

**EFFEECT OF NIMBECIDINE<sup>®</sup> 0.03% AND AGERINE<sup>®</sup> ON THE LARVAE OF LEMON BUTTERFLY (*Papilio demodocus* Esper).**

**BY**

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**ABSTRACT**

A laboratory experiment was conducted at Shambat Research Station to study the effect of Nimbecidine<sup>®</sup> 0.03% (neem oil based) and *Bacillus thuringiensis* Berliner under trade name Agerin<sup>®</sup> against the second larva of *P. demodocus* on lime(*Citrus aurantifolia* Swingle) by using Complete Randomized Design with three replications. Mean mortality number of second instar larvae was recorded daily until the end of experiment. Nimbecidine 0.03% at 4.5 ml/L and 3.6 ml/L showed significantly highest mean mortality number of larva after third day of spray. Than the untreated control, while Agerin<sup>®</sup> at 1.33 g/L and 1.66 g /L showed the same performance after four days of treatment.

To confirm the laboratory experiment results, an experiment was conducted at two different sites under nursery conditions. The effective doses of Nimbecidine 0.03%, Agerin<sup>®</sup> and a mixture of lowest doses of each were tested in a completely randomized design with three replications. No larva was detected at Nimbecidine 0.03% at 4.5 ml/L after two days of spray. The results showed the potential of Nimbecidine 0.03% at 4.5 ml/L and Agerin<sup>®</sup> at 1.33 g/L to 1.66 g/L in the control of *P. demodocus* Larva on lime seedlings under nursery conditions.

**المخلص**

أجريت تجربة معملية بمحطة بحوث شمبات لدراسة تأثير مبيد النيمباسيديين (زيت النيم) و البكتريا الممرضة للحشرات *Bacillus thuringiensis* تحت الاسم التجاري اقربين ضد الطور اليرقي الثاني لأبودقيق الموالح على الليمون باستخدام التصميم العشوائي الكامل بثلاث مكررات. سجل متوسط يرقات أبو الدقيق الميتة يوميا حتى نهاية التجربة. أظهر مبيد

النيمباسيدين بالجرعة 4.5 مل/ لتر اكبر متوسط من اليرقات الميتة بعد اليوم الرابع من المعاملة بينما اظهر الاقرين تحت 1.33 جم / لتر و 1.66 جم / لتر نفس الفعالية خلال أربعة أيام من المعاملة.

كما أجريت تجربة تعضيديّة تحت ظروف المشتل في موقعين مختلفين. اختبرت فيها الجرعات الفعالة من الاقرين ,النيمباسيدين والخليط من الجرعات الصغرى لكل في تصميم عشوائي كامل بثلاثة مكررات. لم تلاحظ يرقات على النيمباسيدين تحت 4.5 مل / لتر و 3.6 مل/ لتر بعد يومين من الرش . أوضحت النتائج فعالية النيمباسيدين تحت 4.5 مل / لتر و الاقرين تحت 1.33 جم / لتر إلى 1.66 جم / لتر في مكافحة يرقات أبو الدقيق على بادرات الليمون تحت ظروف المشتل.

## INTRODUCTION

Lemon butterfly *Papilio demodocus* Esper (Lepidoptera: Papilionidae) was considered only a minor pest of citrus in Sudan but recently high infestations by the larvae of this pest were observed in almost all citrus orchards and nurseries in Khartoum and River Nile States during the rainy and winter seasons (Dawood and Abdelrawf, 2005). The larvae usually attack young nursery plants, but occasionally also defoliate older citrus trees, causing decline on the number and gravity of the fruits (schmutterer, 1969). The larvae feed on the leaves from periphery inwards leaving the midrib and larger veins (Elkhidir, 1968). *P. demodocus* In the Sudan is controlled by hand-picking of caterpillars in small nurseries or individual small citrus trees (schmutterer, 1969). Serious outbreaks may be checked by spraying Malathion 57% EC at 4ml/Litre of water (Dawood and Abdelrawf, 2005).

The use of neem tree *Azadirachta indica* as a source of natural insecticides was discovered approximately 30 years ago (Ascher, 1993). Pest control using extracts from the neem tree is currently practiced in more than 55 countries throughout the world (Stoll, 2000). Neem compounds are most effective against the following orders, Coleoptera (beetles and their larvae), Lepidoptera and Orthoptera (nymph and adults of grasshoppers and locusts). Results against some bugs, leafhoppers, thrips and whiteflies were also good (Stoll, 2000). Elhag (2006) reported that Nimbecidine 0.03% when used against *Thrips tabaci* (Lind) at 4 ml/L gave a good result in reducing *Thrips tabaci* population in onion fields.

The bacteria *Bacillus thuringiensis*, Berliner commonly known as Bt, is the most widely used environment-friendly alternative to chemical insecticides for the biological control of forests and agricultural pests and the vectors of human, animal and plant diseases. Different strains of this bacteria produced crystalline parasporal inclusion bodies composed of one or more toxic protein known as – endotoxin, with high level of specificity against different species of lepidopteran, dipteran and coleopteran insects and certain parasitic nematodes and protozoan pathogen (Peyronnet *et al*; 1997). In the Sudan, many researchers have tested many B.t. products against some lepidopteran species. The acacia bagworm *Auchomphila kordofensis* Rebel showed high susceptibility to Agerin<sup>®</sup> and Dipel<sup>®</sup> DF in the laboratory at 2.5 g/L with 18.2 and 81.6 respective mortality percentages (Mahmoud, 2005). Also, a laboratory investigation showed high performance of Agerin<sup>®</sup> at 4 g/L against *Earias isulana* and *E. vitella* (Eltom, 2006).

This study aimed at assessing the effect of the commercial products Agerin<sup>®</sup> and Nimbecidine 0.03% against *P. demodocus* larvae under laboratory and nursery conditions.

#### **MATERIALS AND METHODS**

The study was conducted at Shambat Research Station and Administration of Horticultural Sector during 2006.

##### **Laboratory experiment**

Lemon butterfly (*P.demodocus*) was reared according to Mardi (2006). Their progeny at the 2<sup>nd</sup> larval stage were used in this study. Two products were tested and their effects on the survival of the second instar larvae were investigated at room temperature range of 25-35°C. These products were: Agerin<sup>®</sup> (*Bacillus thuringiensis*) wettable powder (WP) 32.000 International Unit (IU) /mg), a product of Genetic Engineering Research Institute (GERI), Genetic Engineering Service Unit (BIOGROINT), Egypt. Nimbecidine<sup>®</sup> 0.03% is totally natural neem-oil based product with Azadirachtin as the labeled active ingredient, a product of T. Stones and Co. ltd India. The test was carried out as described by Mardi (2006) with some modifications: simply ten larvae were taken after the first molt (L2, less than 24 h after molt) placed in Petri dishes (9 cm in diameter) lined with moistened filter paper. They were given tender lime (*Citrus aurantifolia* Swingle) leaves containing 0.98 g/L, 1.33 g/L and 1.66 g/L of Agerin<sup>®</sup> and 2.7 ml/L, 3.6 ml/L and 4.5 ml/L of Nimbecidine<sup>®</sup> 0.03%. A control treated with

distilled water only was also considered. Each Petri dish with ten larvae constitutes a replicate. The treatments were arranged in a completely randomized design with three replicates. The mortality number was recorded daily until the end of the experiment.

**Nursery experiment**

This experiment was conducted at two sites to confirm the results obtained from the laboratory experiment. The test was carried out as described by Mardi (2006). Agerin® at 1.33 g/L and 1.66 g/L and Nimbecidine® 0.03% at 3.6 ml/L and 4.5 ml/L and the mixture of the lowest doses of the two products were tested. A control treated with water only was also considered. The treatments were arranged in a completely randomized design with ten replicates. The number of larvae present per seedling was recorded daily until the end of experiment.

**Data analysis**

Data of the experiment were analyzed by analysis of variance (ANOVA), and means were transformed by square root ( $\sqrt{x+0.5}$ ) transformation, when needed. Means separation was made by Least Significant Differences (LSD). A SAS software (SAS Institute, 2000. V.8.1) was applied for data analysis.

**RESULTS**

**Laboratory experiment**

Table (1) shows the effect of Nimbecidine 0.03% on the survival of *P. demodocus* second larva. No mortality was recorded in all tested doses against the second larval instar after one day of treatment. On the second and third day, results showed that, Nimbecidine 0.03% at 4.5 ml /L gave significantly higher mortality than the lower dose and untreated control.

**Table (1): Effect of Nimbecidine® 0.03% on the mortality of the second larval instar of *P. demodocus* Esper in the laboratory.**

| Concentrations        | Mean mortality number |                     |                     |
|-----------------------|-----------------------|---------------------|---------------------|
|                       | Days after treatment  |                     |                     |
|                       | 1 <sup>st</sup> day   | 2 <sup>nd</sup> day | 3 <sup>rd</sup> day |
| 2.7 ml/Litre of water | 0 (0.7) a             | 0 (0.7) c           | 1.8 (1.5) b         |
| 3.6 ml/Litre of water | 0 (0.7) a             | 6 (2.5) b           | 8.3 (3.0) a         |
| 4.5 ml/Litre of water | 0 (0.7) a             | 8(2.9) a            | 10 (3.2) a          |
| Untreated control     | 0 (0.7) a             | 0 (0.7) c           | 0 (0.7) c           |
| C.V %                 | 0.00                  | 8.32                | 6.20                |
| SE±                   | 0.00                  | 0.31                | 0.31                |
| LSD                   | 0.0051                | 0.2663              | 0.2455              |

Means between brackets were transformed to  $\sqrt{x+0.5}$

Means within a column followed by the same letter are not significantly different at P=0.05.

Table (2) shows the effect of Agerin<sup>®</sup> on the survival of *P. demodocus* Larvae. First day after treatment no mortality was detected from Agerin<sup>®</sup> at 0.98 g/L, 1.33g/L and 1.66 g/L against the second instar larvae. As for the results after the second and third days the higher dose caused significantly higher mortality than all other doses includes the untreated control. After four days of treatment the higher and medium doses produced significantly higher mortality than the lower dose and the untreated control.

**Table(2): Effect of Agerin<sup>®</sup> on the mortality of the second larval instar of *P. demodocus* Esper in the laboratory.**

| Concentrations        | Mean mortality number |                     |                     |                     |
|-----------------------|-----------------------|---------------------|---------------------|---------------------|
|                       | Days after treatment  |                     |                     |                     |
|                       | 1 <sup>st</sup> day   | 2 <sup>nd</sup> day | 3 <sup>rd</sup> day | 4 <sup>th</sup> day |
| 0.98 g/Litre of water | 0 (0.7) a             | 1 (1.2) c           | 1.3 (1.5) c         | 2 (1.6) b           |
| 1.33 g/Litre of water | 0 (0.7) a             | 3.3 (2) b           | 4 (2.1) b           | 7.3 (2.8) a         |
| 1.66 g/Litre of water | 0 (0.7) a             | 5 (2.3) a           | 6.3(2.6) a          | 10 (2.9) a          |
| Untreated control     | 0 (0.7) a             | 0 (0.7) d           | 0 (0.7) d           | 0 (0.7) c           |
| C.V %                 | 0.00                  | 3.74                | 7.56                | 13.25               |
| SE±                   | 0.00                  | 0.19                | 0.21                | 0.28                |
| LSD                   | 0.0188                | 0.1031              | 0.2455              | 0.5017              |

Means between brackets were transformed to  $\sqrt{x+0.5}$

Means within a column followed by the same letter are not significantly different at P=0.05.

**Nursery experiment**

Table (3) shows the effect of Nimbecidine 0.03%, Agerin<sup>®</sup> and their mixture on the larva of *P. demodocus*. First day after application Agerin<sup>®</sup> at 1.66 g/L and Nimbecidine 0.03% at 4.5 ml/L recorded significantly lower mean number of larva per lime seedling compared with Agerin<sup>®</sup> at 1.33 g/L , Nimbecidine 0.03% at 3.6 ml/L, the mixture and untreated control. On the second day, no larva was detected on Agerin<sup>®</sup> at 1.66 g/L but, Agerin<sup>®</sup> at 1.33 g/L, Nimbecidine 0.03% at 3.6 ml/L and 4.5 ml/L and the mixture gave significant lower mean number of larvae compared with untreated control. Three days after treatment larvae were detected only on Nimbecidine 0.03% at 3.6 ml/L and untreated control.

**Table (3): Effect of Nimbecidine<sup>®</sup> 0.03%, Agerin<sup>®</sup> and their mixture on the larval stage of *P. demodocus* Esper in the nursery.**

| Treatments   | Mean number of larvae |                     |                     |                     |
|--|-----------------------|---------------------|---------------------|---------------------|
|  | Pre- count            | Count number        |                     |                     |
|  |                       | 1 <sup>st</sup> day | 2 <sup>nd</sup> day | 3 <sup>rd</sup> day |
| Nimbecidine at 3.6 ml/Litre of water                                   | 4.6 a                 | 2.6 (1.8) b         | 1.7 (1.6) b         | 0.9 (1.2) b         |
| Nimbecidine at 4.5 ml/Litre of water                                   | 3.9 a                 | 1.3 (1.3) d         | 1.3 (1.3) c         | 0 (0.7) c           |
| Agerin at 1.33 g/Liter of water  | 4.3 a                 | 2.7 (1.8) b         | 1.3 (1.3) b         | 0.2 (0.8) c         |
| Agerin at 1.66 g/Litre of water  | 4.4 a                 | 1.4 (1.4) cd        | 0 (0.7) d           | 0 (0.7) c           |
| Mixture of (Nimbecidine at 2.7 ml+<br>Agerin at 0.98 g/ Litre of water | 4.3 a                 | 2 (1.5) c           | 1.2 (1.3) c         | 0.3 (0.8) c         |
| Untreated control  | 4.2 a                 | 4.2 (2.1) a         | 4.2 (2.1) a         | 4.2 (2.1) a         |
| C.V %  | 32.32                 | 15.45               | 12.78               | 18.75               |
| SE±  | 0.17                  | 0.05                | 0.06                | 0.07                |
| LSD  | 1.241                 | 0.2303              | 0.1579              | 0.1771              |

Means between brackets were transformed to  $\sqrt{x+0.5}$

Means within a column followed by the same letter (s) are not significantly different at P=0.05.

## DISCUSSION

It was observed from the results presented that Nimbecidine 0.03% and Agerin<sup>®</sup> caused mortality for the second larval instar of *P. demodocus*. Exposure of second larval instar to lime leaves treated with Nimbecidine 0.03% at 4.5 ml/L and 3.6ml/L gave higher mean mortality number than the lower dose and the untreated control. This result agreed with Elhag (2006) who found that Nimbecidine 0.03% at 4 ml/L gave a good result in reducing *Thrips tabaci* (Lind) population in onion fields. Second larva of *P. demodocus* when fed lime leaves treated by Agerin<sup>®</sup> at 1.33 g/L and 1.66 g/L gave significantly higher mean mortality number than the lower dose and untreated control This may indicate that *Bacillus thuringiensis* is effective against lepidopteran and that the commercial product of B.t. Agerin<sup>®</sup> showed in this work high effect against *P. demodocus* second instar larvae. This result agrees with Eltom (2006) who found that Agerin<sup>®</sup> at 4 g/L caused 89% and 94% mortality to *Earias isulana* and *E. vitella* respectively. However, Agerin<sup>®</sup> was ineffective against *Auchomphila Kordofensis* Rebel (Mahmoud, 2005). This different result may indicate that lepidopteran species have different susceptibility degrees to *B. thuringiensis*. The larvae treated in that study by effective doses of Agerin<sup>®</sup> stopped feeding, became inactive and turned to black colour. Also, the head of Larva looked bigger than the normal size. The same observations were listed by Tanada and Kaya (1993) and Eltom (2006). No larva was detected on lime seedlings treated by Agerin<sup>®</sup> at 1.66 g/L after two days of spray. All tested doses of the two products except Nimbecidine 0.03% at 3.6 mL killed all larvae on lime

seedlings after three days of spray. No literature on the use of mixture of Nimbecidine 0.03% and Agerin<sup>®</sup> against *P. demodocus* larva was found.

In this work Agerin<sup>®</sup> at 1.66 g/L was found to be most effective against the larva of *P. demodocus* after two days of spray. A good result was obtained after three days when the lime seedlings treated by Agerin<sup>®</sup> at 1.66 and 1.33g/L, mixture of Nimbecidine and Agerin<sup>®</sup> at (2.6 ml/L + 0.98 g/L ) and Nimbecidine 0.03% at 4.5 ml/L and 3.6 ml/L respectively.

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