

# MANAGEMENT OF THE GREATER WAX MOTH *GALLERIA MELLONELLA* WITH NEEM AZAL- T/S, IN THE LABORATORY AND UNDER SEMI-FIELD CONDITIONS

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## Abstract

Different concentrations of Neem Azal-T/S were used in an artificial diet, to study the mortality of the greater wax moth *Galleria mellonella*. A Neem formulation and different ages of natural beeswax combs were used for the effective management of the wax moth. While the diet was being prepared, Neem Azal-T/S was directly added ensure that the Neem formulation was distributed evenly in the diet at concentrations of 0.25, 0.5, 1, 2, and 4%. The obtained results showed that the different concentrations of Neem Azal-T/S mixed in the prepared artificial diet had a significant efficacy against the tested 2<sup>nd</sup> instar larvae. An outstanding elongation of the 2<sup>nd</sup> larval instar was clear in comparison with the control, even at the least tested concentration (0.25%). Neem Azal-T/S at 4, 2, 1, and 0.5% caused 100% mortality for all tested larvae. When using a 4% concentration, all the tested larvae died in the 2<sup>nd</sup> instar. However, when using a 2% concentration, the larvae died in the fifth instar. When using a decreased concentration of 1.0 and 0.5%, some of the larvae were tolerant and lived till the 6<sup>th</sup> instar. Feeding the larvae on beeswax combs treated with 2% Neem Azal-T/S, caused 100% mortality when fed on very old wax. When the diet was old wax treated with 2% Neem Azal-T/S, a 91% mortality was recorded. When the diet was new wax treated with 2% Neem Azal-T/S, a 90% mortality was recorded. A 4% Neem formulation caused mortality for all larvae during the first week of treatment on the different tested ages of beeswax combs.

**Keywords:** beeswax management, Neem, wax moth

## INTRODUCTION

The greater wax moth (*G. mellonella*, Lepidoptera: Pyralidae) is one of the most serious pests of honey bee colonies. Wax moth larvae feed on the wax comb in weak colonies or during the storage of wax combs in winter. The larvae cause economic loss to the beekeeping industry all over the world (Burgess, 1978; Chang & Hsieh, 1992; Haewoon et al., 1995; Charriere & Imdorf, 1997; Caron, 1999). Wax is one of the most useful products of honey bee colonies. It is used in the pharmaceutical industry and in cosmetics. Wax contains many nutrients, pollen, and honey and is attractive for many pests. The most important pest is the greater wax moth which causes serious damage (Ebadi et al., 1980). The larval stage of the greater wax moth feeds on comb wax and severely damages the wax. The

voracious nature of the larva leads to the destruction of the honey comb and then the death of weak colonies. The maximum infestation of the greater wax moth has been recorded during the summer and autumn months (Brar et al., 1985; Gupta, 1987). Several chemicals and non-chemicals have been used to control the wax moth on stored beeswax combs. Use of chemical and fumigant insecticides such as sulphur dioxide, acetic acid, formic acid, para dichloro benzene (PDCB), methyl bromide or phosphine are harmful to bee populations (Whitcomb, 1967; Calderone, 2000). Some chemicals used for wax moth control, such as PDCB, contaminate honey bee products, such as honey and wax (Wallner, 1991). In the autumn and winter seasons, all empty beeswax combs must be treated and protected against wax moth damage during the storage period. Botanical pesticides are an

important group of naturally occurring, often slow-acting, crop protections. These pesticides are usually safer to humans and the environment than conventional pesticides, and have minimal residual effects (Pavela, 2009).

The objective of the present study is to screen different concentrations of Neem Azal-T/S against the larval mortality of the greater wax moth reared on an artificial diet. Effective management of the greater wax moth by NeemAzal-T/S was done using different aged, natural beeswax combs.

## MATERIAL AND METHODS

### *Galleria mellonella* culture

Greater wax moth larvae were collected from infested beeswax combs in a private apiary of the Belbees region, Zagazig Governorate, Egypt. The emerged adults were released in plastic jars (30x10 cm) for mating. The plastic jar contained a comprised folded paper sheets for the deposition of eggs. The larvae were reared on an artificial diet according to Metwally, 2013 under laboratory conditions of  $28\pm 2^{\circ}\text{C}$ ,  $65\pm 5\%$  R.H, and a 16:8 (L:D) photoperiod.

All experiments and rearing were conducted at constant temperature of  $28\pm 2^{\circ}\text{C}$ ,  $65\pm 5\%$  R.H, and under a 16:8 (L: D) photoperiod. The methods used to prepare and dispense the artificial diet in the containers, and placement of larvae onto the diet, were done according to Büyükgüzel et al. (2010).

### Preparation of the artificial diet, and beeswax Artificial diet

In the first experiment, newly hatched larvae of the greater wax moth were reared on a semi-natural diet. The diet consisted of: wheat flour 350 g, corn flour 200 g, milk powder 130 g, packing yeast powder 70 g, honey 100 ml, and sorbitol 150 ml (Metwally, 2013). These jars were incubated under the previously mentioned conditions till larvae reached the target or proposed instars.

### Beeswax

In the second experiment, different aged combs of beeswax, one month (new wax), one year (old wax), and more than one year (very old wax) were selected as natural diets for feeding

wax moth larvae. Every beeswax comb was cut into pieces (5x5 cm). Every piece of beeswax was put in a plastic container (20x10x10cm). One larva of the greater wax moth (2<sup>nd</sup>) was used for each container. Two concentrations of Neem Azal-T/S (2% and 4%) were sprayed on each tested beeswax piece in the containers. The rest of the beeswax pieces were without treatment and used as the control. Ten larvae or more were used for each wax type and Neem concentration. Five larvae/each wax type acted as the non-treated control.

### Neem formulation

The Neem formulation (Neem Azal-T/S with an azadirachtin content of 1%) was obtained from Trifolio Co., Lahnau, Germany. Neem Azal-T/S was directly put into the prepared diet during preparation to ensure that the Neem formulation at concentrations of 0.25, 0.5, 1, 2 and 4% was evenly distributed in the diet. Larvae reared on a diet without Neem were used as the control in all the experiments. The first experiment was repeated 5 times. For the first experiment there were 4 larvae/each replicate at concentrations 0.25 and 0.5%. In the second experiment, 11 larvae/each replicate were used in 1, 2, and 4% concentrations. Again 5 replicates were done for the control larvae (4 larvae in each replicate).

### Experimental procedures

#### 1- On egg stage

A restricted number of eggs were sprayed with the following concentrations of Neem Azal-T/S (0.25, 0.5, 1.0, 2.0, and 4.0%) using an atomiser. The eggs were left to dry and then put under the above-mentioned controlled conditions of temperature and relative humidity, until hatching. The percentage of hatching was estimated.

#### 2- On larval stage

The experiments were started with 2<sup>nd</sup> instar larvae which were reared on 10 grams of the artificial diet till the sixth larval instar stage. This diet was composed of: wheat flour 350 g, corn flour 200 g, milk powder 130 g, packing yeast powder 70 g, honey 100 ml, and sorbitol 150 ml (Metwally, 2013). The larval diet was changed 2 times during the development of the larva. The survivorship of each larval instar was recorded and any abnormalities were registered. The

Table 1  
Effect of different concentrations of Neem Azal-T/S on some biological aspects of *Galleria mellonella* reared on the treated diet

Neem Azal-T/S concent.	Hatchability of eggs	duration of larval stage in days						duration of pupal stage in days	% adult emergence	% abnormality
		2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	6 <sup>th</sup>			
The control	98.3±1.67 a	3.71±0.18 b	4.14±0.14 c	4.43±0.20 d	4.29±0.29 a	4.29±0.29 c	6.71±0.42	100	0.00	
0.25%	99.291±0.70a	12.75±2.75 a	6.33±0.33 bc	6.67±0.33 cd	9.67±4.67 a	12.6±1.20b	16.3±0.88	100	100.00	
0.5%	97.3±2.67 a	16±4.06 a	8.67±2.67 bc	12.33± 2.1 bc	11.5±0.5 a	15±0.0 b	-	-	-	
1%	96.37±2.202 a	12.3±2.55 a	15±2.57 a	27.6±3.84 a	8.3±2.2 a	61±9.0 a	-	-	-	
2%	97.198±2.13 a	15.7±2.29 a	10.25±0.48 ab	15±2 b	5±0.0 a	-	-	-	-	
4%	96.43±0.94 a	19±2.13 a	-	-	-	-	-	-	-	
F value	0.35	4.706	7.51	33.33	2.32	96.59	128.52	-	-	
Sig.	0.87	0.002	0.00	0.00	0.12	0.00	0.00	-	-	

Treatment means followed by the same letter are not significantly different from each other.

sixth instar larvae (determined according to days) were transferred into a filter-paper lined jar to provide a dry surface for pupation and adult emergence.

**Statistical analysis**

All the different data obtained were subjected to analysis of variance (ANOVA) using the SPSS (Statistical Package for the Social Sciences) computer program. The means were compared using Duncan's Multiple Range Test.

**RESULTS**

**1- Effect of Neem Azal-T/S formulation on the egg stage**

The data obtained in Table 1 show that the percentage of egg hatching was not affected by the different concentrations of Neem formulation used. The majority of eggs hatched even when using the highest concentration (4%). There were no significant differences noted when using the different concentrations of Neem in comparison with the control.

**2- Activity of Neem Azal-T/S formulation against the greater wax moth larva**

The results obtained in Table 1 show that the different concentrations of Neem Azal-T/S mixed in the prepared artificial diet, had significant efficacy against the 2<sup>nd</sup> instar larvae tested. An outstanding elongation of the 2<sup>nd</sup> larval duration is clear in comparison with the control. Even at the least concentration tested (0.25%), the second larval period elongated significantly in comparison with the control (12.75 days compared with 3.71 days for the control larvae. Also, the other larval instars increased significantly in comparison with the control. The total larval duration was elongated significantly, to be 16.3±0.88 days in comparison with the control (6.71±0.42 days), when using the least amount of concentration of 0.25%. Increasing the concentration from 0.5% to 4.0%, the larvae given the treated diet died without being able to moult to the next instar. In the case of using a 2.0% concentra-

tion, the larvae provided with the diet treated with Neem Azal-T/S could proceed to the fifth instar but then failed to moult to the sixth instar (Fig. 1a&b). Those larvae which were provided with a diet treated with 0.5% Neem, succeeded to moult to the 6<sup>th</sup> instar with a significant elongation in the different periods. Only on using Neem Azal-T/S at a concentration of 0.25% did the larvae succeed to moult to the pupal stage. Also, the pupal duration highly significantly increased in comparison with the control. All the other tested concentrations failed to reach the pupal stage.

The results obtained in Table 1 show that the high concentrations used for control of the greater wax moth larvae (4, 2, 1, and 0.5%) caused 100% mortality for all the tested larvae. Using a 4% concentration, all the tested larvae died in the 2<sup>nd</sup> instar stage. However, using the 2% concentration, the larvae died in the fifth instar stage. When the concentration was decreased to 1.0 and 0.5% some of the larvae were tolerant and lived till the 6<sup>th</sup> instar stage. Some of the larvae succeed to the pupae stage and emerged to adults. The obtained adults were very minute and had some abnormalities (Fig. 2a&b) in comparison with the control.

The effects of two concentrations of Neem Azal-T/S (2% and 4%) on the wax moth larvae which were reared on different ages of

beeswax combs, are shown in Tables 3&4. From the results obtained, it is clear that a 2% Neem formulation caused 100% larval mortality when larvae were fed a very old wax diet. Neem formulation caused 91% larval mortality when larvae were fed old recorded wax, and new wax (90%). Only 10% of the tested larvae succeeded to the adult stages when new wax was used, but old wax gave an 8.3% survival (Tab. 3). On using 4% Neem Azal-T/S, all tested wax moth larvae died during the first week of treatment for all the tested different ages of beeswax combs. In contrast, the percentage of adult emergence reached to 100% in the control treatment for new and old beeswax, while very old beeswax resulted in an 83.3% adults emergence (Tab. 3). As shown in Table 4, the data indicated that the highest number of dead larvae which were reared on all different ages of beeswax, was in the 2<sup>nd</sup> and 3<sup>rd</sup> instar stages. Concerning the mortality of treated larvae which moulted to the pupal stage, one pupa that had been reared on new and old beeswax diets was dying.

## DISCUSSION

The above mentioned results showed, that feeding the *Galleria mellonella* larvae diets contains 0.5, 1, 2, and 4% Neem Azal-T/S caused 100% mortality. The larvae could not moult to the



Fig. 1a. An abnormal *Galleria mellonella* larvae which failed to moult to the sixth instar.

Fig. 1b. A healthy larva (the control).

next instar, while 0.25% was the lowest value of the larval mortality. The results also indicated that rearing the *G. mellonella* on the different ages of beeswax combs with 4% Neem Azal-T/S showed that all the tested wax moth larvae died during the first week of treatment. The present findings are in agreement with Surendra et al. (2010) who found that there was a variation in the larval mortality with the different concentrations of plant products used. They added that use of Neem seed extract resulted in the highest mortality rate between the tested *G. mellonella* larvae. Also, Shashidhar & Basalingappa (2000) supported the present findings where the mean larval mortality ranged from 55.33 to 92.23% with use of Neem. The toxicity of the Neem formulation against larvae of the greater wax moth may be due to the presence of tetranortriterpenoid in the Neem formulation. Basedow et al.

(2012) found that the treatment of beeswax with Neem Azal-T/S at different dosages resulted in retarded growth and death of larvae and pupae of *G. mellonella*, and that the mortality was concentration - dependent. The mortality increased from 63% at 20 ppm via 69% at 40 ppm to 100% at 80 ppm Neem Azal-T/S. Larry (2004) stated that high larval mortality with use of Neem extract might have occurred because the extract possesses insecticidal, growth regulatory, and antifeedant properties against insects. Feng & Isman (1995) pointed out that the natural mixtures of azadirachtins in Neem insecticides may usefully mitigate against the development of resistance compared to azadirachtin alone. On the contrary, Vishwas & Gowda (2006) and Yadav et al. (2012) found that Neem oil spray resulted in significantly higher overall mean damage by *G. mellonella* to stored combs (625.31 cm<sup>2</sup>)

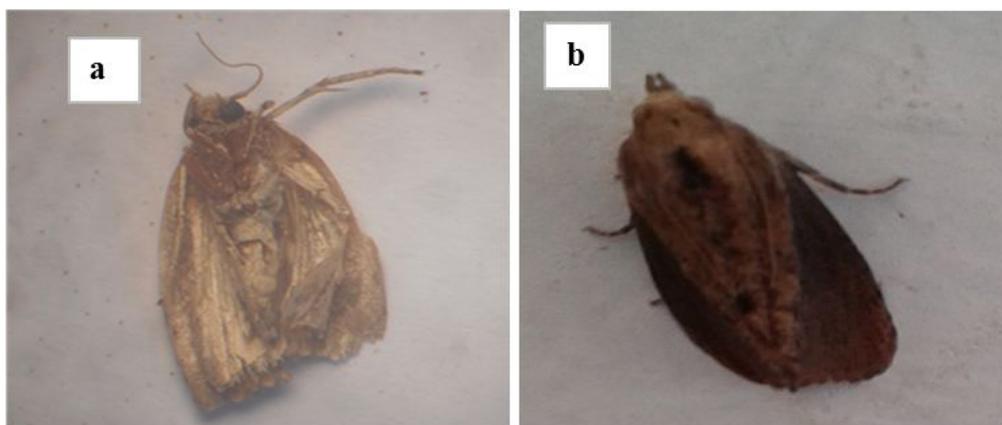


Fig. 2a. An abnormal *Galleria mellonella* adult.  
 Fig. 2b. A healthy adult (the control).

Table 2

Effect of Neem Azal-T/S on larval mortality

Neem concentrations %	Larval mortalities %					Total larval mortality %
	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	
The control	0	0	0	0	0	0
0.25	25	0	0	33.33	0	50
0.5	25	0	33.33	50	100	100
1	30.00	28.57	40.00	33.33	100	100
2	63.64	50.00	50.00	100.00		100
4	100	-	-	-	-	100

Table 3

Effect of Neem Azal-T/S on the total number of grater wax moth dead larvae and adult emergence

Days	No. of dead larvae						No. of emerged adults					
	New wax	%	Old wax	%	Very old wax	%	New wax	%	Old wax	%	Very old wax	%
2% concentration												
1-7 days	1	10%	3	25%	4	40%	0.0	0.0	0.0	0.0	0.0	0.0
8-15 days	2	20%	3	25%	2	20%	0.0	0.0	0.0	0.0	0.0	0.0
16-23 days	2	20%	1	8.3%	4	40%	0.0	0.0	0.0	0.0	0.0	0.0
24-31 days	1	10%	1	8.3%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32-39 days	2	20%	2	16.6%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40-47 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48-55 days	1	10%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
56-63 days	0.0	0.0	1	8.3%	0.0	0.0	1	10%	1	8.3%	0.0	0.0
Total	9	90%	11	91.6%	10	100%	1	10%	1	8.3%	0.0	0.0
4% concentration												
1-7 days	11	100%	11	100%	9	100%	0.0	0.0	0.0	0.0	0.0	0.0
8-15 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16-23 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24-31 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32-39 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40-47 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48-55 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
56-63 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
The control												
1-7 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8-15 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16-23 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24-31 days	0.0	0.0	0.0	0.0	1	16.6%	0.0	0.0	1	20%	3	50
32-39 days	0.0	0.0	0.0	0.0	0.0	0.0	5	100%	4	80%	2	33.3
40-47 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48-55 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
56-63 days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	0.0	0.0	0.0	0.0	1	16.6%	5	100%	5	100%	5	83.3

In the case of a 2% use of Neem Azal -T/S, the total number of tested larvae started with 10, 12, and 10 larva for new wax, old wax, and very old wax diets, respectively.

In the case of a 4% use of Neem Azal-T/S, the total number of tested larvae started with 11, 11, and 9 larva for new wax, old wax, and very old wax diets, respectively.

In the control, the total number of tested larvae started with 5, 5, and 6 larva for new wax, old wax, and very old wax diets, respectively.

Table 4

Effect of Neem Azal-T/S on the number of dead larvae in different instars

Type of wax	Total no. of dead larvae	No. of dead larvae										No. of dead pupae	
		2 <sup>nd</sup>		3 <sup>rd</sup>		4 <sup>th</sup>		5 <sup>th</sup>		6 <sup>th</sup>		2%	The control
		2%	Control	2%	Control	2%	Control	2%	Control	2%	Control		
New wax	7	3	0.0	2	0.0	0.0	0.0	1	0.0	1	0.0	1	0.0
Old wax	10	8	0.0	1	0.0	1	0.0	0.0	0.0	0.0	0.0	1	0.0
Very old wax	10	9	0.0	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

as compared with Karanj oil spray (473.2 cm<sup>2</sup>). Also, they added that dusting stored combs with Neem leaf powder was found to be the least effective treatment against the greater wax moth mortality. Also, Ahmed et al. (2014) revealed that Neem seed extract alone is not a match-effective control of *G. mellonella* while the combination of Neem seed extract + Neem leaf extract was a more effective control.

From the obtained results, it is clear that a mixed Neem Azal-T/S formulation at concentrations of 0.5, 1, 2, and 4% used in the *G. mellonella* artificial diet caused high mortality of larvae in the experiment that was done under laboratory conditions. The greater wax moth larvae fed on the differently aged beeswax combs. When the formulation of 2% Neem Azal-T/S was used, the result was 100% mortality of the moth larvae fed a very old wax diet. Larvae fed an old wax diet had a recorded 91% mortality, and feeding on new wax caused 90% mortality. The 4% Neem formulation caused mortality for all larvae during the first week of treatment on all the beeswax combs of different ages (very old, old, and new wax). The current experimental findings mean that the tested Neem Azal-T/S formulation could be used to control the greater wax moth in storage places.

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