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The Use of *Bacillus Thuringiensis*-based Products in Biocontrol of Tomato Leaf Miner, *Tuta Absoluta* (Lepidoptera, Gelechiidae)

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Abstract* – *Tuta absoluta* (Lepidoptera: Gelechiidae) is a micro-lepidopteran moth with high reproductive potential that attacks crop plants of the nightshade family, but tomato is the main host plant. Experiments have revealed some promising microbial agents for this moth including *Bacillus thuringiensis* (or Bt). *Bt* var. *kurstaki* has exhibited satisfactory efficacy against *T. absoluta* larval infestations in outbreaks. This bacterium produces protein crystals during sporulation which are toxic to insect larvae and cause rapid death of the host. According to this importance, efforts were made to estimate suitable time for using the Bt against *T. absoluta* on tomato. This moth was grown up on tomato plants under suitable temperature and humidity. In order to investigate the toxicity of two Bt strains on this pest, four concentrations were prepared and applied with two methods, leaf dipping and spraying. At the first method, Bt-treated tomato leaf discs were offered to larvae in different instars. At the second method, the bacterial suspensions were sprayed on leaf discs after the larvae were placed on them. For both methods and different ages, values LC_{50}, LT_{50} and total mortality of larvae were estimated. Based on the results, there were no significant differences between LC_{50}s of two bacterial strains for both leaf dipping and spraying methods. But the values of LT_{50} with the range of 37 to 61 hours were significantly different at all of larval stages. Comparison of mortality percentages for 2nd, 3rd and 4th instar larvae caused by 10^6 cells/ml after 24 hours revealed that death rate is significantly higher in the 4th instars than those in the early stages for both Bt application methods. Mortality rates of 2nd and 3rd larvae were affected by Bt application method and was higher significantly at the spraying method.

***Keywords* – Microbial Control, Bacillus Thuringiensis, Tuta Absoluta, Toxicity.**

I. INTRODUCTION

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a very challenging pest to control. Effectiveness of chemical control is limited due to insect's nature of damage as well as its rapid capability of development of insecticide resistant strains. The use of biological factors are still largely under development and not ready to combat this pest effectively and in a cost effective way (TAIN, 2009). The larva feeds voraciously upon tomato plants, producing large galleries in leaves, burrowing in stalks, and consuming apical buds and green and ripe fruits. It is capable of causing a yield loss of 100%. Tomato is the main host plant, but *T. absoluta* also attacks other crop plants of the nightshade family, including potato, eggplant, pepper and tobacco (Desneux, 2010). The adult moth has a wingspan around one centimeter. In favorable weather conditions eight to ten generations can occur in a single year. Some populations of *T. absoluta* have developed resistance to organophosphate and pyrethroid pesticides (Lietti *et al*., 2010).

Among the biocontrol agents, *Trichogramma* wasps, *Macrolophus*, *Nesidicoris* and *Nabis* bugs are potential agents against this devasting pest. *Bacillus thuringiensis* (Bt) which is a gram-positive spore forming bacterium and produces toxic crystal proteins (Glare, 2000), is considered another biocontrol agent for the larvae of this pest. The fungus *Baeuveria bassiana* (strain GHA) was tested alone and combined with *B. thuringiensis* in tomato farms of Spain (Torres Gregorio et al, 2009). Compared to the controls, both treatments reduced the frequency and severity of fruit losses. In Algeria, the susceptibility of *T. absoluta* larvae was examined against a native strain of the fungus *Baeuveria* sp. Three fungal doses were applied. In the high dose treatment, 4.75×10^7 conidia/ml, all larvae (100%) died within 3 days, while low doses led in fatality rate of 87% within 4 days. More than 80% of the control larvae reached the pupation stage. The results of *in-vitro* investigations indicated this strain efficiency (Ikram and Abdallah, 2011).

By applying pure crystals from five Bt strains taken from dead or diseased larvae, against *Homoeosoma nebulella*, Apolyu *et al.* showed that Bt-*kurstaki* and Bt-*aizawai* had the most toxicity (Apolyu *et al*., 1995). Hernandez-Fernandez *et al*. (2010) examined the toxicity of local Bt samples taken from soil on *T. absoluta* larvae and compared it with one commercial Bt. The result was that the toxicity of local variants were three times better than commercial ones.

In order to examine the toxicity of *B. thuringiensis*, two Bt- based products were tested on the tomato leaf miner moth larvae in this study.

II. MATERIALS AND METHODS

The larvae and pupae of the tomato leaf miner moth were collected from tomato fields and greenhouses in Qazvin province, being transferred to the laboratory. The germinator was used to establish appropriate conditions of growth in the Plant Protection laboratory of Qazvin. Environmental conditions of the incubator was set as 16 hours of light, 8 hours of darkness, relative humidity of 60 ±10% and the temperature of 24 ±1 °C. The pots were placed in cages. Each cage was allocated to a larval stage of the tomato leaf miner. Water and 10% honey in plastic containers were used for adult nutrition. Cylindrical cottons were placed inside the containers to provide the moths with feed solution. Pupae collected in plastic containers were transferred to the cages of adult insects. In this study, the effects of two domestic microbial products

containing spores and crystals (*Bt. Biolep* and *Bt.AzLP*) were examined on the mentioned pest. These products are used in two application methods of formulation and culture suspension. For both strains of *Bt*, four concentrations 10^3, 10^4, 10^5 and 10^6 cells/ml were tested in three replications. Variance analysis of the mortality data were conducted by GLM procedure. With significantly different treatments ($p < 0.05$), F-LSD test was used to compare their means. Calculating LC_{50} and LT_{50} was performed by POLO-PC and POLO-PLUS, respectively.

III. Results and Discussion

A. *Toxicity by Dipping Method*

Through dipping method, the toxicity test was performed on tomato leaf miner moth larvae. Mean mortality rates of *Bt. Biolep* (Fig 1) and *Bt. AzLP* (Fig 2) have been indicated on different larval instars. The highest

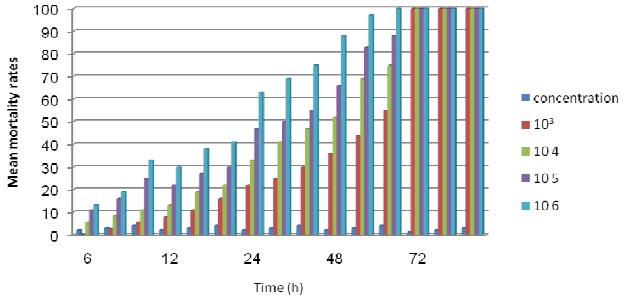

Fig. 1. Mean mortality rates (percent) induced by Bt. Biolep dipping in different larval instars with various times and concentrations.

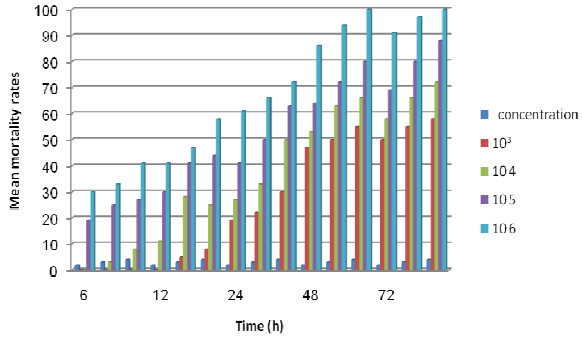

Fig. 2. Mean mortality rates (percent) induced by Bt.AZ dipping in different larval instars with various times and concentrations.

B. *The Comparison Between LC_{50} and LT_{50} of Bt. Biolep and Bt. Az strains in Dipping Method*

In dipping method**,** no significant differences were found between the effect of *Bt. Biolep* and *Bt. Az* on different larval instars. Mortality process has promoted slower in the second larval instars than the third and fourth ones.

As shown in Table 1, comparing the LC_{50} and LT_{50} of two strains in dipping method showed no significant differences between LC_{50} values. In all larval instars, LC_{50} values were higher for *Bt. Biolep* strain. The maximum LC_{50} values were exhibited by second larval instars with *Bt. Az* as 61 hours while it was 37 hours for *Bt. Biolep*.

Table 1. Comparison between LC_{50} and LT_{50} of Bt. Biolep and Bt. Az bacteria in dipping method

Larval instar	Bt product	Relative potencies (LC_{50})	Relative potencies (LT_{50})
2	Biolep	1.7(0.2-12)	0.5(0.3-0.6)*
	AZ		
3	Biolep	0.9(0.13-6.4)	0.4(0.3-0.6)*
	AZ		
4	Biolep	0.8(0.1-6.9)	0.6(0.4-0.8)*
	AZ		

C. *Toxicity by Spraying Method*

Through spraying method, the toxicity test was performed on tomato leaf miner moth. To this end, two strains of *Bt. Biolep* and *Bt. Az*were used in different larval instars. Mean mortality rates were induced by *Bt. Biolep* spraying on different larval instars in various times and concentrations. Figure 3 shows mean mortality rates induced by *Bt. Biolep* spraying on different larval instars in various times and concentrations. Figure 4 shows mean mortality rates induced by Bt. Az spraying on different larval instars in various times and concentrations.

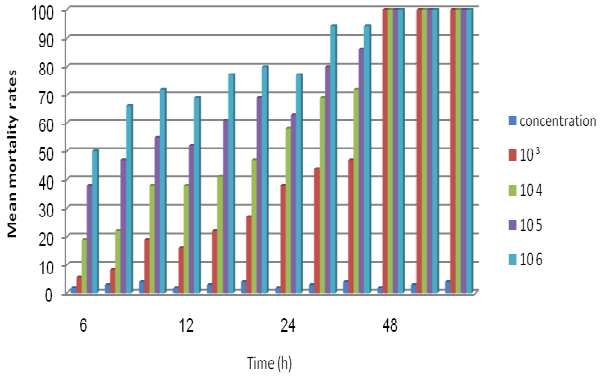

Fig. 3. Mean mortality rates (percent) induced by Bt. Biolep spraying on different larval instars in various times and concentrations.

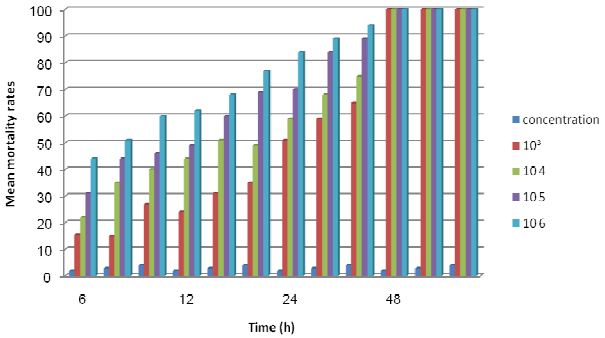

Fig. 4. Mean mortality rates (percent) induced by Bt. Az spraying on different larval instars in various times and concentrations.

D. *The Comparison Between LC_{50} and LT_{50} of Bt. Biolep and Bt. Az Strains in Spraying Method*

No significant differences were found between the effect of *Bt. Biolep* and *Bt. Az*on different larval instars. Therefore, different larval instars have shown similar vulnerability to bacterial toxicity. Compared to the third

and fourth larval instars, mortality process has promoted slower in the second one.

As shown in Table 2, the comparison between LC_{50} and LT_{50} of Bt. Biolep and Bt. Az bacteria in spraying method within 12 hours showed no significant differences in LC_{50} values. The LC_{50} value of the second larval instar was 14 hours for *Bt. Biolep* strain while it was 21 hours for *Bt. Az* strain.

Table 2. Comparison of LC_{50} and LT_{50} of Bt. Biolep and Bt. Az bacteria by spraying method within 12 hours

Larval instar	Bt product	Relative potencies(LC_{50})	Relative potencies(LT_{50})
2	Biolep	1.2 (0.1-8)	0.7 (0.4-1.3)
	AZ		
3	Biolep	0.5 (0.09-2.9)	1.3 (1-1.7)
	AZ		
4	Biolep	0.9 (0.15-5.5)	1.1 (0.8-1.5)
	AZ		

E. *The Comparison Dipping and Spraying Methods in Bt. Biolep and Bt. Az Products*

In order to compare these two methods, mortality rate in the maximum concentration (10^6) were evaluated through t-test. As shown in Table 3, comparing the dipping and spraying methods in *Bt. Biolep* strain shows significant differences between the second and third larval instars, indicating higher mortality rates in the spraying method.

Table 3. Comparison of mean mortality rates (±SE) of the tomato life miner larval instars induced by dipping and spraying methods

Instar larvae	Spraying	Dipping	P
2	77.6±2.6	63.7±2.8	0.02*
3	94.4±2.8	69.4±2.8	0.00*
4	94.4±5.5	74.9±4.8	0.06

*Differences are considered significant by Tukey test (P<0.05).

Comparing the dipping and spraying methods in *Bt. Az* strain, as shown in Table 4, shows significant differences between all larval instars, indicating higher mortality rates in the spraying method.

Table 4 Comparison of mean mortality rates (±SE) of the tomato life miner larval instars induced by dipping and spraying methods

Instar larvae	Spraying	Dipping	P
2	88.8±2	60.6±2.6	0.001*
3	88.7±4.2	66.4±0.1	0.006*
4	90.8±2.4	72±3	0.008*

*Differences are considered significant by Tukey test (P<0.05).

According to the results of the severity of pathogen on tomato leaf miner moth by immersion method in two bacteria of Bt.Az and Bt.Biolep for LC_{50} and LT_{50} of the two bacteria at 48 hours, there was a significant difference between the two isolates of bacteria in LC_{50} values. LC_{50} value in all larval stages was less for isolate of Bt. Biolep, its maximum value for bacteria Bt. Az was 61 hours, while it was 37 hours for Bt. Biolep. Moreover, according to the results of the severity of pathogen on tomato leaf miner moth by spraying method in two bacteria Bt.Az and Bt.Biolep for LC_{50} and LT_{50} of the two bacteria at 12 hours, there was no significant difference in LC_{50} values. LC_{50} value in bi-larval age for isolate of Bt. Biolep was 14 hours, while it was 21 hours for isolate of Bt.Az. It seems that this is due to the leaf miner larvae at the second instar.

Comparison of spraying and dipping methods in Bt. Biolep, showed that there is a significant difference between the mortalities of the highest concentration of 10^6 in 24 hours for each method between the larvae of ages of two and three so that mortality rate was higher in spraying method. Comparison of spraying and immersion methods in the bacteria of Bt. Az, showed that there is a significant difference between larvae of ages of two and three and four so that mortality rate was higher in spraying method.

The results of the relative assessment of tomato leaf miner moth pest damage reduction is as follows: in the first phase, after spraying by Bt.Biolep, the percentage of mortality was calculated after 24 hours and only 39 larvae came out from the initial 160 eggs. In the second phase, i.e., 96 hours after the first spray, the second spray was performed, and the percentage of mortality in the larvae remained from the first phase was calculated after 24 hours, which was equal to 100 percent mortality. Through dipping and spraying methods, bioassays were conducted for two *Bt.Biolep* and *Bt. Az* strains in different larval instars of *T. absoluta* pest. Accordingly, the best results have been achieved by the spraying method especially for the Bt-AzLP. Based on the results, *Bt.Biolep* strain showed different pesticidal activities in different larval instars of *T. absoluta.*

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