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Evaluation of *Bacillus thuringiensis aizawai* and Neem for Controlling the Larvae of the Greater Wax Moth, *Galleria mellonella* (Lepidoptera: Pyralidae)

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ABSTRACT

Third instar larvae of *Galleria mellonella* were fed for five days on beeswax dipped for 20 sec into 0.5, 1 and 2 g/L water concentrations of the commercial preparations of *Bacillus thuringiensis aizawai* viz. XenTari® and 20, 40 and 80 ppm azadirachtin A, viz. NeemAzal®-T/S. The larvae were then given *ad libitum* access to untreated artificial diet. Effects on wax consumption, larval growth and mortality were determined. The speed of action of the two bio-insecticides was determined as well. Beeswax treated with NeemAzal T/S was consumed at all concentrations tested uniformly; on the contrary XenTari-treated wax (20 mg/L) was almost not consumed. The growth of larvae was delayed by the treatments, especially in XenTari. The highest concentration of NeemAzal T/S (80 ppm) caused 100% mortality within a month; while XenTari (20 ppm) reached 68% corrected mortality within 10 days (whereas NeemAzal T/S had induced 25% corrected mortality). The final corrected mortality, reached by XenTari (20 mg/L), after four weeks, was 77%. The emergence of moths was delayed by four days in XenTari-treatments, even when no mortality occurred. The significance of the findings for the protection of honey bees is discussed, concerning the efficacy against the greater wax moth and the additional effect on the *Varroa* mite.

Key Words: NeemAzal®-T/S; XenTari®; *Galleria mellonella*; Beeswax; *Varroa* mite

INTRODUCTION

The Greater Wax Moth, *Galleria mellonella* (L.), is a worldwide serious honey bee pest, especially in warm climate regions of the world (Calvert, 1982; Hachiro & Knox, 2000). Larvae are mostly found in old combs of honey bees, feeding on beeswax, wax residues of honey, insect skin and pollen (Hachiro & Knox, 2000). The larvae of the wax moth cause considerable damage to unattended combs by bees and to combs in storage (Caron, 1992). Adults and larvae are capable of transferring serious bee diseases, such as foulbrood (Charriere & Imdorf, 1999). Several chemical and non-chemical methods have been developed to control wax moth in stored combs including freezing, heating and CO_2. The chemicals include fumigants, such as *P*-dichlorobenzene (PDB), acetic acid, calcium cyanide, ethylene dibromide, methyl bromide and phosphine (Hood *et al*., 2003). Recently, male sterile technique was used to control the greater wax moth (Jafari *et al*., 2010). Despite all these efforts, a safe, low-cost method of wax moth control is needed, especially for commercial beekeepers operating in the tropics and subtropics. Therefore, alternatives are to be used. The *Bacillus thuringiensis aizawai* (*B.t.a.*) is registered now in the European Union as Mellonex®. However, since several neem products were shown to be not harmful to bees (Schmutterer & Holst, 1987; Naumann & Isman, 1996; Leymann *et al*., 2000) and were used to protect bees against the *Varroa destructor* (Liu, 1995; Melathopoulos *et al*., 2000). It was of interest to compare the effects of both *B.t.a*. and Neem on *G. mellonella.* The *B.t.a*. was effective against several Lepidoptera (Tabashnik *et al*., 1994; Liu *et al*., 1998), and against *G. mellonella* (Li *et al*., 1987). The objective of this study was to use B.t.a. and neem as alternatives to synthetic insecticides against the *G. mellonella* that damage honey bee combs both in the hives and during storage.

MATERIALS AND METHODS

Rearing of *G. mellonella* larvae: Larvae of *G. mellonella* were kept at 32°C for 24 h in an incubator in darkness. They were fed on Haydak diet consisting of oat flakes, honey, glycerol, yeast, milk powder and vitamins (Haydak, 1943). Before treatments, larvae were starved for 24 h.

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Test materials: NeemAzal®-T/S, a commercial formulation, containing 1% Azadirachtin A, produced by Trifolio-M GmbH company Lahnau, Germany, was used at three different concentrations of 20, 40 and 80 mg/L Azadirachtin A. The subspecies of *B.t.a.* was chosen, since B.t. (*kurstaki*) has been shown to be ineffective against *G. mellonella* (Vandenberg & Shimanuki, 1990) and the B.t.a. proved to be more efficient against several lepidopteran species than B.t. (*kurstaki*) (Schnepf *et al*., 1998). A commercial preparation (XenTari®) was used, which is recommended to be used with 0.6 to 1 kg/ha. We used suspensions of 0.5, 1 and 2 g/L water containing 0.1% TritonX-100 as non-ionic detergent. The active ingredient in XenTari is a pure, potent strain of *B.t.a*, which composed of protein crystal and viable endospores. Xen Tari is produced by Valent Bioscience Corporation, USA.

Treatment of *G. mellonella* larvae with bio-insecticides: Square plates (18.5 cm²) of beeswax (2 g) were dipped for 20 sec into the respective concentrations of XenTari® and NeemAzal®-T/S as mentioned above and then allowed to dry for 20 min on a clean metal grid. Twenty four larvae were used for each treatment and were kept in 12 glass jars (two larvae per jar) to avoid cannibalism. In spite of this precaution, cannibalism occurred and sometimes only 20 out of 24 larvae remained. A round glass jar of 430 mL (10 cm high & 8 cm in diameter) closed with a metal lid was used to keep every two larvae. The lid has a circular opening with 1.7 cm in diameter, covered by metal gauze. A filter paper, daily moistened with tap water, was used to maintain the humidity. The larvae were fed on treated beeswax for 5 days followed by free-feeding on untreated diet, while larvae of control were fed with wax dipped for 20 sec into tap water containing the same concentration of Triton X-100. Larvae were weighed initially and daily until they reached the pre-pupal stage. The treated beeswax was weighed for the first five days, prior to the addition of artificial diet. Mortality of larvae and pupae was recorded daily over a period of 30 to 40 days as well as up to the time required by adults to emerge.

There was an unavoidable larval mortality of 24 to 27% in the untreated control presumably caused by microbial contamination. However, it was corrected according to Schneider-Orelli (1947).

Statistical analysis: In all experiments, treatments were replicated 12 times and the non-parametric tests (Kruskall-Wallis, Mann-Whitney) were used to analyze the data.

RESULTS

Consumption of treated beeswax by the larvae of *G. mellonella:* Table I and II show the amount of beeswax consumed by *G. mellonella*. The larvae which were feeding on the untreated beeswax consumed most: the six- to 10-fold of their initial weight (in the neem- & *B.t.a.*-experiment, respectively), within 5 days. In all neem-treatments, larvae consumed more than the five folds of their initial weight (Table I), while in B.t.a-treated wax, the feeding declined with the concentration: The 5.5-fold of the initial body weight in the lowest concentration of XenTari (0.5 g/L), 3.3-fold in the second concentration (1 g/L) and the 1.5-fold in the highest concentration of XenTari (2 g/L), within five days.

Growth of larvae after feeding on the treated beeswax: Table I show that the growth of larvae, when fed for five days on neem-treated beeswax, was retarded depending on the concentration, whereas the larvae in the control grew well. Larval growth was less in NA 20 and 40 mg/L, while in NA 80 ppm it was negative after five days and only slightly positive after nine days.

In the experiment with XenTari (Table II) larval growth was much more retarded than in the neem experiment, while it was considerable up to nine days in the untreated control. Larval weight diminished when XenTari-treated beeswax was given. At 0.5 and 1.0 g/L larval growth recovered after nine days, but at 2 g XenTari per litre the decline in larval weight continued.

Larval and pupal mortality: Table I shows that the treatment of beeswax with NeemAzal T/S at different dosages resulted in retarded growth and death of larvae and pupae of *G. mellonella* and the mortality was concentration-dependent. The corrected mortality increased from 63% at 20 ppm via 69% at 40 ppm to 100% at 80 ppm NeemAzal T/S.

Table II shows that the lowest concentration of XenTari (B.t.a.) (0.5 g/L) had no lethal effect on larvae of *G. mellonella*, while the medium concentration (1 g/L) caused a 25.0% corrected mortality, and the highest concentration (2 g/L) resulted in 77.1% corrected mortality at the end of the experiment.

The speed of action of the two bio-insecticides: In the neem treatment, it took four weeks until the final mortality of *G. mellonella* was reached and the slope of increase was rather flat. However, the curve was quite different in the treatment with *B.t.a*. (Fig. 1), where the slope of increase was initially rather steep. Thus, XenTari at 2 g/L induced a mortality of 71% (67.8% corrected mortality) after ten days, adding up to a corrected mortality of 77.1% after 32 days). At the same time, NeemAzal T/S at 80 ppm showed a mortality of 29%, only, adding up to 100% after 32 days.

The effect on eclosion of surviving moths: In the treatments with NeeemAzal T/S few individuals survived, and no differences were observed in the time of adult eclosion. But in the treatments with XenTari, the delay in larval growth (Table II) resulted in a delay of eclosion of surviving moths, when compared with the untreated control (Fig. 2). It is remarkable that also the treatment with the lowest concentration, which did not induce mortality, delayed the emergence of adults. The eclosion of adults, from immature stages fed with XenTari-treated beeswax, started four days after eclosion from the untreated control, where more than 50% of adults had already emerged.

Table I: Effects of feeding beeswax treated with NeemAzal T/S (NA) at different concentrations on the larvae, pupae and adults of the greater wax moth *G. mellonella*

Parameter	Untreated (n = 22)	NA 20ppm (n = 24)	NA 40ppm (n = 24)	NA 80ppm (n = 24)
Initial larval weight (g)	0.048	0.042	0.057	0.073
Weight of consumed Beeswax within 5 days (g)	0.33	0.24	0.29	0.37
Larval weight gain after 5 days (g)	+ 0.005	+ 0.003	+ 0.002	- 0.006
Larval weight gain (g) after 9 days with added (day 5) artificial diet *ad lib*.	+ 0.063	+ 0.038	+ 0.035	+ 0.007
Ratio of consumed beeswax per initial larval weight (after 5 days)	6.875	5.714	5.088	5.068
Number of dead larvae after 30 days	6	15	18	23*
Number of dead pupae after 40 days	0	3	1	1
Number of emerged adults after 33 days	16	6	5	0
Corrected larval mortality (%)	-	63.3	69.4	100.0

*Significant difference from untreated at p = 0.01 (Kruskall-Wallis-test)

Table II: Effects of feeding beeswax treated with different concentrations of *B.t.a.* on the larvae, pupae and adults of the greater wax moth *G. mellonella*

Parameter	Untreated (n = 21)	*B.t.a.* 0.5 g/L (n = 20)	*B.t.a.* 1 g/L (n = 21)	*B.t.a.* 2 g/L (n = 23)
Initial larval weight (g)	0.028	0.028	0.034	0.044
Weight of consumed beeswax within 5 days (g)	0.278	0.154	0.113	0.067
larval weight gain after 5 days (g)	+ 0.003	- 0.004	- 0.012	- 0.031
Larval weight gain (g) after 9 days with added (day 5) artificial diet *ad lib*.	+ 0.054	+ 0.022	+ 0.001	- 0.029
Ratio of consumed beeswax per initial larval weight (after 5 days)	9.93	5.50	3.32	1.52
Number of dead larvae after 30 days	5	4	9	19*
Number of dead pupae after 40 days	0	3 [1)]	3 [1)]	0
Number of emerged adults after 40 days	16	13	9	4
Corrected larval mortality (%)	-	0	25.0	77.1

*significant difference from untreated at p = 0.01 (Mann-Whitney-test)

1) Death of some late pupating larvae was not induced by XenTari, but by mould originating from the artificial diet, infecting the silken cocoons of the pre-pupae

Fig. 1: Cumulative uncorrected mortality (%) of *G. mellonella* larvae fed on beeswax treated with XenTari and NeemAzal-T/S

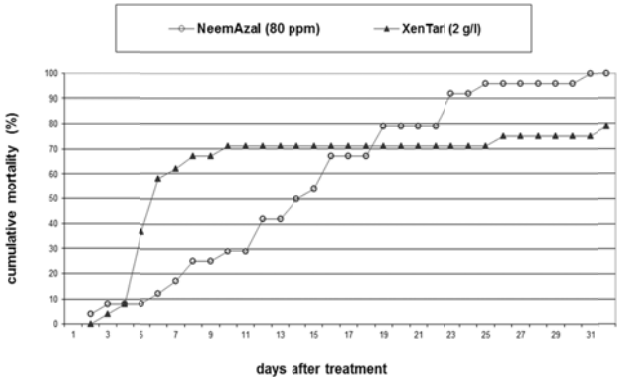
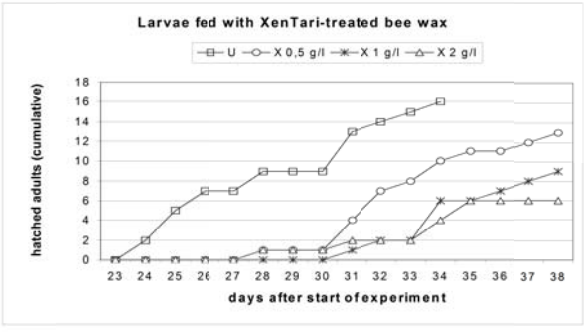


Fig. 2: The time of hatching of adult wax moths after the larvae had been feeding on XenTari-treated beeswax, as compared with "untreated" (U)



DISCUSSION

In the experiment with XenTari, a late low mortality of pupae was observed, which could not be attributed to XenTari (no mortality of pupae was noticed at the highest concentration). At the end of the experiment, mould originating from the synthetic diet infected the silk cocoons of the late pupae, so that no cleaning was possible without damaging them. It cannot be excluded that pupae had been weakened by the XenTari-food, so that they were affected by mould. But since this could not be confirmed, this pupal mortality was not added to the larval mortality, which is caused by *B.t.a.* (XenTari).

The B.t.a. (Mellonex®) is recommended and registered in the European Union to protect inhabited bee hives or empty combs against *G. mellonella*, However, the results from our investigation indicate that B.t.a. is required in the highest concentration (20 ppm) since only this concentration resulted in a 77.1% larval mortality (Table II). The lowest concentration of XenTari used in the present experiments proved to be sub- lethal. If the B.t.a. is to be used, it must

not be used too often, to avoid the wax moth resistance (Shelton *et al.*, 1993; Gaughey & Johnson, 1994). Thus, ways have to be found to avoid unnecessary applications of B.t.a. Though a treatment with NeemAzal T/S® takes four weeks to reach 100% mortality, the larvae do not feed a lot in this time, and NeemAzal T/S has the advantage of a higher efficacy than XenTari and of controlling simultaneously the *Varroa* mite *Varroa destructor* (Liu, 1995; Melathopoulos *et al*., 2000), without being harmful to bees (Leymann *et al*., 2000). When Xen Tari was applied with NeemAzal-T/S against the 4th instar larvae of *Spodoptera exigua*, a mortality of 99% was reached within 5 days (Basedow *et al*., 2008). This could also be applicable in the case of the greater wax moth. Combination of NeemAzal with Xen Tari seems to be a very good tool for partly replacing the use of synthetic insecticides to control the wax moth. This will also reduce the risk of further rise of resistance in the greater wax moth due to application of Xen Tari alone. In the light of the above-mentioned, the registration of NeemAzal T/S and Xen Tari for protection of honey bees against wax moths and *Varroa* mite should be considered.

In conclusion, both NeemAzal-T/S and Xen Tari proved to be boirational insecticides and can be safely used for the control of greater wax moth and varroa mites infesting honey bee colonies, particularly in organic beekeeping.

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