LARVICIDAL ACTIVITY OF *BACILLUS THURINGIENSIS* KURSTAKI AGAINST *TUTA ABSOLUTA* (LEPIDOPTERA: GELECHIIDAE)

Abdelkader harizia[1], Abdelkader Benguerai[1], Yahia Boukhari[2]
[1]Laboratory Research on Geo-Environment and Spaces Development, Faculty of Nature and Life Sciences, University of Mascara, Algeria
[2]Research Laboratory on Geomatics, Ecology and Environment, Faculty of Nature and Life Sciences, University of Mascara, Algeria

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ABSTRACT

Harizia, A. Benguerai, A. Boukhari, Y. 2019. Larvicidal activity of Bacillus thuringiensis kurstaki against *Tuta absoluta* (Lepidoptera: Gelechiidae). *Lebanese Science Journal.* 20(3): 352-362.

A commercial formulation of Bacillus thurengiensis var Kurstaki (BTK) (IAB Bt) has been shown to be effective in controlling Tuta absoluta Zeller larvae under laboratory conditions. Five concentrations are tested ((200, .350; 500; 650 et 800 ppm) by ingestion, on the various larval stages (L1, L2, L3, L4 and L5). The larval mortality caused by BTK swelled with increasing concentrations. The high concentrations of 650 and 800 ppm give 100% mortality rates for the early stages L1, L2, L3 and L4. The acute toxicity (LC50) of BTK calculated for all larvae is 517 ppm. The lethal efficacy of BTK is noted 02 days, after the ingestion treatment, for the young stages (L1 and L2) and 04 at 6 days for the older larvae (L3 and L4) with a LT50, all larval stages combined equal to 2.6 days.

Keywords: BTK, *Tuta absoluta*, biological control, acute toxicity, LT50.

INTRODUCTION

The tomato leaf miner *Tuta absoluta*, native from South America (Siqueira *et al*., 2000), is a quarantine pest (EPPO, 2008) that has invaded all tomato growing regions in the world. The Mediterranean coasts are a favorable habitat for the multiplication of *T. absoluta* (Desneux *et al*., 2010), it was first reported in Spain in 2006 (Urbaneja, 2007) and then spread to other countries such as Morocco, Algeria, Tunisia and France (Guenaoui, 2008, Roditakis *et al.*, 2010, Mohammed, 2010). Larval attack occurs over the entire aerial part of the plant (Filho *et al*., 2000) and can cause between 70% and

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100% loss (Pratissoli and Parra, 2000). The use of intensive chemical applications , up to 36 sprays per season, yielded very variable results depending on the active ingredients while remaining ineffective for the complete eradication of the pest (Da Cunha *et al.*, 2006 ; Luna *et al.*, 2007). The misuse of certain active ingredients, such as abamectin and permethrin in Brazil, has led to the development of resistance in populations of *T. absoluta* and the destruction of useful auxiliary fauna (Siqueira *et al* . 2000 ; Siqueira *et al* ., 2001 ; Miranda *et al*, 2005). In addition, toxic residues in harvested fruits cause a serious human health problem (Medeiros *et al*., 2006; Pereira *et al*., 2008). With these multiple risks **,** many researchers have studied the alternatives as well as the effective combinations of means of fight against insect pests by using natural enemies (Filho *et al*., 2000 ; Marchiori *et al*., 2004 ; Salvo and Valladares, 2007), Entomopathogenic fungi (Shalaby *et al*., 2013, Harizia and Lazreg, 2016) plant extracts (Yankova *et al*., 2014, Bouchikhi *et al*., 2010, Bouchikhi-Tani *el al*., 2018) and bacteria (Youcef and Hassan, 2013, Noujeim *et al*., 2015). More than 250 biopesticides, representing 4.2% of the pesticide market, are sold worldwide, 80% of which are based on *Bacillus thuringiensis* (Bt) (Mark and Whalon, 2003; Ibrahim et *al*,. 2010). Most Bt insecticides contain delta-endotoxin crystals and spores that synergistically enhance the toxicity of crystals mainly against epidopteran, dipteran and coleopteran larvae. (Schnepf *et al*., 1998). Only 2 subspecies of Bt, kurstaki and aizawaï have been developed as an insecticide to control lepidopteran pests. The most common trade names for these products include Dipel ® Dipel, Javelin®, Thuricide ®, Worm Attack®, Caterpillar Killer® et Bactospeine®, but many small businesses sell similar products under various trade names (Sanchis and Bourguet, 2008).

Bt insecticides based on the Kurstaki or BTK subspecies, whose spectrum of action is limited to Lepidoptera, are widely used in the fight against forest defoliating caterpillars. (Martin, 2006; Roversi, 2008) especially in Algeria against the defoliating caterpillar corkoak *Lymantria dispar L* (Lepidoptera, Lymantriidae), the date moth *Ectomyelois ceratoniae* Zeller and the pine processionary *Thaumetopea pytiocampa* Schiff (Lepidoptera, Notodontidae) (Zammoum *et al*., 2013, Bouzar *et al*., 2014; Oumane *et al*., 2017). In order to be able to develop a strategy for the integrated protection of glass house tomato crops. This work aims to study, in the laboratory, the insecticidal efficacy of a commercial formulation of *B. thuringiensis* BTK (IAB-BT) on the different larval stages of *T. absoluta.*

MATERIALS AND METHODS

Tuta absoluta

Individuals of *T. absoluta* used in the experiments were reared on untreated greenhouse tomato plants (Lycopersicon esculentum Mill) in the experimental station of the Faculty of Naturel and Life Sciences, (University of Mascara). The greenhouse rearing was conducted in optimal conditions with a temperature of 25 ± 2 ° C, a hygrometry of 70 ± 10% and a photoperiod of 12 h: 12h.

Baccilus therungiensis var Kurstaki (BTK)

The sample of *B. thurengiensis* var Kurstaki (tradename: IAB Bt), used in this study, is a microbiological insecticide which is available in the Algerian market. It is registered for the control of the pine and cedar caterpillar *Thaumetopoea pityocampa* (Denis &Schiffermüller) (Lepidoptera: Notodontidae). The patchy bombyx *Lymantria dispar* L. and the date moth *Ectomyelois ceratoniae* Zeller. Bio insecticide is a wettable powder with a concentration of 32000 IU g^{-1}.

Larvicidal assay

BTK larvicidal efficacy testing on *T. absoluta* involves the larvae being tested at concentrations of 200, 350, 500, 650 and 800 ppm for ingestion toxicity. Larvae were individually placed in petri dishes (6.5 cm x 2.5 cm) containing a Whatman filter paper moistened with distilled water and fed on fresh tomato leaves discs dipped in each of the five concentration. BTK treated leaves are used once, the larvae are then fed on untreated tomato leaves. Control larvae receive tomato leaves dipped in distilled water. The experiment was a randomized complete block design with replications consisting of 08 larvae per stage (L1, L2, L3, and L4) and concentration replicated three times. The experiment was carried out under controlled conditions in the laboratory at a temperature of 30 ° ± 0.5 ° C, a relative humidity of 70 ± 10% and a photo period of 14 hours. Larval mortality was checked every day for six days.

Statistical analysis

Data were analyzed by 1-way analysis of variance with ANOVA- test. The mortality rate of the larvae exposed to test was corrected using Abbott's formula (Abbotts, 1925):

$$\text{Abott's formula:} \quad \text{CM } \% = \frac{M2-M1}{100-M1} x\ 100$$

With CM% = % corrected mortality. M2 = % mortality in treated larvae. M1 = mortality among controls.

Statistical significance of differences in mortality was examined by using the Chi-square test (χ^2). The level of significance was $P<0.05$. The probit regression was used to calculate the lethal concentrations (LC50, LC90 and the median lethal time (LT50, including their confidence limits, at the various concentrations. The logistic regression model gives the LC50 and LT50 with their 95% confidence intervals after the probit transformation of cumulative mortality rates. XLSTAT software was used for statistical analysis.

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RESULTS

Larvicidal efficacy

The larval mortality of *T. absoluta* vary significantly among the *B. thurengiensis* var kurstaki treatments and increased with increasing concentrations ($F_{3.88} = 22.55$, $P = 0.00008$) (Table 1).

Table 1. *Tuta absoluta* larval corrected mortality (%) after exposure to tomato leaflets treated with *Bacillus thurengiensis* var kurstaki at various concentration.

Larvae	Concentration (ppm)	2 days	4 days	6 days
	200	0	0	0
	350	0	0	16.6
L1	500	0	16,5	33
	650	54,6	81,8	100
	800	100	100	100
	200	0	0	0
	350	0	0	0
L2	500	36,44	76,11	83,89
	650	77,77	100	100
	800	100	100	100
	200	0	0	0
	350	0	0	0
L3	500	35,11	53,55	53,55
	650	62,89	75,22	100
	800	72,22	86,11	100
	200	0	0	0
	350	0	0	0
L4	500	0	10,71	20,59
	650	66,43	77,62	100
	800	47,02	73,45	100

Three replicates per concentration and per larval stage. The results are significantly different at $P < 0.05$.

The lower concentration (200 ppm) had no effect on the different larval stages. 6 days after treatment, the concentration of 350 ppm revealed a lower mortality rate of 16.6% for the L1 larvae but remained without lethal effect on the other stages. At 500 ppm, the results show mortality rates of 33%, 83, 89%, 53, 55 % and 20, 59 for L1, L2, L3 and L4 respectively. The ingestion treatment with the highest concentrations, 650 and 800 ppm, gives 100% mortality of all the larvae stages. The young larval stages (L1-L2) are more sensitive to BTK than the old stages (L3 - L4). All larval stages die within 2 to 6 days after ingestion treatment at concentrations of 650 and 800 ppm. The young stages L1 and

L2 die after 2 days after exposure to the highest concentration of 800 ppm. While the total mortality of older larvae L3 and L4 occurs 6 days after treatment for concentrations 650 and 800 ppm ($F_{3.056}$ = 6.60; P =0.003). The mortality of the control larvae did not exceed the rate of 5% throughout the duration of the experiment.

Acute toxicity and median lethal time

The estimated LC50 value shows that BTK is more toxic to young larval stages (L1 and L2) than the older ones. In fact, the results recorded in Table 2 show that the lowest value of the LC50 is obtained for L1 larvae with 409 ppm and the highest for L4 with 554 ppm. The other intermediate stages L2 and L3 have respective estimated LC50 value of 449 and 516 ppm. The same trend is observed for CL90. The LC50 and the LC90 values and their confidence interval, for all larvae stages, are 517 ppm (478 - 0.557) and 670 ppm (621-748) respectively (r^2= 0,783, chi^2=48.41, p<0.0001) (Figure 1). The respective lethal times TL50 and TL90 and their 95% CI, calculated for all larval stages, are 2.601 days (2.454- 2.759) and 3,679 days (3,415-4,046) (r^2= 0,761, chi^2=136.21, p<0.0001) (Figure 2).

Table 2. Estimates of *Bacillus thurengiensis* var kurstaki lethal concentration 50 and 90 (LC50 and LC 90) for *Tuta absoluta,* 6 days post treatment.

Larvae	LC50 (CL 95%)	CL90 (CL 95%)	r^2	chi^2 (Wald)
L1	409 (371-438)	471 (442-513)	0,887	25,38
L2	449 (419-475)	533 (505-576)	0,864	36,99
L3	516 (479-549)	654 (614-714)	0,744	47,54
L4	554 (517- 585)	671 (634-729)	0,769	36,83

The low value of chi2 expresses a better fit to the linear regression model, and hence the reliability of the LC50. LC50 is considered valid when p ≥ 0.05% (ddl= 1; p< 0,0001).

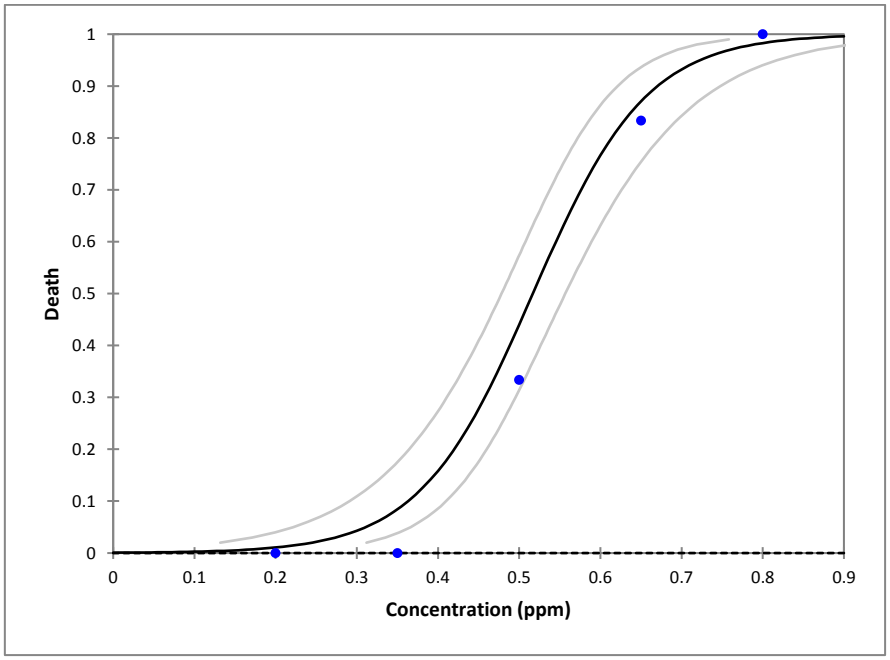

Figure 1. Probit model: dose-death dependence of *Tuta absoluta* treated with BTK at various concentration. Gray lines represent 95% confidence intervals.

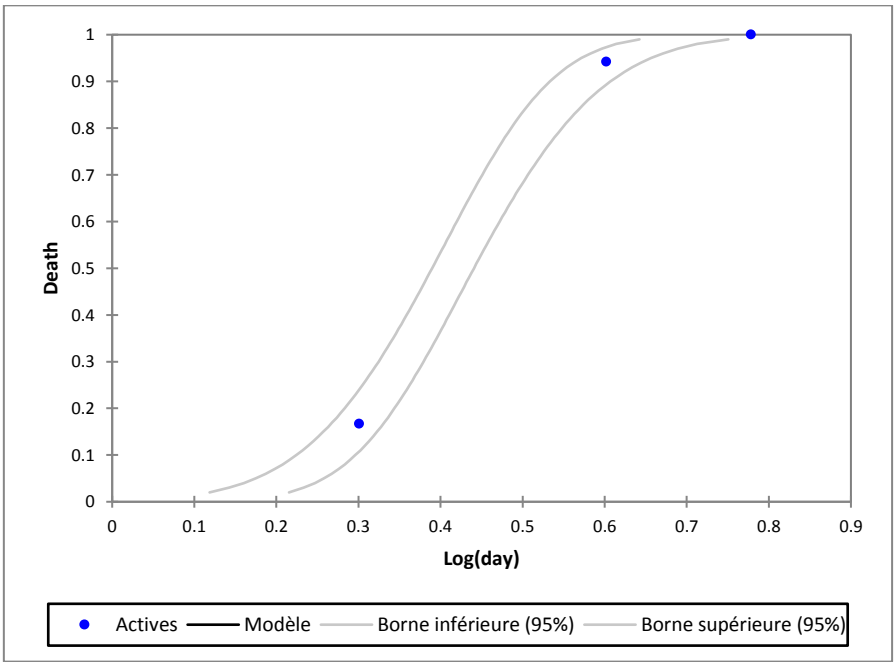

Figure 2. Probit model: day – death dependence of *Tuta absoluta* larval treated with BTK at various concentration. Gray lines represent 95% confidence intervals.

DISCUSSION

The results obtained show that *B. thurengiensis* kurstaki has an insecticidal effect on the different larval stages of *T. absoluta*. The total mortality of the different larval stages of *T. absoluta* occurs after 6 days after the application of the highest concentrations of BTK (650 and 800 ppm).The biocidal potential of the commercial formulation of BTK in crops control is well known as a key element of integrated pest management programs (Roh *et al*., 2007). The results obtained in this study are in agreement with those obtained by Cabello *et al*. (2009), Gonzalez-Cabrera *et al*. (2011), Shalaby *et al*. (2013) et Alsaedi *et al.* (2017). These authors reported that *B. thurengiensis* kurstaki has sufficient efficacy against the four larval stages of tomato leaf miner. Aronson *et al*. (1986) specify that *B. thurengiensis* acts by ingestion, a few hours after the absorption of the product the caterpillar cannot feed itself because of the paralyze of the jaws then die a few days later. Our finding show that the larval mortality rate varies with the applied BTK concentrations and the age of the larvae. Indeed McGaughey (1985) states that the concentration to be applied depends on the size of the larva. A large larva requiring a higher concentration. In the present experiment, the young larval stages L1 and L2 are more sensitive and die after 02 days and 6 days for the old stages L3 and L4, treated at high concentrations (650 and 800ppm). In this regard, several authors emphasize on the excellent effectiveness of the bacterium in young stages of Lepidoptera. (Giustolin *et al.*, 2001, Gonzalez-Cabera *et al*., 2011, Shalaby *et al*., 2013). Giustolin *et al.* (2001). In the same context, they point out that the speed of mortality of young stages compared to older stages is related to differences in feeding behavior. On the other side, Narmen and Hassan, (2013) reported that 53.5% to 100% mortality of larvae treated in the laboratory by the BTK die between 1 and 5 days. In general, Bt loses 50% of its insecticidal potential within 1 to 3 days. However, some researchers report a longer residual activity (7 days, 10 days and 45 days) on tomato leaves (Theoduloz *et al*., 2003. Torres Gregorio *et al*., 2009). The toxic intensity of BTK with respect to lepidopteran pests is related to the strains and proportions of δ-endotoxins contained in the different commercial formulations (Sanchis and lereclus, 1999).

Most Lepidoptera are sensitive to crystals produced by kurstaki strains (Aronson et al., 1986). During sporulation, BTK synthesizes crystal inclusions consisting essentially of one or more cytotoxic (Cry) and cytolytic (Cyt) cytotoxic proteins. These δ-endotoxins are very specific to the target insect. They first require solubilization as well as toxin activation by the proteolytic enzymes of the insect (Jenkins *et al*., 2000). Then they bind specifically to membrane receptors located in the intestinal cells. Finally, this toxin becomes oligomerized. It forms a membrane pore, inducing intestinal paralysis and the death of the insect (Höfte & Whiteley, 1989; Mark & Whalon , 2003; Bravo *et al*., 2007). For most Lepidoptera, protein crystals are solubilized by the alkaline pH (10-11) of the insect's gut (Hofmann *et al*., 1988).

The results obtained, in this work, show that the formulation of *B. thurengiensis* var kurstaki has a high insecticidal efficacy on all larval stages of *T. absoluta* and in particular on the young stages. BTK could be a key element in developing a rational

strategy for controlling tomato leaf miner. Further research will have to be undertaken in the area of combining BTK with other means of control.

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