BIOLOGICAL EFFECTS OF NEEM OIL ON GRAPE BERRY MOTHS, *Lobesia botrana* and *Eupoecilia ambiguella* (Tortricidae: Lepidoptera)

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ABSTRACT

The present study was carried out in 2005/2006 to determine the biological effects of commercial neem oils on the biology of both grape berry moths (GBM) *Lobesia botrana* and *Eupoecilia ambiguella* (Tortricidae: Lepidoptera). Results showed that, longevity of GBM varied significantly from 6.6 ±1.1 to 12.1 ± 0.6 and from 7 ± 0.9 to 17.5 ± 0.43 days for females of *Lobesia* and *Eupoecilia* at 0.1 and 1 % neem concentrations, respectively. The longevity of both GBM males was less affected by neem treatment than females. The highest reduction in longevity was for *Lobesia* females and recorded 66.5 %, while it reached 56 % for *Eupoecilia* females. Regarding the egg laying capacity, the number of eggs was significantly lower for neem-treated moths compared to control. The mean numbers of eggs / female were 49.8 and 35.4 for those treated with neem and 149 and 168 for control of *L. botrana* and *E. ambiguella*, respectively. The hatchability of eggs obtained from treated GBM females with Neemazal-T was significantly deterred, recording reductions ranged from 26.8 to 78.04 % for *L. botrana* and 30.9 to 90.6 % for *E. ambiguella*. The emergence rate was significantly decreased to 39 and 34.1 % by increasing neem doses to 1 % for *Lobesia* and *Eupoecilia*, respectively. The reduction of adult emergence ranged from 10.7 to 58.06 % in *Lobesia* at neem concentrations 0.1 and 1 %, respectively. Concerning the larval treatments, larval mortality and adult emergence of GBM were adversely affected in varying degrees when larvae were reared for the first 2 weeks of larval life on medium saturated with 0.5% of neemazal-T. Adult emergence was reduced to 18 and 27.7% for *L. botrana* and *E. ambiguella*, respectively. The effects of neem oil on 2^{nd}, 3^{rd} and 4^{th} larval instars of both GBM species were investigated at concentration of 0.5%. Second instars of both GBM species which were fed on a diet of treated with neem oil were the most sensitive to neem oil than other larval instars. Mortality percentage of 2^{nd} larvae reached 82 and 72.3 % for *L. botrana* and *E. ambiguella*, respectively. Moreover, female longevity, egg laying

capacity and hatchability of survived females were negatively affected in comparison with control.

INTRODUCTION

In many countries, a national goal in plant protection is to reduce the use of synthetic chemical pesticides. One approach toward this goal is to replace such chemicals with botanically based insecticides, such as those extracted from the neem tree, *Azadirachta indica* (A. Juss). The triterpenoids, different azadirachtins of this tree, have been recognized for their insecticidal properties, i.e., antifeedant and growth disruptant effects (Arnason *et al.*, 1985; Isman *et al.*,1990; Villanueva-Jime´nez *et al.*, 2000), as well as adverse effects on reproduction (Pathak and Krishna, 1986; Riba *et al.*, 2003; Shimizu, 1988), which have been found among several insect species treated with neem extracts. Neem oils have also been found to be oviposition deterrents to noctuid moths (Naumann and Isman, 1995) and the citrus pest *Ceratitis capitata* L. (di Ilio *et al.*, 1998), reducing egg viability in the webworm *Crocidolomia binontalis* Zeller (Rebollos, 1994) and egg hatching of bruchids (Chinwada and Giga, 1993; Reddy and Singh, 1998).

The grape berry moths (GBM) *Eupoecilia ambiguella* Hb. and *Lobesia botrana* Schiff. (Tortricidade: Lepidoptera) are the most economic pests for the viticulture. They cause considerable losses in both quality and yield of grapevine (KAST 1990). In many of the vine growing areas a striking shift of the two species has been recorded. The economic importance of GBM depends strongly on the developmental stage of the grapevine.

Extracts of neem (*Azadirachta indica* A. Juss) are used in the developing world for many purposes including management of agricultural insect pests (Nathan *et al.*, 2007).

One of the most promising natural plant compounds for insect control is neem oil (Azadirachtin). It is isolated from the seeds of the neem tree, *Azadirachta indica*. Azadirachtin has a complex structure which was initially proposed by (Zanno *et al.*1975). Seeds of *A. indica* were found to contain up to 0.48% azadirachtin (Zhang *et al.* 1992). It discourages feeding by making African Violets unpalatable to such pests as Aphids, Beetles, Caterpillars, Mealy Bugs, Mites, Thrips and Whiteflies. Neem is considered virtually non-toxic to humans. It is classified for general use by the "US Environmental Protection Agency" EPA.

Many previous studies have shown the importance of neem extracts in controlling insect pests. Insect pests which proved susceptibility to plant extracts, mainly to neem ingredients reached 413 species/subspecies belonging to 15 orders, but most of them are Lepidoptera (136), Coleoptera (79), Homoptera (50) and Diptera (49). It is estimated that about 450-500 insect species have been tested with botanical products specially neem up till now (kelany 2005). However, insufficient research has been done on the biological effects of neem on insect pests (Schmutterer, 1990; Durmusoglu *et al.*, 2003).

The aim of this work was to study the effect of neem extracts on different stages of the life cycle of both GBM species.

MATERIAL AND METHODS

2.1. Used chemicals (Neemoil)

The NeemAzal-T is used in concentrations as follows:

1. For larval treatments 0.5% was used
2. For adult treatments 0.1, 0.2, 0.5, 0.7 and 1% were used.

2.2. Laboratory mass culture of grape berry moths (GBM):

Several hundred pupae of both *Eupoecilia ambiguella* and *Lobesia botrana* were collected from the field, classified into two species and kept in a climatic cabinet at a temperature of 24°C. Emerged adults were kept separately according to species in plexi-glass cylinders with 25 cm length and 15 cm in Ø. The inner walls of these cylinders were lined with polyethylene foil, where the females lay their eggs. For the feeding of the adults serves tap water, which can be supplied to the adults by using a soaked cotton ball with water with different concentrations of neem oil as follows: of 0.1, 0.2,0.5,0.7 and 1%. Whereas for the control, served only tap water for feeding of the adults. The experiments were conducted in two separate lines in the same time for *E. ambiguella* and for *L. botrana*.

Experiments were carried out in 5 treatments, each treatment consisted of 6 replicates and each replicate included 20 adults (10 ♀: 10 ♂). The experiments carried out under 25±1 °C, 70–80 % RH and 16 h light.

2.3. Adult Treatments:

2.3.1. Longevity of the adults

The longevity of adults was estimated from emergence to death in both treated and untreated adults.

2.3.2. Egg laying capacity

The adults were left for egg laying for its entire life. For the withdrawal of the freshly laid eggs of GBM, adults were stupefied by CO2 and converted into new Plexiglas cylinders. Dead adults were segregated. The laid eggs are counted and observed up to the hatching of larvae. The average number of eggs per female was determined in each treatment.

2.3.3. Hatchability

The counted eggs are marked with a red pen, while the black head stages and hatched larvae with black. These eggs were given in plastic cups for observation.

2.3.4. Emergence potential

The foil with the GBM eggs was cut in ca. 3 cm broad strips and put on medium in an 8 cm high plastic box. The medium consisted of 17 ingredients (according to Ibrahim 2004). Before the completion of the larval development, corrugated paper was settled, in whose curvatures the developed larvae could pupate. Briefly before emergence, the pupated larvae in the corrugated paper were moved in another clean plastic box with the masses 13x21x8 cm. Two times per week, fully occupied corrugated paper with Prepupae was taken and replaced by new ones. Loose pupae from emergence boxes were collected, as well the emerged moths, the bowls were covered additionally with a very fine-mesh curtain. The adults emerging out of the rolled up cardboard can now, as are anesthetized. The covers should be likewise provided with large vents, which must be sealed with bonded gauze. Afterwards, the emerged adults were precisely counted.

2.4. Larval treatment:

The previous described medium was used after dissolve 0.5 ml neem oil in 100 ml water. This concentration is equal to the recommended dose (2 L./Feddan) for using in the field. Each artificial medium unit was surface sprayed with 2.5 ml and left to be dried. Three larval 2nd, 3rd and 4th instars of both GBM species were treated. One thousand of each larval instar of both GBM species was divided into five replicates (200 Larvae/replicate) and added separately to the treated artificial medium bowls. An untreated medium was served as control for each larval instar. Dead larvae were daily removed and counted. Briefly before pupation, 5 stripes of corrugated paper were added as pupation shelters. Later pupae were classified into males and females according to Stelwaag 1928 and kept separately in Petri dishes. Malformed pupae were segregated. After adult emergence, 24 h old healthy ones were oriented to 5 Plexiglas cylinders for egg laying. Adults were transferred daily to new cylinders in order to determine the total number of laid eggs. The tested parameters were larval mortality, adult emergence, egg laying capacity, hatchability, and longevity of the females' of both GBM species.

Statistical analysis

Statistical analysis was performed by using the statistical software *STATISTICA* V6.0 (StatSoft, 2001). After ANOVA multiple mean comparisons were made by the Tukey-HSD-Test ($P < 0.05$). Percentage data were arcsine transformed prior to analysis. Data from mortality experiments were subjected to analysis of variance (ANOVA of arcsine, logarithmic and, square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test. Mortality was corrected using Abbott's (1925) formula if it was necessary.

RESULTS AND DISCUSSION

The search for alternative ways of controlling agricultural insect pests has led to the investigation and re-examination of plant sources for naturally occurring compounds which may have toxic, repellant, anti-feedant or anti-hormonal characteristics (Thomas and Callaghan 1999). Neem has been used for centuries to control agricultural insect pests (Immaraju 1998).

One of the most desirable properties of neem is its low degree of toxicity. It is considered almost nontoxic to humans and animals and is completely biodegradable (Schmutterer 1990). Neem also works as a systemic pesticide; it absorbed into the plant and carried throughout the tissues to be ingested by insects when they feed on the plant (Vietmeyer 1992). This may make it effective against certain piercing/sucking or rasping insects such as leafhoppers and thrips (Schmutterer 1990).

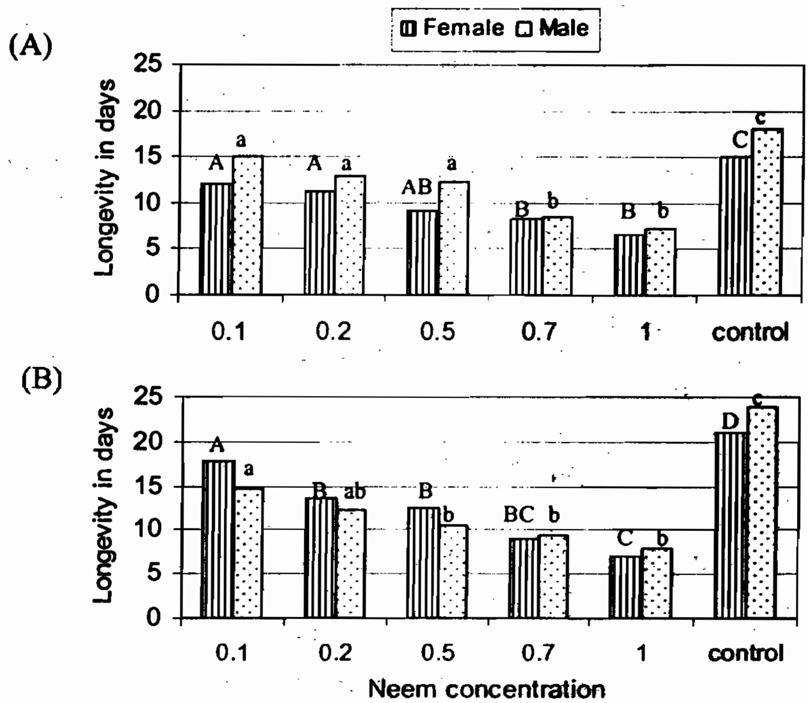

Fig. (1) Effect of NeemAzal on the longevity of both grape berry moths *L. botrana* (A) and *E. ambiguella* (B). Different letters indicate significant differences (P< 0.05, Tukey, HSD-Test).

A. Adult treatments:

1. Longevity

The longevity of both grape berry moths differed significantly depending on neem concentrations. Longevity of GBM varied significantly from 6.6 ±1.1 to 12.1 ± 0.6 and from 7 ± 0.9 to 17.5 ± 0.43 days for females of *Lobesia* and *Eupoecilia* at 0.1 and 1 % neem concentrations, respectively (Fig. 1 A & B). The longevity of both GBM males was less affected by neem treatment than females. The highest reduction in longevity was for *Lobesia* females and recorded 66.5 %, while it reached 56 % for *Eupoecilia* females. As shown in (fig 1 A & B), increasing concentration of neem negatively affected longevity of GBM adults.

2. Egg laying capacity

Figure 2 shows the effect of various neem concentrations on egg laying capacity of both grape berry moths *L. botrana* (A) and *E. ambiguella* (B). Statistical analysis showed significant differences in reductions of egg laying potential at 0.1 and 1 % neem concentrations. Reductions of *L botrana* eggs ranged from 45.6 to 75.8 %, whereas, in *E. ambiguella* eggs it ranged from 71.4 to 85.1%.

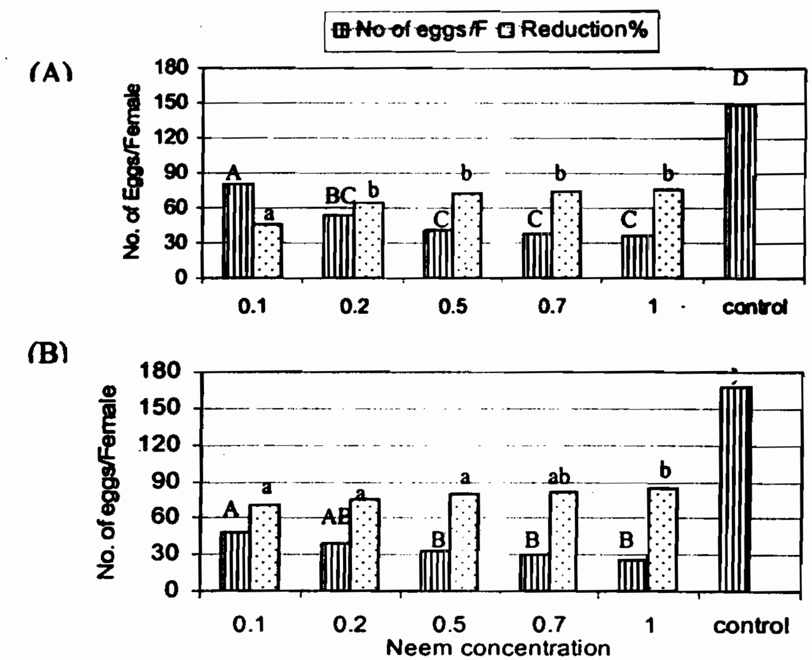

Fig. (2) Effect of NeemAzal on egg laying capacity of both grape berry moths *L. botrana* (A) and *E. ambiguella* (B). Different letters indicate significant differences ($P < 0.05$, Tukey, HSD-Test).

In general, the number of eggs was significantly lower for neem-treated moths compared to control ($P < 0.05$) (Fig. 2). The mean numbers of eggs / female were 49.8 and 35.4 for those treated with neem and 149 and 168 for control of *L. botrana* and *E. ambiguella*, respectively.

Gajmer *et al.* (2002) found that adults of *Earias vittella* fed on a neem extract-containing sucrose diet laid significantly fewer eggs with poor hatching. There was no egg laying when the moths were fed on a sucrose diet containing 6, 8 and 10% neem extracts.

Naumann and Isman (1995) found a similar reduction in oviposition by the noctuid moth Spodoptera litura on neem-treated plants. Several neem extracts have insecticidal properties on larvae, but it is not certain that all of these have repellent effects on adults. Our experiments show that Neem Azal-T is among the preparations that could be expected to have an insecticidal effect against GBM. The extracts also manifested repellent activity by reducing the number of eggs laid even when the ovipositing females were not in direct contact with the extracts.

3. Hatchability

In the present investigation, treated GBM adults with Neemazal-T deterred the hatchability by GBM, recording significantly less hatchability in treated females than the control ones. In *L. botrana*, reductions of egg hatchability ranged from 26.8 to 78.04 % at neem concentrations of 0.1 and 1 %. Whereas for *E. ambiguella*, reductions in egg hatchability were 30.9 and 90.6% at 0.1 and 1 % neem concentrations.

The results of this investigation are in agreement with the earlier findings on the ovipositional deterrent effect of Neemark on bhendi fruit borer, *E. vittella* (Sojitra and Patel, 1992; Patel *et al.* 1994). In general, egg hatchability obtained from treated females of *E. ambiguella* were more affected than others obtained from females of *L. botrana*.

Earlier, Verkerk and Wright (1993) reported that azadirachtin at 100 μg concentration effected 48 percent mortality of *Plutella xylostella* L. eggs.

Similarly, neem oil inhibited the hatchability of eggs of rice leaf folder, *Cnaphalocrosis medinalis* Guenee and *S. litura* (Saxena *et al.* 1981; Ramachandra Rao *et al.* 1990).

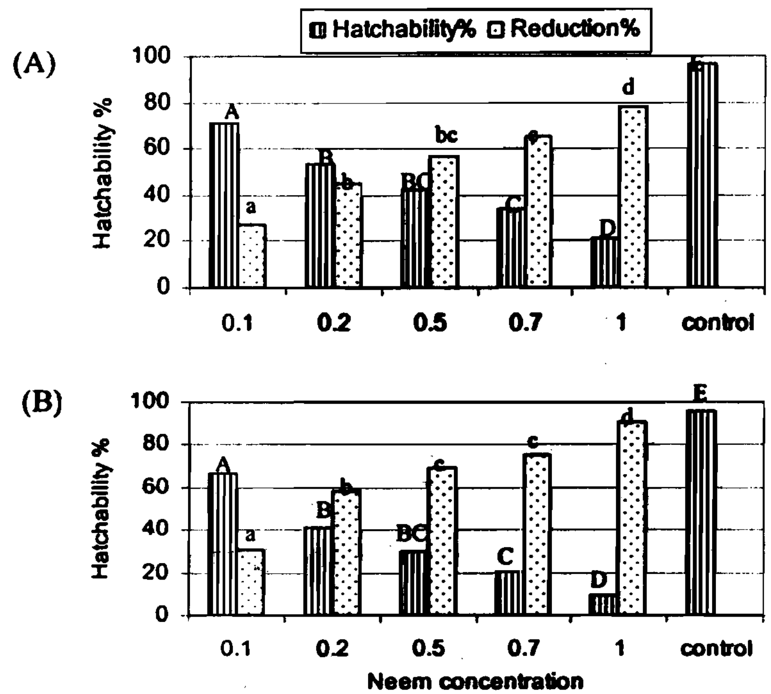

Fig.(3) Effect of NeemAzal on hatchability of both grape berry moths *L. botrana* (A) and *E. ambiguella* (B). Different letters indicate significant differences (P< 0.05, Tukey, HSD-Test).

4. Emergence potential

Emergence rates differed significantly between GBM species (P < 0.05, Tukey HSD-test). The emergence rate was significantly decreased to 39 and 34.1 % by increasing neem doses to 1 % for *Lobesia* and *Eupoecilia*, respectively. The reduction of adult emergence ranged from 10.7 to 58.06 % in *Lobesia* at 0.1 and 1 %, respectively (Fig. 3 A & B). Whereas in *Eupoecilia* it varied from 12.2 to 62.3 % at the same neem doses.

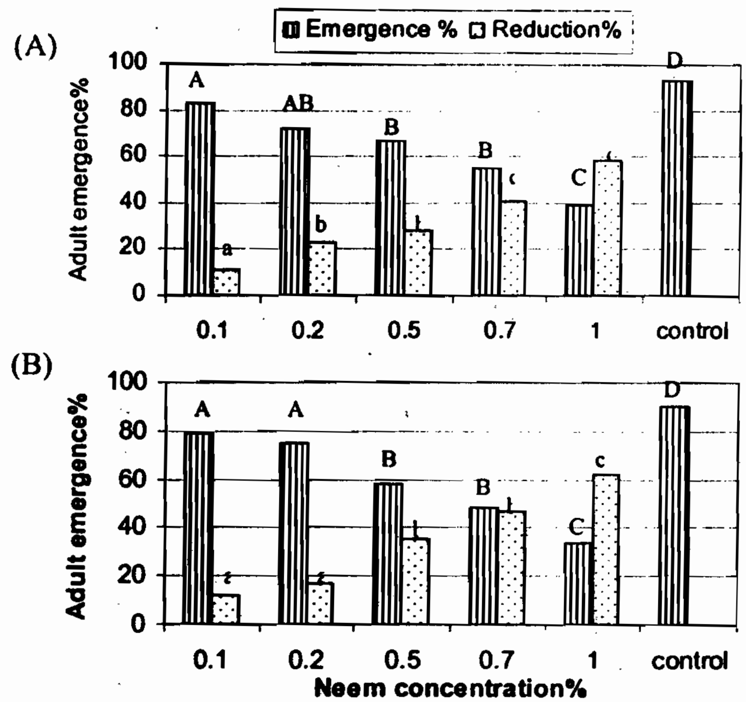

Fig.(4) Effect of NeemAzal on emergence rate of both grape berry moths *L.* botrana (A) and E. ambiguella (B). Different letters indicate significant differences (P< 0.05, Tukey, HSD-Test).

B. Larval treatments

Larval mortality and adult emergence of GBM were adversely affected in varying degrees when individuals were reared for the first 2 weeks of larval life on medium saturated with 0.5% of neemazal-T. Adult emergence was reduced to 18 and 27.7% for *L. botrana* and *E. ambiguella*, respectively (table1).The effects of neem oil on 2nd, 3rd and 4th larval instars of both GBM species were investigated at concentration of 0.5%. Second instars of both GBM species which were fed on a diet of treated with neem oil were the most sensitive to neem oil than other larval instars. Mortality percentage reached 82 and 72.3 % for *L. botrana* and *E. ambiguella*, respectively (table 1)

Neemazal, added to the medium of 2nd, 3rd and 4th instars larvae of GBM, adversely affected oogenesis and reproductive maturation in subsequent female moths. Moths obtained from such treated larvae failed to mature their oocytes, probably as a result of interference of azadirachtin with vitellogenin synthesis and/or its uptake by developing oocytes. Such larval treatment also caused substantial decreases in egg laying capacity, hatchability and female longevity and although, emerging larvae was less viable, less than control ones (table 2).

Table 1: Larval mortality percentage and adult emergence of *L. botrana* and *E. ambiguella*

Parameters	Mortality and adult emergence of *L. botrana*							
	2nd larval Ins.		3rd Larval Ins		4th Larval Ins		Total	
	C	T	C	T	C	T	C	T
Mortality%	13.2	82 ± 5.1	10.5	65.7 ± 2.3	15.1	71.8 ± 4.2	12.9	73.2 ± 2.3
Emergence%	86.8	18 ± 1.1	89.5	34.3 ± 1.2	84.9	28.2 ± 0.2	87.1	26.8 ± 1.1
	Mortality and adult emergence of *E. ambiguella*							
Mortality%	13.5	72.3 ± 3.2	22	63.1 ± 4.1	25.5	65.1 ± 5.2	28	67 ± 3.5
Emergence%	86.5	27.7 ± 1.3	78	36.9 ± 1.1	74.5	34.9 ± 1.4	72	33 ± 2.1

Table 2: Longevity, egg laying capacity and number of laid eggs /day of *L. botrana* and *E. ambiguella.*

Treatment	Data of *L. botrana*			
	Female Longevity	No. of eggs /Female	Average number of eggs/day/F	Hatchability %
2nd larval Instars	13.5	66.7	4.9	79.1
3rd Larval Instars	12.3	60.8	4.8	65.5
4th Larval Instars	13.8	32.7	2.4	57.1
Control	14.5	128.4	8.9	98
Treatment	Data of *E. ambiguella*			
2nd larval Instars	10.7	90.7	8.5	69.6
3rd Larval Instars	10.3	66.3	6.4	49.4
4th Larval Instars	13	93.5	7.2	68.2
Control	15.3	156	10.2	97

A marked decline in the reproductive potential, in terms of egg output and egg hatchability, of the moth was observed when the larvae were reared for the first 15 days in the presence of 0.5% concentrations of Neem oil.

In most instances, adults obtained from 2nd instar larvae treated were severely affected than other ones. Although both methods of SWE treatment caused a significantly high mortality rate, there was no difference in dose response in the range studied. Thus SWE at a concentration of 5 g l^{-1} was shown to be as active as that at 20 g l^{-1}. The results indicate that neem is a potent antifeedant and also has toxicity against GBM.

This study demonstrated a high mortality of the larvae; but the egg and adult stages were the least among the developmental stages of this insect to be seriously affected by the application of neem products. DORN *et al.* (1987) reported that the hatchability of the eggs from large milkweed bugs injected with AZA was close to normal. Earlier instars of sweetpotato whitefly were more susceptible to Margosan-O compared to later instars and there was little mortality to eggs or adults (Price and Schuster 1990).

Other studies also confirm a decreased ability for larvae to develop normally with increasing levels of azadirachtin (Sharma 1992).

Trials under field conditions are important, whereby additional factors have to be considered.

So, by using NeemAzal-T plants can be protected from damage for at least three weeks if applied before oviposition, or at least two weeks if applied against 2nd instar larvae. In these studies, neem inhibited oviposition, larval development and feeding, and greatly increased mortality.

Results of laboratory experiments suggest a potential for the best NeemAzal doses/formulations to be used in vineyards. However, additional studies are needed to evaluate the potential impact of neem extracts in vineyards. It is useful to incorporate neem extractions in biocontrol strategies of grape berry moths, since this will minimize insecticide costs and problems.

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الملخص العربي

التأثيرات البيولوجية لزيت النيم علي ديدان ثمار العنب

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أجريت هذه الدراسة بهدف توضيح التأثيرات البيولوجية لمركب النيم ازال علي بيولوجي ديدان ثمار العنب كذلك تقدير التأثير الابادي لها علي يرقات هذه الآفات. .وقد تناولت الدراسة تأثير النيم علي الحشرات البالغة بشكل مباشر وذلك بتغذية الفراشات علي محلول يحتوي تركيزات متباينة من النيم وتم تقدير كل من طول عمر، كفاءة وضع البيض، نسبة الفقس والخروج من العذراء وقد تناولت الدراسة أيضا تأثير النيم علي يرقات العمر الثاني والثالث والرابع لهذه الآفات وذلك باضافتة بتركيز ٠,٥ % إلي البيئة الصناعية التي تتغذي عليها هذه اليرقات وتم تقدير النسبة المئوية للموت، طول عمر الحشرات الناتجة، كفاءة وضع البيض، نسبة الفقس والخروج من العذراء. أوضحت النتائج عند معاملة – تغذية - الحشرات البالغة بالنيم أن طول العمر الإناث قد تباين من6.6 إلي ١٢,٢ يوما في إناث حشرة اللوبيزيا بينما تراوح بين ٧ إلي ١٧,٥ يوما في إناث حشرة ايبوسيليا، وقد لوحظ أن فترات طول عمر ذكور النوعين من الحشرات كانت أقل تأثرا بالنيم عنها في الأناث. وقد بلغت أعلي نسبة للخفض في طول عمرالي ٦٦,٥% في اناث اللوبيزيا بينما كانت ٥٦% في ايبوسيليا. كذالك فإن كفاءة وضع البيض قد انخفضت في الإناث المعاملة لتصل الي متوسط ٤٩,٨ و٣٥,٤ بيضة/انثي في كل من حشرات لوبيزيا وأيبوسيليا علي التوالي. كذالك فان نسبة فقس البيض قد تأثرت سلبيا هي الاخري حسب التركيزات المستخدمة وتراوحت نسب الخفض من ٢٦,٨ الي ٧٨,٠٤% في بيض حشرة اللوبيزيا، بينما تراوحت ذات النسبة في بيض حشرة أيبوسيليا من ٣٠,٩ إلي ٩٠,٦%. هذا بالإضافة إلي انخفاض نسبة الخروج الحشرات الكاملة من العذراء عند تركيز ١% من النيم لتصل الي ٣٩ و٣٤,١% في حشرة اللوبيزيا وأيبوسيليا علي التوالي. علي الجانب الأخر في معاملات اليرقات، فان يرقات العمر الثاني كانت الاكثر حساسية للنيم وبلغت نية الموت فيها ٨٢ و ٧٢,٣% في يرقات اللوبيزيا وأيبوسيليا غلي الترتيب. كذالك فان فترات طول العمر الإناث، كفاءة وضع البيض ونسب فقس البيض للحشرات الناتجة من اليرقات المعاملة قد تاثرت سلبا نتيجة للمعاملة مقارنة بالكنترول.