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EFFECT OF *Bacillus thuringiensis* AND DIMILIN ON THE DATE MOTH *Ectomyelois ceratoniae* ZELLER

Fatima Zohra BISSAAD[1], Farid BOUNACEUR[2], Bahia DOUMANDJI-MITICHE[3]

[1]University M'Hamed Bougara, Faculty of Science, Department of Biology, BP 35000 Boumerdes, Algeria
[2]University Ibn Khaldoun Tiaret, Faculty of Agricultural and Veterinary Sciences, Algeria
[3]National School of Agronomy, ENSA Department of Agricultural and Forest Zoology

Corresponding author email: bissaad@yahoo.com

Abstract

The date moth is a pest that causes damage to the date palm and decline the quality walking of this production. For this, in this study, the 4th instar larvae of Ectomyelois ceratoniae three tests were performed. *In the first, we tested a biopesticide Bacillus thuringiensis kurstaki only with 3 concentrations (0.25 g/0.5l, 0.50g/0,5l, 0.75 g/0,5l). In the second test, we added 1% of dextrin to each bacterial concentration. In the third test, 3 doses the Diflubenzuron (0.2 g/l, 0.4 g/l, 0.6 g/l of.*
The toxicity of B. thuringiensis showed a highly significant mortality on the added media and larval stages. Mortalities recorded with the addition of 1% dextrin were relatively higher compared to biopesticide only.
Regarding the toxicity of Diflubenzuron, it should be noted that the percentages of mortality recorded for this growth regulator were not very important for the three doses tested. The dose D3 (0,6 g/l) had superior mortalities to those of doses D1 (0,2 g/l) and D2 (0,4 g/l).

Key words*: Bacillus thuringiensis, Ectomyelois ceratoniae,* Diflubenzuron, mortality, LD50, TL50.

INTRODUCTION

The date palm, *Phoenix dactylifera* is for the populations of the Sahara the welfare of the tree that provides not only dates, but also a large number of different productions that are very useful for families. It is also the backbone of which are true oasis of greenery and islets of life in the desert (Louvet et al., 1970). It is a perennial plant species of great importance in the economy of many hot countries (Baaziz and Bendiab, 1994).
The date production in Algeria is a more important place in marketing. However, it is constantly subject to phytosanitary problems, among which the moth date: *E. ceratoniae* constitutes the main constraint on exports whose larvae are worms that grow inside of dates (Dridi, 1999). The damage it causes is estimated between 7.24% and 28.69% (Adila, 2006), and can reach up to 29% of the date production (Feliachi, 2005) Polyphagia of this species and its wide distribution on various hosts make it difficult to develop an effective chemical control (Biliotti and Daumal, 1969).
Faced with this worrying situation, it is necessary to move towards the application of biological control using entomopathogenic microorganisms.
It is in this context, we have tested the effect of the biopesticide (*Bacillus thuringiensis*) only then added to the dextrin as the biological control and insect growth regulator (dimilin) as chemical control against the 4th instar larvae of the date moth *Ectomyelois ceratoniae*.
We associate the dextrin to the biological control in other to facilitate the ingestion of a food containing with the bacteria, which allows to increase its digestion and toxicity of the biopesticide.

MATERIALS AND METHODS

Biological material

Ectomyelois ceratoniae: The *E. ceratoniae* larvae were from a mass rearing conducted within the unity of the INPV Biskra by two techniques: individual and mass rearing livestock.
Individual farming involves spreading of wormy dates in plastic film in a breeding room,

dropping below a card to collect chrysalises when needed. Dates were kept under controlled conditions to allow better adult emergence. While for the artificial medium on mass-rearing was to recover adults from the individual housing in plastic bottles with fine-mesh tulle chiffon for aeration by keeping within filter paper serve nesting support for female borer. After 24 hours mating occurs, the eggs laid on the filter paper in the support were collected and deposited on an artificial medium in plastic boxes of 24x12x10 cm in dimensions. These boxes were previously disinfected with ethanol 70% (v/v). Control of these boxes was made every 3 to 4 days. The eggs hatch after a few days of incubation and the emergence of the larvae (L1). The breeding was conducted under a temperature of 25°C to 30°C, relative humidity ranging from 60-75% and a photoperiod of 16 hours light and 8 hours of darkness.

Bacillus thuringiensis var *kurstaki*: The preparation of different doses of biopesticide (Biobit DF-E-phy) treatment was made from a commercially available powder, consisting of a freeze-dried bacterial strain, as described by the producer. In this study, two tests were carried out. Test 1: the biopesticide was used added only at distilled water, three concentrations were chose BH1 (0.25 g/0.5l), BH2 (0.50g/0.5l) and BH3 (0.75 g/0.5l). The test 2: we added 1% of dextrin and distilled water at each concentrations (BHS1; 0.25 g/0.5l+1% dextrin, BH2:0.50g/0.5l +1% dextrin and BH3: 0.75 g/0.5l+1% dextrin.

Insecticide: The selection of the growth regulator doses the Diflubenzuron (Dimilin SC Syngenta) of this trial was made from the approved dose of 40g/hl: Three doses were choose :DIF1=0.2 g/l, DIF2=0.4 g/l, DIF3=0.6 g/l.

Treatments

The 4th instar larvae were spread over 10 boxes of experiments at 5 individuals per box with three repetitions to avoid cannibalism. In each box, 80g of culture medium was put. The control groups were treated only with sterile distilled water. To determine the daily mortality rate after treatment, the dead individuals both in treated and in control series sets was counted within 14 days of observation.

Calculation of percentage mortality

Percent mortality was calculated for reated larvae of *E. ceratoniae* compared to control by the formula of Abbot (1925).

Data analysis

To estimate the efficacy of each treatment we calculated LC50 (fifty percent lethal concentrations) and LT50 (time required to achieve 50% mortality) by Probit-analysis (Finney, 1971).

RESULTS

1. Effect of *B. thuringiensis* var kurstaki on 4th instar larvae of *E. ceratoniae*

The corrected cumulative mortalities on the larvae (L4) appeared weak during the first days after treatment especially for the dose BH1; the mortality rate increased slightly to reach its maximum (100%) at the end of day 12 for dose BH2; and after the 11th day for the dose BH3. However, the dose BH1 showed a rate of 86.73% for the 14th day. It was found that the corresponding mortalities dose BH3 were greater than BH1 and BH2 doses. Indeed mortalities recorded exceed 50% after the 3rd day and were of the order of 99% after the 10th day. Daily observations allowed us to note also that the first mortalities occurred on the first day after treatment with the bacteria (Figure 1).

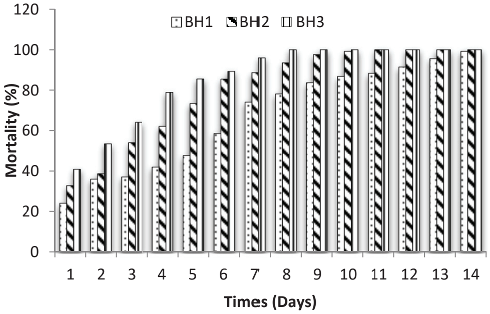

Figure 1. Larval mortality rate of the 4th instar larvae of *E. ceratoniae* treated with *B. thuringiensis* var *kurstaki*

1. Effect of *B. thurigiensis* treatment associated with dextrin on 4th instar larave of *E. ceratoniae*

The mortalities caused by the first dose (BHS1) ranged between 24.16% and 78.19% during the first week after treatment, reaching 99.12% at the end of the last day of monitoring. The percentages of deaths recorded by the dose BHS2 were in the order of 32.88% for the first day increased slightly to reach 50% from the 3rd day; the maximum rate was 100% mortality wasobtained after the 11th day. The third dose BHS3 recorded a rate of 40.94% from 1 day to reach 100% by Day 8. It should be noted that mortalities recorded with the addition of 1% of dextrin are relatively higher compared to one biopesticide (Figure 2).

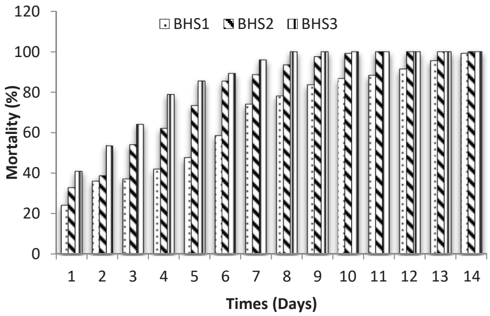

Figure 2. Mortality rate of the 4th instar larvae of *E. ceratoniae* treated with *B. thuringiensis* var *kurstaki* associated Dextrins

An examination of Figure 2 shows a significant evolution in daily mortalities recorded after 14 days of observation. BHS1 dose proved less virulent by comparing it with the other two doses. Indeed, registered mortality peaks are of the order of 24.16% at the 1st day remain between 73.48 and 85 61% for the 5 days following treatment, they remain between 60% and 80% for days with the exception of 14 days or there is a total mortality is 100%.

Parallel both doses BHS2 and BHS3 do not appear to exhibit wide variations there between, it should be noted that the peaks of mortalities were obtained after the 9th day after ingestion of the product associated with 1% of dextrin.

3. Effect of Diflubenzuron on the 4th instar larvae of *E. ceratoniae*

Mortalities were insignificant for DIF2 and DIF3 doses during the first three days after treatment application, and exceeded the fourth day dose DIF1. The latter causes a mortality that ranges from 1.33% for the first days or a rate of about 1.33% to 53.33% and 5th day after the 14th day. However, a percentage of about 3.33% was recorded from the 4th day for the dose DIF2, the latter increases to reach 68.89% after the 14th day. The DIF3 has a mortality rate mortality rate of around 74.81% and after 14 days of follow-up. It should be noted that mortalities percentages recorded for this insecticide or growth regulator are not very important and those for the three doses tested. DIF3 dose has superior mortalities those doses DIF1 and DIF2 (Figure 3).

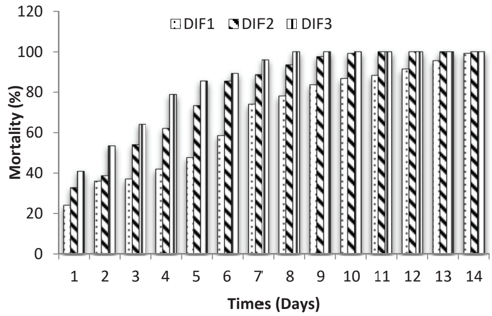

Figure 3. Mortality rate of the 4th instar larvae of *E. ceratoniae* eller treated with Diflubenzuron

The highest toxicity is obtained with the bacteria added to the dextrin (0.13g/0.5l) and then only with the bacteria (0.23g/0.5l). The lower toxicity is obtained with diflubenzuron (1.03g/0.5l).

Table 1. LC50 values of the treatments applied in larvae of the fourth stage of *E. ceratoniae*

Traitements	BH	BHS	DIF
LC50 (g/0.5l)	0.23	0.13	1.03

The review of correlation coefficients showed that they were significant for all products and doses used except dose D1du Diflubenzuron, with relatively low value or R^2 = 0.492. The mortality rate and time vary in the same direction (Table 2).

The table 6 brings together the LT50 which are calculated from the regression line equations of each dose tested in the 4th instar larvae of *E. ceratoniae* Zeller. These show that the highest LT50 14.2 days is recorded by the dose (DIF1); by against the weakest time of 1.81 days is observed for the dose (BHS3).

Table 2. LT50 values in larvae of 4th stage of *E.ceratoniae*

Concentrations	LT 50 (days)
BH1	6.09
BH2	2.92
BH3	2.35
BHS1	3.16
BHS2	2.15
BHS3	1.81
DIF1	14.2
DIF2	11.6
DIF3	10.71

DISCUSSIONS

The results showed that the toxicity of the entomopathogenic: *B. thuringiensis* var *kurstaki* for the 4th instar larvae of the moth dates is greater than that of the insecticide Diflubenzuron. It can be returned to the mode of action the latter, whose ingestion crystals *B. thuringiensis* var *kurstaki* containing endotoxin causes direct mortality on the larvae against Diflubenzuron takes time; these results are confirmed by Jardak and Ksantini (1986), they stated that the integrated control framework, control test against the 3rd generation *Prays oleae*, *Bacillus thuringiensis* resulted a larval mortality rate approaching 50%, while that diflubenzuron net effect was not compared to the control. However, control of the pupae in the traps corrugated showed some effectiveness of this product tested which result in a reduction of emerging populations of about 74-79% compared to the control. It should be noted that *B. thuringiensis* affects only the larval stages, while Diflubenzuron has a slow action that runs until pupation stage (pupae mortality can reach 40%).

The bacterial strain of *B. thuringiensis* has proven very virulent against *E. ceratoniae* causing larval mortality up to 86.73% and 100% thereof are in agreement with the results obtained by Jerraya (2003). In examining the results from treatments with bacteria alone or associated with dextrin, we note that the mortality rate observed in our experiment is justified as follows: the scale and speed of poisoning vary with the stage we examined larval mortality noting that the latter is strongly evident from the first day after treatment.

According noticed mortalities during the single *Bacillus* tests are assumed due mainly to the effect of the ingestion of the crystals of this bipopesticide containing endotoxin. This hypothesis just been arguing with the studies of other authors in particular those of: Frankanhuyzen et al. (1992). Which report that the *B. thuringiensis* kurstaki is effective against caterpillars of various lepidopteran pests in fruit trees and forest crops. It is one of the best selectively toxic strains for a wide variety of lepidopteran larvae. For his part, Abdel Razek (1998), used formulations of *B. thuringiensis* and var. *indiana*. and var. *morrisoni*, the latter have proven effective with respect to *Cadra cautella* Walker and *Tribolium confusum* Duval respectively. Moreover Saadawi et al., (2007), showed a hyper toxicity of a strain of *B. thuringiensis* var *kurstaki* on insect larvae which allowed him to confirm that this strain has a hyperactivity against them. Our results are comparable to Zouiouech and Rahim (2008), who tested a biopesticide from the same family ie Dipel 8L, the results they led, shows high efficiency of this biological insecticide on hatched larvae (L1). Similarly Kardi and Rouici (2007) showed in laboratory conditions that the sputtering of a bacterial suspension after a local strain based *B.thuriengiensis* applied to the artificial culture medium for the larvae feed of neonates *E. ceartoniae* causes mortality percentages of about 64% in L3 and 40% in 5th instar larvae of *E. ceratoniae*.

Moreover Dhouibi (1992), noted that mortality among this same pest occurs at any larval stages but it is very important for young stages and even older caterpillars die when she received the lethal dose, the caterpillar reached by *B. thuringiensis* stops eating due to metabolic disruption while causing narrowing of the body of the larvae and this bacterium has the advantage of being compatible with the auxiliaries and other biological agents, on the other hand he noticed the Bactospein does not present a knockdown against E.ceratoniae.

Simpson et al., (1971), Gary and Karl (1992) and quoted by Charles Coderre (1992), reported that *B. thuringiensis* toxin produced several important effects, including death of the larvae before moulting and the cessation of all power after moulting, sub-lethal doses generally produce teratology including malformations of the mouthparts, eyes, antennae and wings. According to Taoufik, (1993) cited by Brahmi, (1998), after the inclusion of endotoxin by the insect, the protease active protoxin which then causes swelling in the digestive tract cells; therefore breaks at the perithrophic membrane with a destruction of the basement membrane, which opens the way for the invasion of pathogens and other secondary parasites found in the digestive tract, they causes paralysis of midgut in general and induces the word of the insect.

The toxicity of the growth regulator diflubenzuron showed relatively low mortality rates for the three doses tested; D1, D2 and D3, on the larvae of the moth stage (L4) relative to the testing of the tested entomopathogenic. Given the unavailability of bibliographical works available on this growth regulator against borer dates, we sum focused to compare our data with the literature on other pests.

The mode of action of this insect growth regulator reduced significantly the lipids contents, the proteins and carbohydrates from the 4th day after ingestion. Boudebous and Djoumeh (1995) demonstrated this effect on adult female *Tenebrio molitor* treated by ingestion of 10 mg/g. Soltani-Mazuni (1994) its side observes of the same alterations for pupae of this beetle.

At the base of doses applied on L4 larvae *Ectomyelois ceratoniae* Zeller, CPA has identified four groups with the doses tested, including the Prime group (Gp1) and the fourth group (Gp4) are not correlated with the period treatment, the second group (Gp2) correlated to the last days of monitoring mortality, and the third group (Gp3) correlated to the early days the application of biopesticide.

CONCLUSIONS

This work mainly devoted to the study and assessment of toxicity compared to a strain of a marketed entomopathogen *B. thuringiensis* alone or with 1% dextrin added and a growth regulator Diflubenzuron on the 4th instar larvae of *E. ceratoniae,* a potential pest of dates.

It was found that the corresponding mortalities high doses are greater than the middle and low doses. The highest adjusted mortality relate to corresponding doses 3 and those for the three products which were the subject of our follow.

The LD50 of *B. thuringiensis* treatment was 0.23 g/0.5l; and 0.13 g/0.5l when dextrin was added. For Diflubenzuron the value of LD50 as 1.03 g/0.5l.

It seems interesting to us to see the action of these entomopathogenic on target cells, thereby broadening of such treatment for applications on date-palms for sustainable phoeniculture in environmental conservation framework and biodiversity. It can be concluded that the doses currently used in the field to these insecticides could be reduced to an economic interest and for good protection of the environment against pollution.

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