Toxic and sub toxic effects of *Bacillus thuringiensis* svar. *kurstaki* toward *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae).

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Abstract

The objective of this study is to determining mainly, the toxicity of *Bacillus thuringiensis* svar. *kurstaki* (Bt) on the first larval instar of *Ectomyelois ceratoniae* and also to study its deferred effect on other biological parameters such as the development and reproduction of this pest. We paired the treated larvae, The concentrations used are: 0,25 *g/L* (250 ppm), 0,5 g/1L (500 ppm), 1 g/L (1000 ppm), 1,5 g/L (1500 ppm) and 2 g/L (2000 ppm) (6 pairs for each dose used), and placed 6 control pairs on separate Petri dishes to count the number of eggs laid. We then tallied the number of eggs that hatched after their incubation. For Bt svar. kurstaki, the variable being measured is the rate of larval mortality. The results showed that there was a strong and positive link between the doses used and the adjusted mortality of the larvae across a range of bioinsecticide exposure times, the five *Bt* svar. *kurstaki* concentrations used had generated a corrected mortality of *E. ceratoniae* first instar larvae which varied between a minimum of 50.78% and a maximum of 97.92%. *Bacillus thuringiensis* svar. *kurstaki* becomes more and more toxic each time the larvae were exposed to the biopesticide. Thus, the LC_{50} of *Bt* svar. *kurstaki* for *E. ceratoniae* larvae, calculated at the concentrations of 250, 500, 1000, 1500, 2000 ppm were inversely proportional to the different lethal times. On the other hand, the Bt treatment decreased female insects' reproductive rate and egg viability. Therefore, the *BT* had a negative influence on the growth and reproduction parameters of *E. ceratoniae*.

Key words: *Bacillus thuringiensis*, *Ectomyelois ceratoniae*, Mortality, Fecundity, Fertility.

INTRODUCTION

The Date palm, scientifically known as *Phoenix dactylifera* L., is an important fruit crop with strategic significance (Bedjaoui and Benbouza, 2020). Arid and semiarid regions have long relied on it as a crucial component of their economic and social fabric (Ben chaaban et al. 2019). The *Ectomyelois ceratoniae* is still one of the most formidable pests that attack Algerian palm trees. The significant losses caused by this pest cannot be effectively mitigated using chemical management methods, as its biology and feeding behaviour make it resistant to such measures, rendering date production vulnerable. The larvae are nourishing and maturing within the date fruit, where they are securely shielded (Lebdi Grissa et al., 2011; Peyrovil et al., 2011), causing a significant decline in both their quality and their worth (Jouve et al. 2006). The indiscriminate and irrational application of pesticides, coupled with farmers' lack of awareness about their hazards, exacerbates their detrimental impact on human health, animals, and the environment. Additionally, this practice contributes to the depletion and destruction of beneficial fauna (Ben Saad, 2010; Lhoucine, 2010; Bisaad et al., 2011). The use of synthetic pesticides leads to the build-up of residues in the food chain, pollution, and the emergence of pest resistance over several generations (Bélanger and Musabyimana, 2005; Abdullah, 2009; Richard, 2010). An inherent drawback of utilizing these novel synthetic compounds lies in their lack of biodegradability. These molecules therefore reproduce the initial substances that made them up and concentrate in organisms and are transmitted throughout food chains (Escoubet, 2011). This makes it an urgent referral to other control methods that use natural compounds given from the living world (plant or micro-organisms), which can be at the origin of preventive and curative treatments. Developing these biological potentials allows for efficient management of insect populations and mitigates. The potential for environmental pollution resulting from the usage of synthetic and non-biodegradable substances (Mossini and Kemmelmeier, 2005).

. In this context, biologists have focused on the creation of a new generation of biopesticides based on natural oils, pathogenic bacteria, insect growth regulators (IGR), pheromones, nematodes and marine toxins (Abdullah, 2009). To this effect, our work aims to study the toxicology of *Bacillus thuringiensis* var. kurstaki, with the object of determining mainly, the toxicity of this molecule on *E. ceratoniae* and also to study its delayed effect on the growing and reproduction of this pest, under laboratory conditions.

MATERIALS AND METHODS

Mass-Rearing of Carob Moth: The study of the date moth biological parameters in the laboratory requires their mass rearing. The mass rearing of *E. ceratoniae* was conducted with the strain from infested dates of Biskra palm groves. The infested dates were placed in cages in a rearing room with controlled ambience (T = 27 ± 2 ° C, RH = 65 ± 10%) and a photoperiod (16: 8) (L: D) (Al-izzi et *al*. 1987). Emerged adults of *E. ceratoniae* were captured using a test tube and then they placed inside a mating pot regardless to the sex. After mating, the females laid their eggs inside the pot coupling; the laid eggs were dumped through the fine mesh tulle into large plastic boxes containing an artificial diet (Mediouni and Dhouibi, 2007). After eggs hatching, the first instar larvae were collected for our bioassays.

Bio pesticide preparation: Dipel DF was used, as biological insecticide, which contains the active substances (spores and crystals) of *Bacillus thuringiensis* subspecies *kurstaki* strain ABTS-351. It had a WG formulation (water dispersible granules) titrating 32000 IU/Mg. The *kurstaki* variety is selective on lepidopteran larvae against which it acts by ingestion (Dhouibi, 1993). After several preliminary tests of the bio pesticide approved doses, five concentrations were chosen in such a way that each concentration was twice the previous one. The selected concentrations were weighed and added with distilled water, following the procedure for use of this bio pesticide (0.1-1*kg/Ha*). The approved concentrations are: 0,25 *g/L* (250 ppm), 0,5 g/1L (500 ppm), 1 g/L (1000 ppm), 1,5 g/L (1500 ppm) and 2 g/L (2000 ppm).

Study of Bacillus thuringiensis svar. kurstaki toxicity on the date moth larvae: Twenty larvae of first instar were placed in Petri dishes containing the artificial diet treated by 5 concentrations of *Bt* (250, 500, 1000, 1500 and 2000 ppm), plus a control, all in three repetitions. The Petri dishes were strictly closed and placed in the rearing room. Observations were made daily for counting dead larvae using a binocular loupe.

Effect of Bacillus thuringiensis var. kurstaki on females and eggs' fertility: The larvae that survived the toxic impact of *Bacillus thuringiensis* svar. kurstaki were transferred to an artificial diet in order to complete their development into adult stage. Upon reaching adulthood, 30 pairs of treated larvae (6 pairs for each dose) and 6 pairs of control larvae were individually placed in separate Petri dishes to quantify the number of eggs laid. The quantity of eggs that successfully hatched following the process of incubation was tallied.

Statistical Analysis: For *Bt* svar. kurstaki, the variable being measured is the rate of larval mortality. The mortality rate was adjusted using Abbott's formula (1925), which provides an estimation of the actual toxicity of the bioinsecticide. The various rates undergo an angular

metamorphosis in accordance with the Bliss tables (Fischer and Yates, 1975). The normalised data were analysed using one-way analysis of variance (ANOVA).

To characterize the insecticidal effect of the molecule used, the lethal concentration 50% (LC_{50}) was determined. Corrected mortality rates obtained were transformed into probits and permit establishing a linear regression line according to the decimal logarithms of the doses used. Using regression equation, all the remarkable doses, according to the mathematical procedures of Finney, (1971) were determined. The method of Swaroop *et al.*, (1966) was used to calculate the LC_{50} confidence interval.

Abbot's formula: Corrected mortality Percentage (%) = X-Y / X x100.

Where: X = Number of living in the control lot,

Y = Number of living in the treated lot.

Parametric tests were used to compare the means. The computations were performed using the XLSTAT software.

RESULTS

Mortality study of E. ceratoniae larvae exposed to Bacillus thuringiensis svar. Kurstaki:

Table 1. Corrected mortality rate of first instars larvae of *Ectomyelois ceratoniae* treated with *Bacillus thuringiensis* svar. *Kurstaki*

Exposure time (hours)	**250 *ppm***	**500 *ppm***	**1000 *ppm***	**1500 *ppm***	**2000 ppm**	***DOF***	***F***	***P***
24	38,86±6,78	50,88±5,23	54,04±11,48	57,54±6,56	64,39±10,06	4	3,748	0,0410
48	43,95±6,64	56,05±6,64	60,88±12,29	66,05±3,54	76,23±7,82	4	6,678	0,0070
72	50,78±6,76	58,38±9,93	63,74±7,52	70,86±3,70	83,33±16,67	4	3,754	0,0408
96	54,58±7,08	62,31±5,93	69,83±8,36	81,05±6,82	90,63±11,51	4	8,419	0,0031
120	62,89±5,20	78,38±3,71	88,21±0,69	96,06±3,43	97,92±3,61	4	33,074	<0,0001
144	82,90±10,49	89,67±9,20	95,82±3,64	100,00±0,0	100,00±0,00	4	4,346	0,0271

After the exposure of *E. ceratoniae* first instars larvae to *Bt* svar. *kurstaki* during 24, 48, 72, 96, 120 and 144 hrs., the corrected mortality rates revealed significant differences among the five concentrations tested; $P = 0,0410$; $P = 0,0070$; $P = 0,0408$; $P = 0,0031$; $P < 0,0001$ and $P = 0,0271$, respectively (Table 1).

After a lethal time of 24 and 48 hours, the lowest corrected mortality rates (38.86 and 43.95%, respectively) were recorded in the larvae treated with the lowest concentration (250 ppm), while

it exceeded 50% for concentrations of 500, 1000,1500 ppm and reached, respectively a maximum of 64.39 and 76.23% at the concentration of 2000 ppm (Table 1). For an exposure time of 72, 96 and 120 hours, the five *Bt* svar. *kurstaki* concentrations used had generated a corrected mortality of *E. ceratoniae* first instar larvae which varied between a minimum of 50.78% and a maximum of 97.92%. Also, the high concentrations (1500 and 2000 *ppm*) induced the highest mortalities (100%) in a longer lethal time (144 hours) (Table 1).

Toxicological parameters of Bacillus thuringiensis svar. Kurstaki study:

Table 2. Toxicological parameters of *Bacillus thuringiensis* svar. *kurstaki* after different intervals

Exposure time (hours)	**Regression equation**	**R^2**	**LC_{16}**	**LC_{50}**	**LC_{84}**	**LC_{90}**	**Slope**
24	Y = 3,28 + 0,624 * X	0,942	14,49	568,60	22312,99	64365,60	39,24
48	Y = 2,872 + 0,833 * X	0,932	22,95	358,61	5604,02	12392,45	15,63
72	Y = 2,74 + 0,919 * X	0,845	23,83	287,87	3478,16	7140,92	12,08
96	Y = 2,243 + 1,152 * X	0,832	33,88	247,30	1805,14	3204,27	7,30
120	Y =0,703 + 1,893 * X	0,971	55,54	186,18	624,16	885,04	3,35
144	Y = -2,41 + 3,323 * X	0,822	85,24	169,79	338,21	412,66	1,99

As indicated in table (2), the highest LC_{50} and LC_{90} (568.60 *ppm* and 64365.60 *ppm*, respectively) were recorded at the exposure time of 24 h with $R^2 = 0.942$ and a regression equation $y = 0.624 x + 3.28$ with a Slope of 39.24, while the lowest LC_{50} and LC_{90} (76.86 and 2320.54 ppm, respectively) were obtained for the exposure time of 144 h with $R^2 = 0.822$, a regression equation $y = 3.323 x - 2, 41$ and the Slope was 1.99.

Bacillus thuringiensis svar. *kurstaki* becomes more and more toxic each time the larvae were exposed to the biopesticide. Thus, the LC_{50} of *Bt* svar. *kurstaki* for *E. ceratoniae* larvae, calculated at the concentrations of 250, 500, 1000, 1500, 2000 ppm were inversely proportional to the different lethal times (24, 48, 72, 96, 120 and 144 hrs.). Table (2) showed that the LC_{50} of *Bt* svar. *kurstaki* calculated at the longest mortality duration (144 h) was lower (169.79 ppm) than that recorded at the lethal time of 24 h (568.60 ppm).

Study of E. ceratoniae female and eggs fertility:

Table 3. Average number of eggs lay per female and hatchability

	Control	Concentrations (ppm)				*F*	*P*
		250	500	1000	1500		
Average number of eggs laid per female	145,00	46,00	34,50	32,17	24,17	27,497	< 0,0001
Standard deviation	11,54	15,10	2,98	6,05	7,14		
Average rate of eggs hatched (%)	91,24	55,02	50,80	39,30	37,82	5,392	0,003
Standard deviation	1,93	6,21	5,45	12,69	12,52		

Control females laid the highest number of eggs with (145.00 ± 11.54) whereas those treated with the different concentrations of *Bt* svar. *kurstaki* showed a very low egg laying number, which oscillated between 24.17 ± 7.14 at the concentration of 1500 ppm and 46.00 ± 15.10 at the concentration of 250 ppm (Table 3).

Data presented in table (3), showed that the highest average hatching rate of eggs was recorded for control females (91.24 ± 1.93%). On the other hand, it was lowest in case the eggs of the females from larvae treated with the different *Bt* svar. *kurstaki* concentrations (between 37.82 ± 12.52% and 55.02 ± 6.21%). Analysis of the variance of the eggs number means lay per female and average rate of *E. ceratoniae* eggs hatched in a lot treated by the four *Bt* svar. *kurstaki* concentrations (250, 500, 1000, 1500 ppm), Obtained results showed significant differences with P <0.0001 and P = 0.003, respectively (Table 3). The results indicated that *Bt* svar. *kurstaki* diminish female fertility by 83.34% and egg hatching by 58.55%, whatever the concentration used.

DISCUSSION

In order to prepare an integrated pest management program against the date moth *E. ceratoniae*, the toxicological effect of *Bacillus thuringiensis* svar. *kurstaki* was evaluated. *Bt* svar. *kurstaki* is used to control of lepidopteran caterpillars harmful to cultivated plants and forest species (Habbachi, 2013). Tests carried out to determine *Bt* svar. *kurstaki* toxicity towards *E. ceratoniae* larvae seemed conclusive. The first mortalities rates became evident 24 hours after the larvae were exposed to the substance. The survival rate of *Euprosterna elaeasa* caterpillars (Lepidoptera: Limacodidae) was assessed 48 hours after exposure to Bt-strains at a concentration of 0.84 mg mL−1. The survival rate decreased from 99.9% in the control group to 52.79% with SA-12 var. kurstaki, 51.37% with GC-91 var. aizawai, 35.62% with HD-1 var. kurstaki, and 23.12% with ABTS-1857 var. aizawai (Plata-Rueda et al. 2020). According to Chaufaux (1995), the death of the insect occurred within 24-48 hours after ingestion of the *Bt* crystals. The bacteria produce a

toxin which, when ingested by the larvae, destroyed its digestive system and the larvae stopped feeding and dies few days, following the treatment (Lambert, 2010). The *kurstaki* subspecies is toxic against larvae, but to varying degrees depending on the species of the larvae (Anonymous, 2012). The same author indicated that the insects against which *Bt* was toxic cease feeding in less than a few hours and die after 2-5 days. The mortality rate was lower at a short exposure time (24 and 48hrs), regardless of the concentration used. From the information available and cited above, the mortality seen in these young larvae (L1) following their exposure to *Bt* was substantial. Hence, the efficacy of treatments utilising *Bt* svar. kurstaki was found to be higher when administered during the youngest larval stages (Chaufaux, 1995). In their study, Lereclus and Chaufaux (1986) observed that when a larva in its early stage consumed crystals, they were quickly broken down and the resulting toxin led to paralysis of the digestive tract. As a result, insects treated with the toxin died from either toxaemia or septicemia within a few days. Most lepidopteran are sensitive to the crystals produced by the *kurstaki* and *aizawai* strains (Drummond and Pinnock, 1994). For Lambert (2010), the *Bt* subspecies *kurstaki* (*Btk*) was effective only on young larvae of the gypsy moth (*Lymantria dispar* Linnaeus). On *Tuta absoluta*, Mazollier (2018), found out that the *Bt* subspecies *kurstaki*, was active only by ingestion and on young larvae. Therefore, young larvae are more sensitive to *Bt*, so it is important to target the early larval instars (Anonymous, 2012). The mortality rate observed in our experiment was probably related to the quantity and the duration of food taking. Ghy's study (1971), on the *Bt* action against the growth and development of the migratory locust (*Locusta migratoria*), indicated that when the toxin was ingested by the locust at the beginning of a larval stage, the slowdown in development was manifested at this instar and the retard persists all the longer time that quantities of toxin ingested were greater. The same author observed that most mortalities occurred between the second and sixth days after treatment, in fact, 30% of mortalities caused by high doses of *Bt* toxin occur in the first three days, following treatment. 50% of mortalities were reached after four days, 60% after five days and 80% after six days. However, the corrected mortality rates increased gradually with augmentation of the bio pesticide concentration and the exposure duration to reach its maximum after 6 days (100%) in lots treated by high concentrations (1500 and 2000 ppm). So, the larval mortality rate was significantly correlated with the period of exposure to the biopesticide. Obtained results evinced that the LC_{50} gradually decreased with time. So, the *Bt* becomes more and more toxic as the exposure duration of the larvae to the product increased. The toxicity of *Bt* may also be due to the age of *E. ceratoniae* larvae treated (L1). *Bt* appeared less toxic to *E. ceratoniae* larvae than on the last larval stages of *Simulium vittatum* with an LC_{50} between 1 and 1.1 *ppm* after 24 hours of exposure. Abid *et al.*, (2021), It has been shown that biological control is the most

ecologically friendly and superior choice for combating E. ceratoniae. The lipopeptide biosurfactant generated by *Bacillus subtilis* SPB1 shown high efficacy against the insect that was infesting preserved dates. *Bacillus thuringiensis* (*Bt*) toxins have demonstrated significant efficacy in managing dangerous insects that impact human health and agriculture. These toxins are utilised as the primary biological component in the production of bio insecticides, owing to their ability to selectively target certain insect orders. Chapa et al. (2019).

CONCLUSION

Under laboratory conditions, the assessment of the larvicidal activity of *Bacillus thuringiensis* svar. kurstaki on the first larval stage of E. ceratoniae demonstrated a significant susceptibility to Bt. The sensitivity, which is directly proportional to the fatal period, was higher when the concentration was higher. The LC50 value demonstrated a negative correlation with the length of time that the larvae were exposed to the bio pesticide. The duration of lethality was prolonged during periods of weakness and shortened during periods of strength. The collected results demonstrated that *Bt* svar. kurstaki exhibits promising larvicidal activity against *E. ceratoniae*.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Competing interests

On behalf of all co-authors, I hereby confirm that I have reviewed and complied with the relevant Instructions to Authors, the Ethics in Publishing policy, and Conflicts of Interest disclosure.

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