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Evaluation of some microbial agents, natural and chemical compounds for controlling tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract: Solanaceous plants have a great economic impact in Egypt. These groups of plants include potatoes, tomatoes and eggplants. The new invasive pest of tomatoes, *Tuta absoluta* (Meyrick) causes the greatest crop losses which can range from 60 to 100%. After its detection in Egypt during the last half of 2009, it spread quickly to all provinces in the country. We aiming to propose a sustainable control program for this devastating pest. In this research we tested three groups of control agents. The first was microbial and natural, the second – plant extracts and the third – chemical insecticides. Our results showed that the impact of *T. absoluta* can be greatly reduced by the use of sustainable control measures represented by different insecticide groups. Bioassay experiments showed that this devastating pest can be controlled with some compounds that give high mortality rates. Of these compounds, spinosad and *Beauveria bassiana*, microbial control agents, followed by azadirachtin, gave the best results in controlling *T. absoluta*. Of the chemical insecticides, lambda-cyhalotrin was the most effective, followed by lufenuron and profenofos. In conclusion we encourage farmers to use microbial and natural control measures in combating the tomato leafminer, *T. absoluta,* in Integrated Pest Mangement (IPM) programs.

Key words: biopesticides, IPM programs, natural insecticides, tomato leaf miner

Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is considered to be one of the most serious pests of tomatoes all over the world. The female adults lay their eggs on different parts of the plant and even on the tomato fruits themselves when there is heavy infestation (Eppo 2005). The damage to tomato crops comes from hatched larvae feeding on the vegetative parts of the tomato plant, causing delayed plant growth. In severe infestation the larvae can attack the tomato fruits (Caceres 1992; Cely *et al.* 2006). In some countries such as Spain and Brazil, tomato crop losses due to infestation by this pest has reached up to 90% (Korycinska and Moram 2009). This pest has 10–12 generations per year (Mahmoud *et al.* 2015).

The control strategies of this pest are mainly based on using chemical insecticides. The crops are sprayed several times during the plantation period, leading to resistance to these chemical insecticides in many countries (Siqueira *et al*. 2000; Torres *et al*. 2002). Additionally, these chemical insecticides cause adverse environmental effects including water pollution, eradication of beneficial wildlife and human health problems (Abd El-Ghany *et al.* 2016). In this research, three groups of control agents were evaluated. The first group included different biopesticides such as, *Bacillus thuringiensis* (Bt), *Beauveria bassiana, Heterorhabditis bacteriophora,* and spinosad**.** The second group included natural plant extracts, garlic, neem and green miracle. The third group included trade formulations of the most popular chemical insecticides used against this pest i.e. lufenuron, profenofos, lambda-cyhalotrin, cyfluthrin.

The aim of this research was the selection of the most effective and safest group that could be used in controlling this pest alone or even in Integrated Pest Management (IPM) programs as a safe and ecofriendly alternative to the hazards of chemical insecticides.

Materials and Methods

Insects

Tomato plants were grown in a greenhouse at National Research Centre in pots (20 cm diam.) with fertilized soil. They were watered three times a week. These studies were done in the Laboratory of Microbial Control, National Research Centre, Egypt. The colonies of *T. absoluta* had been reared with tomato leaves under laboratory

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conditions [25±1°C; 60±5% relative humidity (RH), photoperiod: 14 : 10 h (L : D)].

Compounds evaluated

In these studies three different groups (microbial, natural plant extracts and chemical) of insecticides were evaluated against *T. absoluta* larvae inside and outside the mines. The first group included nine different biopesticides (Bt subsp. *kurstaki*, Bt subsp. *entomocidus*, combination of Bt subsp. *kurstaki* + *entomocidus*, Bt subsp. *aizawai*, spinosad, *B. bassiana, H. bacteriophora*, azadirachtin and M-Pede. Three concentrations of each of the mentioned agents were used. For Bt preparations, 0.02, 0.01, and 0.005 g · l^{-1} were used. Spinosad concentrations were 0.6, 0.3 and 0.15 cm^3. Concentrations of 5 × 10^5, 2.5 × 10^5 and 1.25 × 10^5 spore · m^{-2} were prepared for *B. bassiana* and 5 × 10^5, 2.5 × 10^5 and 1.25 × 10^5 IJ · m^{-2} for *H. bacteriophora.* For azadirachtin concentrations of 1.0, 0.5 and 0.25 ml · · l^{-1} were used. M-Pede concentrations were 3.65, 1.88 and 0.94 ml · l^{-1}. The second group included combinations of water plant extracts from garlic + neem + green miracle (1 : : 2 : 1) and from garlic + basil (2 : 1). The concentrations of the first combination were, 1.0, 0.1, 0.05 ml · l^{-1} followed by 3.0, 1.5, 0.75 ml · l^{-1} for the second combination of garlic + basil water extracts. The third one included trade formulations of the most popular chemical insecticides used against this pest i.e. lufenuron (1.0, 0.75, 0.5 ml · l^{-1}), profenofos (4.0, 3.0, 2.0 ml · l^{-1}), lambda-cyhalothrin (2.5, 1.25, 0.63 ml · l^{-1}) and cyfluthrin (0.5, 0.25, 0.125 ml · l^{-1}).

Biopesticides

1) *Bacillus thuringiensis* subsp. *kurstaki* [DiPel® 2X DF, wettable powder containing the HD-1 isolate with 32,000 international units (IU) · mg^{-1}, Nufarm];
2) *Bacillus thuringiensis* subsp. *entomocidus* (an isolate HD-635 obtained from H.D. Dulmage collection in Egypt);
3) *Bacillus thuringiensis* subsp. *kurstaki* + *entomocidus*;
4) *Bacillus thuringiensis* subsp. *aizawai* (2014-XEN-0001 XenTari, form 04-7782-R9, CA & NY from Valent BioSciences Corporation);
5) spinosad (Tracer®, suspension concentrate, 480 g · l^{-1}, Dow AgroSciences, Franco da Rocha, SP, Brazil);
6) *Beauveria bassiana* (Bio-Power, 1 × 10^9 cfu · ml^{-1}, T-Stanes & Company Limited);
7) *Heterorhabditis bacteriophora* (an entomopathogenic Egyptian nematode isolate);
8) azadirachtin (Nimbecidine® EC, cold pressed, 0.03%, T-Stanes & Company Limited);
9) M-Pede (M-Pede® Insecticide Miticide Fungicide, potassium salts of fatty acids, 49%, Gowan Company, USA).

Natural compounds

Water extracts of crop products from these plants were prepared:

1) garlic + neem + green miracle;
2) garlic + basil.

Chemical insecticides

1) lufenuron (Match®, emulsifiable concentrate, 200 g · l^{-1}, Syngenta Crop Protection Limited);
2) profenofos (Celeron®, emulsifiable concentrate, Excel Crop Core Ltd., Maharashtra);
3) lambda-cyhalothrin (Lambda Select™, synthetic pyrethroid, emulsifiable concentrate, 13.0%, Syngenta Group Company);
4) cyfluthrin (Bulldock® 25 EC, synthetic pyrethroid, emulsifiable concentrate, beta-cyfluthrin 25 g · l^{-1}, Bayer, Germany).

Bioassay studies

Fresh tomato leaves from greenhouse plants were sprayed with three concentrations of each of the previously mentioned agents from the three groups. The leaves were left for a few minutes to dry, and then introduced to the third instar larvae of *T. absoluta*. Control groups were sprayed with water alone.

Three independent replicates for each bioassay (10 larvae/replicate) were done. After treatments, the larvae were placed in 15 cm^3 glass Petri dishes in a controlled environment room at 25±1°C, 60–70% relative humidity (RH) with a photoperiod of 14 : 10 h (L : D). Mortality was recorded 24 h after treatment under a stereoscopic microscope (10×). Larvae were considered as dead when they were not able to move back to the ventral position after being placed on their dorsum.

Statistical analysis

Mortality percentages were reported and corrected according to Abbott (1925). The median lethal concentrations LC_{50} of each tested compound were computed through probit analysis within 95% confidence limits using the Propan program.

Results

The data in Figure 1 show that larval mortality percentages of all tested bioagents (microbial and natural) were higher outside the mines than inside them, where the larval mortality percentages of spinosad ranged from 84 to 92% inside the mines in comparison to approximately 80 to 100% outside the mines at concentrations of 0.15, 0.3, and 0.6 ml · l^{-1}. Entomopathogenic fungus, *B. bassiana*, was second. Its larval mortality ranged from 20 to 60% inside the mines and 33 to 83% outside the mines. Larval mortality of the entomopathogenic nematode, *H. bacteriophora*, was similar and had approximately the same percentages inside the mines, while outside the mines the larval mortality percent ranged from 47 to 53% at the two highest concentrations only. On the other hand, larval mortality percentages did not exceed 60% either inside or outside the mines with all the tested strains of *B. thuringiensis*. Moreover, the very weak mortality effect of different strains of *Bt* was more pronounced inside the mines than outside where it reached zero percent inside the mines with *B. thuringiensis* subsp. *aizawai* while the larval mortality percentages of the other three tested Bt strains did not exceed 50% inside the mines. This percent was recorded with the combination of two strains of *B. thuringiensis* subsp. *kurstaki* + subsp. *entomocidus*, at the higher concentration of 0.02 mg · l^{-1}. A higher mortality percent was obtained with M-Pede® (100% mortality) at its higher concentration outside the mines. On the other hand, azadirachtin gave the highest larval mortality percent (92%) of all tested natural compounds inside the mines in comparison to 70% mortality with M-Pede. In general, both azadirachtin and M-Pede were the most effective natural compounds against the larval stage of *T. absoluta* both inside and outside the treated mines.

Moreover, the LC$_{50}$ for the mentioned microbial agents inside and outside the mines were calculated (Table 1). The data of LC$_{50}$ for different *Bt* strains indicate that, the conjugated effects of both *B. thuringiensis* subsp. *kurstaki* + subsp. *entomocidus* gave the lowest LC$_{50}$ value compared with individual strains. Furthermore, there were significant differences inside and outside mines between each

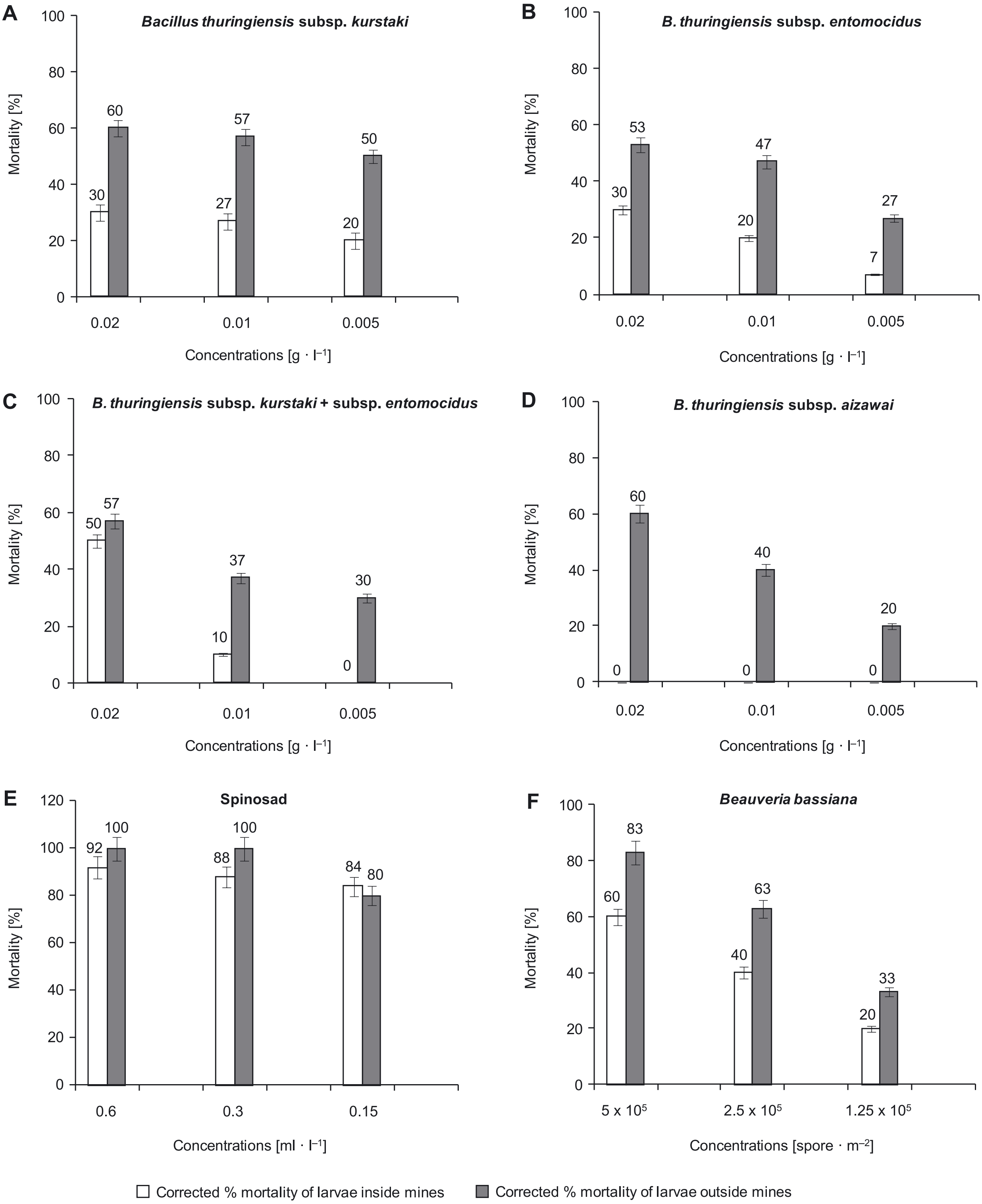

Fig. 1. Effects of different microbial control agents (A–I) against tomato leaf miner, *Tuta absoluta*

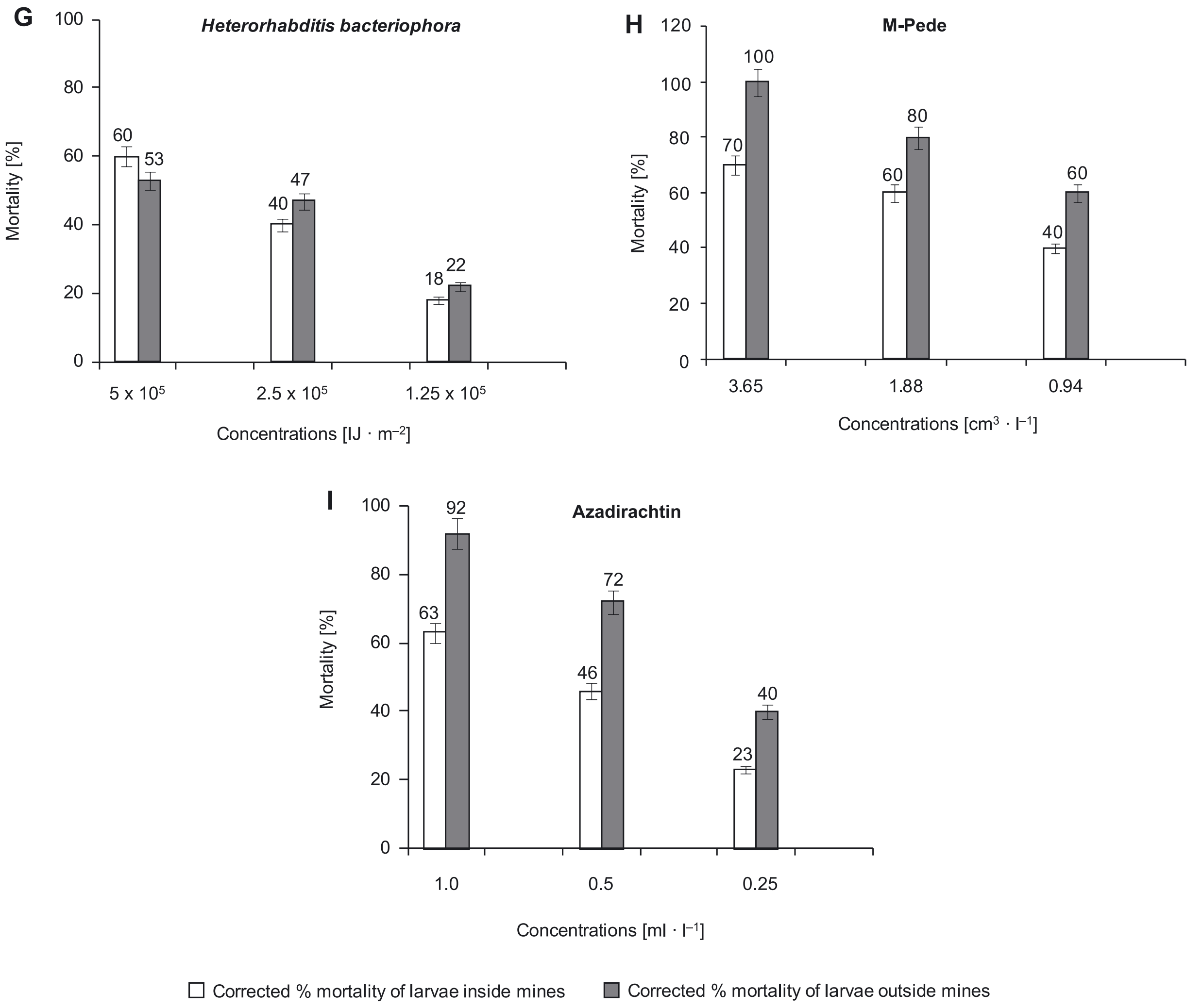

Fig. 1. Effects of different microbial control agents (A–I) against tomato leaf miner, *Tuta absoluta – continuation*

Table 1. LC_{50} inside and outside mines at confidence limits (95%) of different microbial, natural and chemical control agents against tomato leaf miner, *Tuta absoluta*

Compound	LC_{50} inside mines	Slope±SE	LC_{50} outside mines	Slope±SE
Bacillus thuringiensis subsp. *kurstaki*	0.186 g · l^{-1}	1.02±0.98	0.005 g · l^{-1}	0.86±0.48
B. thuringiensis subsp. *entomocidus*	0.041 g · l^{-1}	1.72±1.05	0.0.015 g · l^{-1}	0.67±0.76
B. thuringiensis subsp. *kurstaki* + *entomocidus*	0.019 g · l^{-1}	0.97±0.86	0.012 g · l^{-1}	1.17±1.28
B. thuringiensis subsp. *aizawai*	0.00	0.00	0.014 g · l^{-1}	1.67±0.28
Spinosad	0.14 ml · l^{-1}	0.72±0.40	0.13 ml · l^{-1}	0.72±0.40
Beauveria bassiana	3.57 spore · m^{-2}	1.81±0.32	1.89 spore · m^{-2}	2.32±0.33
Heterorhabditis bacteriophora	3.53 IJ · m^{-2}	1.67±0.28	3.74 IJ · m^{-2}	1.22±0.27
M-Pede	1.37 ml · l^{-1}	1.33±0.98	0.83 cm^3 · l^{-1}	3.07±1.42
Azadirachtin	0.62 ml · l^{-1}	1.76±0.31	0.31 ml · l^{-1}	2.75±0.36
Garlic + neem + green miracle	1.87 ml · l^{-1}	1.15±0.83	0.43 ml · l^{-1}	1.38±·,28
Garlic + basil	10.0 ml · l^{-1}	2.39±1.39	5.98 ml · l^{-1}	0.71±0.31
Lufenuron	0.61 ml · l^{-1}	2.87±0.61	0.26 ml · l^{-1}	1.66±0.65
Profenofos	3.72 ml · l^{-1}	2.52±0.68	2.17 ml · l^{-1}	2.96±0.74
Lambda-cyhalothrin	0.49 ml · l^{-1}	1.83±0.35	0.56 ml · l^{-1}	3.07±1.08
Cyfluthrin	0.73 ml · l^{-1}	2.37±1.39	0.29 ml · l^{-1}	1.85±0.31

Bt subspecies and conjugated ones. However, no significant differences were found between LC$_{50}$ values inside and outside the mines for spinosad and *H. bacteriophora*. The LC$_{50}$ value outside the mines for *B. bassiana* is was recorded as 1.89 spore · m^{-2} which show significant difference than inside mines (3.57 spore · m^{-2}). Data in Table 1 indicate that there was a significant difference in the LC$_{50}$ value between larval mortalities inside and outside the mines; the value outside mines was lower (0.83 ml · l^{-1}) than insides mines (1.37 ml · l^{-1}). For azadirachtin, the LC$_{50}$ values inside and outside the mines were double fold significance as 0.62 and 0.31 ml · l^{-1}, respectively.

Two combinations of of different water plant extracts were evaluated against the larval stage of *T. absoluta* inside and outside the treated mines (Fig. 2). A higher mortality percent was recorded for a combination of garlic + neem + green miracle extracts, while the combination garlic + basil gave the lowest mortality (40%). The LC$_{50}$ values for a combination of three natural compounds (garlic + neem + green miracle) were also determined and significant differences were found between inside and outside the mines. The lowest potency was recorded for the combination of garlic and basil 10.0 ml · l^{-1} inside the mines and 5.98 ml · l^{-1} outside the mines, respectively.

Four widely recommended chemical insecticides were evaluated in controlling *T. absoluta* (Fig. 3). An evaluation of these insecticides was made to compare their mortality effects with that of both microbial agents and natural compounds. The data revealed that lambda-cyhalothrin insecticide gave the highest larval mortality percentages both inside and outside mines; they ranged from 60 to 100% and from 60 to 91%, respectively. On the other hand, the lowest larval mortality percentages were recorded with both profenofos and cyfluthrin insecticides inside mines while lufenuron insecticide gave moderate larval mortality percent both inside and outside mines especially at its high concentration.

Concerning LC$_{50}$ values, of all tested chemical compounds lufenuron was found to be the most potent compound followed by lambda-cyhalothrin. For lufenuron, the LC$_{50}$ value inside mines was 0.61 ml · l^{-1}, whereas a lower value of 0.26 ml · l^{-1} was recorded outside the mines. On the other hand, profenofos was the weakest tested compound with a LC$_{50}$ value of 3.72 ml · l^{-1} and 2.17 ml · l^{-1} inside and outside the mines, respectively.

Discussion

Tomato is one of the most important vegetable crops in Egypt which is considered to be the fifth largest tomato producer in the world (WPTC 2011). In recent years, this crop has been so heavily attacked by the tomato leaf miner, *T. absoluta*, that some infested areas appeared as a divested desert (Mahmoud *et al.* 2015). Due to indiscriminate and intensive use of insecticides in controlling this pest, the resistance of *T. absoluta* to these insecticides has frequently been reported (Salazar and Araya 1997; Siqueira *et al.* 2000; Salazar and Araya 2001; Abdel-Razek and Abd El-Ghany 2014; Mahmoud *et al.* 2015). Among various bio-rationale insecticides, *B. thuringiensis, Beauveria bassiana*, spinosad and azadirachtin are popularly used for controlling many insect pests (Merdan *et al.* 2010; Salama *et al.* 2012; Abdel-Razek *et al.* 2014; Abd El-Ghany *et al.* 2015). This study evaluated the efficacy of different bio-rationale control agents, natural and chemical, for controlling this serious pest alone or even in IPM programs. The results of this research indicated that spinosad exhibited a satisfactory efficacy against the larval stage of *T. absoluta* inside and outside the treated mines, where all larvae were killed outside the mine in Egypt. Spinosad is used for controlling many lepidopterous pests of vegetable crops (Michaud and Grant 2003; Galva *et al.* 2005; Gamal *et al.* 2013). Hilal (2006) suggested that spinosad is very effective in controlling *Spodoptera littoralis*. The entomopathogenic fungus, *B. bassiana*, was second in its effectiveness after spinosad. It caused more than 50% larval mortality outside mines. On the other hand, the lower toxicity effects of different Bt strains against the larval stage of *T. absoluta* may be due to the insect rearing behavior and/or higher sensitivity of Bt to environmental factors such as temperature and relative

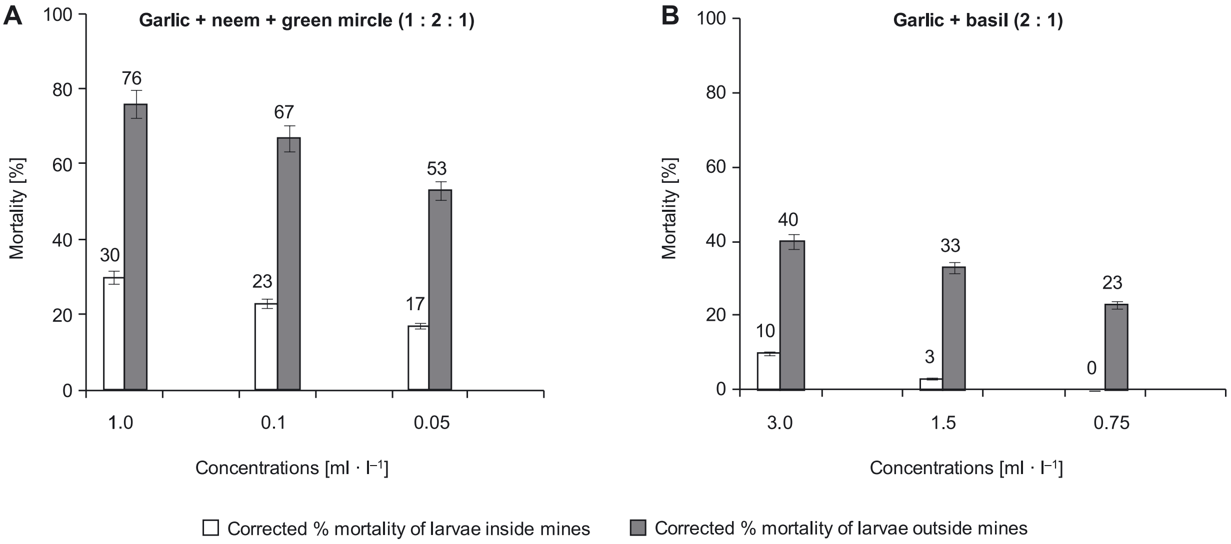

Fig. 2. Effects of different natural control agents (A–B) against tomato leaf miner, *Tuta absoluta*

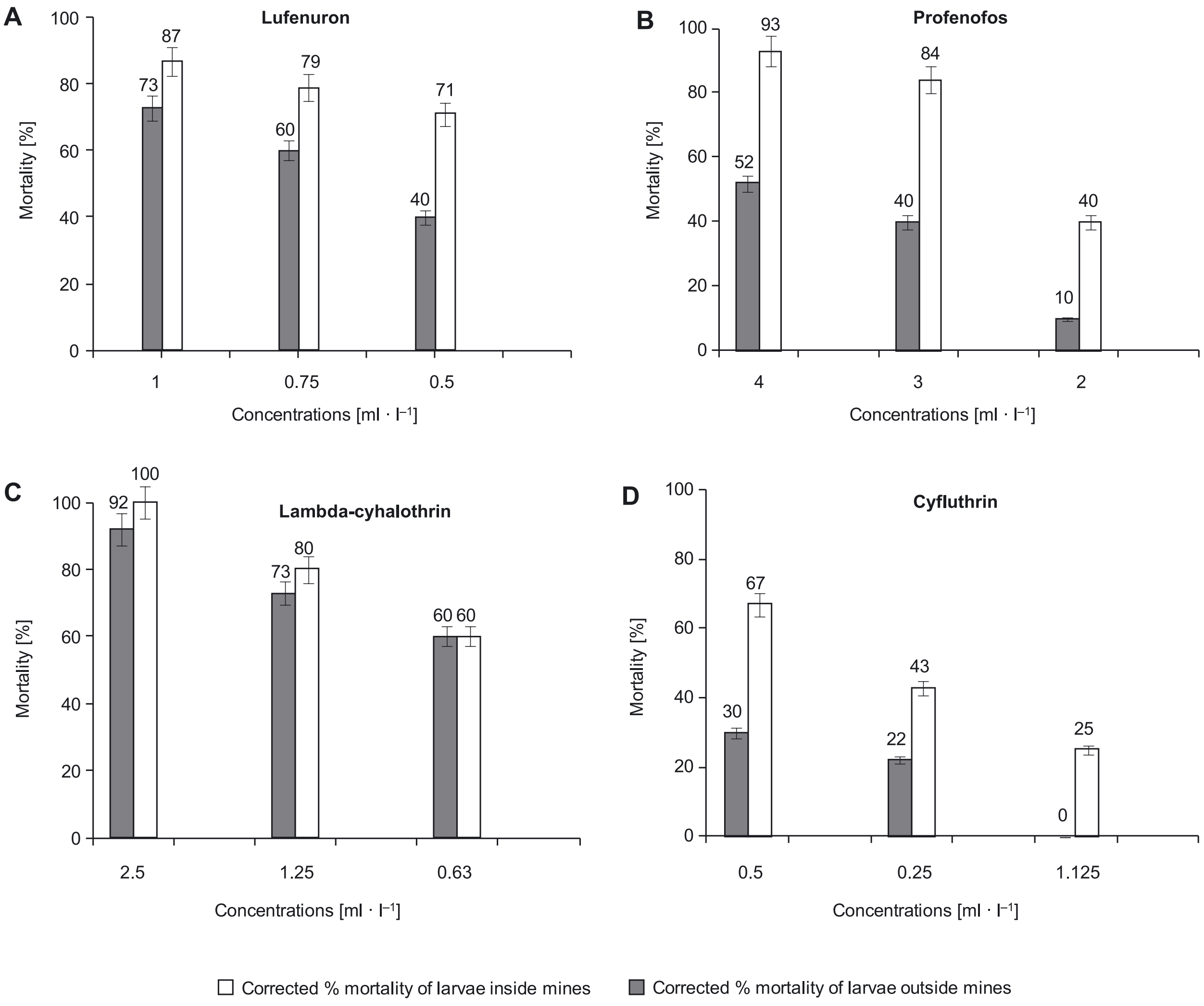

Fig. 3. Effects of different chemical insecticides (A–D) against tomato leaf miner, *Tuta absoluta*

humidity (Khidr *et al.* 2013). Sow and Diarra (2013) reported that Bt alone is not highly effective as a control measure of lepidopterous pests but it can help and complement integrated control measures.

Biopesticides derived from natural plant extracts, are widely used for controlling many families of insect pests because they are friendly to the environment, humans and natural enemies (Nilahyane *et al.* 2012; Braham and Hajji 2012; Ghanim and AbdelGhani 2014). The tested natural compounds provided promising results in controlling the larval stage of *T. absoluta* inside and outside the mines especially azadirachtin and M-Pede. The effects of these two compounds were very similar to the insecticides, and caused almost complete eradication of the larvae while the other two combinations had no larval mortality especially inside mines. Many authors consider neem extract to be an insecticide due to its high toxicity against the target pest (Kleeberg 2001; Isman 2006; Hiiesaar *et al.* 2009). The high effectiveness of neem extract comes from its mode of action where it can act as a contact and systemic insecticide against the larval stage of *T. absoluta* (Gerrasio and Vendramin 2007). Yankova *et al.* (2014) recorded high mortality percentages in early larval stages of *T. absoluta* even 14 days after the tomato plants had been sprayed.

Chemical insecticides are one of the most common and widely used methods for controlling *T. absoluta* around the world because they have rapid action and strong toxicity against the target pest. Much research has been done on using chemical insecticides for controlling *T. absoluta* (Colomo *et al.* 2002; Molla *et al.* 2011; Shalaby *et al.* 2012; Mahmoud *et al.* 2014; Deleva and Harizanova 2014). Of the tested chemical insecticides lambda-cyhalothrin was the most effective in controlling *T. absoluta.* It caused complete mortality of the larval stage of *T. absoluta* inside and outside the treated mines especially at its high concentration. Match was second in effectiveness after lambda-cyhalothrin. It caused 75% larval mortality outside the mines and more than 50% inside the mine. The other tested insecticides, profenofos and cyfluthrin showed low mortality effects against *T. absoluta* larvae. Their toxicity on the larvae inside the mines was so minimal that it can be neglected. Mahmoud *et al.* (2014) stated that lambda-cyhalothrin caused considerable mortality against early larval stages of *T. absoluta*.

Generally, the results of this research confirmed that spinosad is one of the most effective biological control agents used for controlling *T. absoluta*. This biological agent can fully protect tomato plants from infestation with *T. absoluta*. The natural compounds M-Pede or

Nimbecidine EC can be used alone for controlling *T. absoluta*. A comparison of the percentages of larval mortality of the three types of formulations shows that spinosad, M-Pede and Nimbecidine EC can be recommended as very good, safe alternatives for controlling the tomato leaf miner, *T. absoluta*, especially in IPM Programs.

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