomopathogenic microorganisms (fungi or bacteria) was tested on the larval stage of the tomato leafminer inside a Petri dish. Both *B. bassiana* and *B. thuringiensis* exhibited higher insecticidal toxicity than the other bioagents tested (Table 4).

Additionally, there was a strong correlation between the length of exposure and LC_{50} values, and thus, minimum values (682340.58, 995679.42, 1456327,32 and 1912867.45 conidia/ml) were attained 7 days treatment with *B. bassiana*, *B. thuringiensis*, *M. anisopliae and V. lecanii*, respectively (Table 3).

Likewise, the interaction between entomopathogenic agents and exposure time was also significant ($F_{30.503}$ = 74.6, P <0.001). Consequently, maximum insect mortality rate (92.5 & 85.83, Figure 5-A) was reached by using both biopesticides (Bb and Bt), respectively, seven days after treatment. Moreover, interaction of treatment type and extract concentration was also significant ($F_{30.503}$ = 6.77, P <0.001). Nonetheless, the aqueous extract concentration was not affected by how many days the larvae were exposed to the biological agents ($F_{18.503}$ = 0.8, P= 0.70, Figure 5-B).

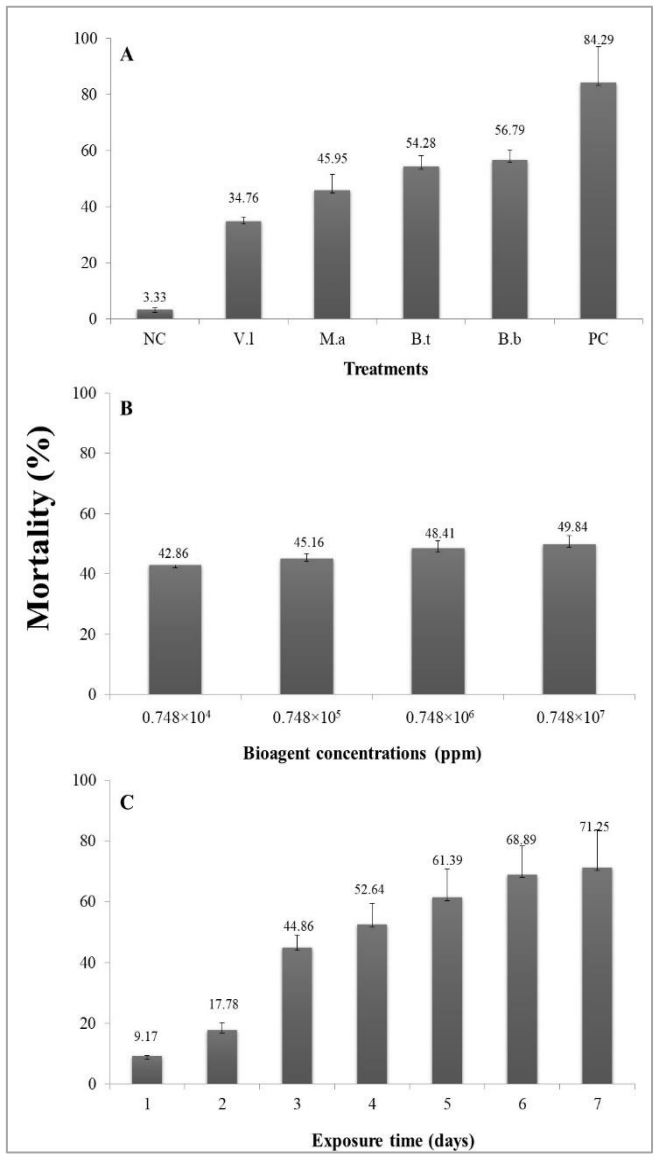

Figure 4. Mortality rate of *T. absoluta* in response to (A) treatment types (B) spore concentrations (C) exposure time.

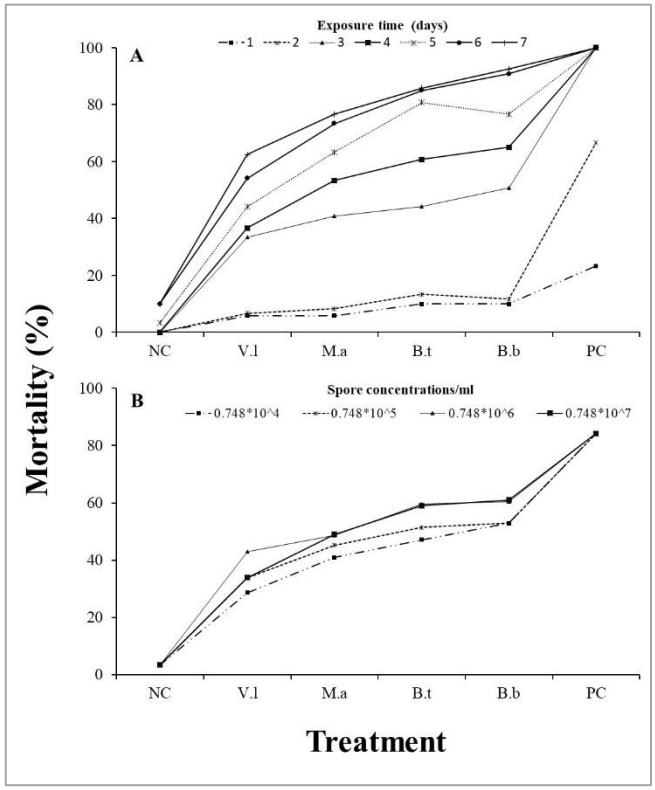

Figure 5. Mortality rate of *T. absoluta* larvae in response to interaction between (A) treatment types and exposure times, and (B) treatment types and spore concentrations.

Discussion

The species-specific PCR approach proved effective in distinguishing *Tuta absoluta* species in Iraq via designing six specific primers from mitochondrial cytochrome c oxidase 1 with amplified product size ranging between 164 and 560 bp, which subsequently can be utilized in DNA sequencing, evolutionary ecology as well as in applied agricultural research. The species-specific primers mt-COI have been utilized successfully earlier for species verification and taxonomical studies (Hebert *et al.*, 2003; Khidr *et al.*, 2017). The findings that *T. absoluta* displayed susceptibility towards some bio-agents while showing resistance to other types would be of value in establishing the use of biopesticides against tomato leafminers to reduce the further spread of this potential pest. The variation in the level of susceptibility is not only due to the type of entomopathogenic microorganism used but also to the kind of toxin released by the pathogenic species (González-Cabrera *et al*., 2011; Youssef, 2015). Likewise, the plant extract types, mode of action, route of exposure and secondary plant components can influence the ability to disrupt essential metabolic pathways and hence can contribute to the toxicity level (Kumrungsee *et al.*, 2014; Radulovic *et al.*, 2013). In addition, the larval stage and the type of the target insect species can play a significant role in the vulnerability to the biological agents, whether plant extracts or microbial pathogens were used (Campolo *et al.*, 2017; González-Cabrera *et al.*, 2011; Shiberu & Getu, 2018).

Table 3. The LC_{50} values (conidia/ml) for the adults of *T. absoluta* for using different bioagents after exposure to different periods of time.

Time (days)	LC_{50} (conidia/ml)	Average
B. bassiana		
1	16382793.44	6077462.2
2	15418237.02	
3	4052907.63	
4	3728594.88	
5	1295021.212	
6	982340.58	
7	682340.58	
B. thuringiensis		
1	16203367.79	6331213.1
2	14488894.78	
3	5470051.62	
4	4917639.97	
5	1208171.49	
6	1034686.66	
7	995679.42	
M. anisopliae		
1	22394093.18	8933055.7
2	19911917.00	
3	7394187.83	
4	5739755.99	
5	3701002.77	
6	1934105.68	
7	1456327.32	
V. lecanii		
1	22462605.02	9898575.9
2	20087429.85	
3	7908120.46	
4	7543555.23	
5	6039302.50	
6	3336150.75	
7	1912867.45	

Results of this study indicated that *B. bassiana* was the most effective species against TLM compared with other entomopathogenic fungi used in this study, and thus can be deployed as an efficient biocontrol agent for the management of TLM. This study's findings were in agreement with earlier reported research (Shalaby *et al*., 2013; Youssef, 2015). The effectiveness of the fungal entomopathogens utilized in this study is likely due to the activity of several enzymes (such as chitinases) that are essential in the breakdown of the insect integuments and improve conidial adhesion to the host for successful penetration across the host hemocoel that eventually led to the insect death (Feng *et al*., 1994; Roy *et al*., 2009). Likewise, the effectiveness of entomopathogenic bacteria, *B. thuringensis* might reflect on the type of proteinaceous protoxins that are found in the crystalline structure of the bacterial cell that is highly specific to the insect gut and thus results in the quick death of the target insect species (Roh *et al*., 2007). At the same time, the Cry1 endotoxin binding site might play a significant role in the susceptibility of various lepidopteran larval stages, since a positive correlation was found between toxicity and

availability of binding sites (Gilliland *et al*., 2002; Lee *et al*., 1999; Rajamohan *et al*.,1996).

Furthermore, the results obtained indicated that the mortality rate was dose-dependent and increased gradually with increasing concentrations, which is in agreement with earlier reports (García *et al*., 2011; Giustolin *et al*., 2001; Tadele & Emana, 2017). In addition, the results obtained revealed that the entomopathogenic fungi had no significant influence on the larvae of *T. absoluta*. until three days after exposure which is in agreement with earlier findings (Shiberu & Getu, 2018). Additionally, among the plants used in the experiment, the extract of eucalyptus species appeared to be the most toxic, which is in agreement with an earlier report (Pinto *et al.*, 2020).

Plant-derived active substances have played a significant role in sustainable pest control; in particular, the plant's secondary metabolites which enhance resistance to herbivores and pathogen attacks (Pagare *et al.*, 2015). Moreover, the main constituents of volatile eucalyptus components (i.e. terpenes, benzene derivatives and hydrocarbons) have antimicrobial and anti-insect activity against various diseases and pests (Batish *et al*., 2008, Marzoug *et al*., 2011). It could be concluded from this study that it is possible to design control programs based on either biological control alone or combined with other control techniques to reduce the use of chemical pesticides. Hence, further studies under field or greenhouse conditions are needed to confirm the importance of these findings at a large scale. Besides, in order to achieve more accurate identification, molecular-based methods are seen as a crucial complementary method to conventional methods, and designing various primers for improved identification of agricultural pests is highly recommended.

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الملخص

خضر، ساهاند وسارة صالح عبد الله. 2023. التحديد الجزيئي لحافرة البندورة/الطماطم (*Tuta absoluta*) ومكافحتها باستخدام بعض المستخلصات النباتية المائية والعوامل الحيوية الميكروبية في ظروف المختبر. مجلة وقاية النبات العربية، 41(4): 427–436.
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أجريت هذه الدراسة لتقييم فاعلية بعض المبيدات الحيوية (مستخلصات نباتية مائية وكائنات ممرضة للحشرات) ضدّ حشرة حافرة البندورة/الطماطم (*Tuta absoluta* Meyrick) كبديل أو رديف للمبيدات الكيميائية. أظهرت نتائج التجارب المختبرية أن مستخلصات نبتة اليوكالبتوس (*Eucalyptus camaldulensis*) حققت أعلى سمية قاتلة، وسجلت نسبة موت بحدود 57.78% ليرقات الحشرة، وتلاها مستخلص نبتة السرو العمودي (*Cupressus sempervirens*) بنسبة 46.67% وذلك بعد سبعة أيام من التعرض لهما. كانت فاعلية كلا المستخلصين النباتيين كمبيدات حشرية متساوية احصائياً عند استخدامهما بالتركيزين 5000 و 25000 جزء بالمليون وسجلتا أعلى نسبة موت، أما عند استخدامهما بنسبة 12500 جزء بالمليون فقد سجلت أقل نسبة موت في مجتمع الحشرة. من جهة أخرى، كانت اليرقات أكثر تأثراً بالكائنات الممرضة للحشرات المختبرة. ومع ذلك إختلفت نسب التأثر والمقاومة بحسب أنواع الكائنات الدقيقة الممرضة المستخدمة في التجربة، فقد أشارت النتائج إلى أنّ كلاً من الفطر *Beauveria bassiana* والبكتيريا *Bacillus thuringiensis* أظهرا أقصى فاعلية قاتلة بنسب تقدر بـ 92.5 و 85.83%، على التوالي، بعد سبعة أيام من التعرض لهما، كما ازدادت النسبة السمية القاتلة لجميع أنواع العوامل الحيوية مع زيادة التركيز. وكانت قيمة التركيز النصفي القاتل (LC_{50}) للعوامل الحيوية سابقة الذكر 68.23×10^4 و 99.57×10^4 بوغ/مل، على التوالي، خلال اليوم الأخير للتعرض. أظهر المبيد indoxacarb 15% كفاءةً عالية مقارنة بالشاهد (الماء المقطر) الذي لم يكن له تأثيرٌ على الحشرة . تم تأكيد تشخيص نوع الحشرة باستخدام تقنية فحص الحمض النووي، حيث تم تصميم بادئات مختلفة محددة للأنواع المذكورة أعلاه وبحجم منتج يتراوح ما بين 164–560 زوج قاعدي ليتم استخدامها في البحوث الزراعية التطبيقية.

كلمات مفتاحية: التركيز النصفي القاتل، مدة التعرض، استخلاص الحمض النووي DNA، الجرعة القاتلة النصفية.

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