

# EFFECTS OF AQUEOUS NEEM AND *MELIA AZEDARACH* EXTRACTS ON THE EARLY DEVELOPMENT OF *EUREMA BLANDA* (LEPIDOPTERA: PIERIDAE)

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## ABSTRACT

*Aqueous Neem and Melia azedarach seed extracts were applied on the eggs of Eurema blanda. The effects on the egg and early larval development were assessed and compared with the effects of the commercial Neem product, Neem Azal-T/S. All treatments resulted in a significant lower survival rate of the larvae than of the control larvae treated with water only. 100% mortality was reached 6 to 7 days after the treatment with Neem seed extracts and Neem Azal-T/S. The treatment with Melia seed extracts took 14 days to kill 100% of the caterpillars.*

**Key words:** *Azadirachta indica*, biological activity, plant-derived insecticide.

## INTRODUCTION

Most pesticides are toxic, persist in the environment, affect non-target organisms and sooner or later lose their efficacy against the pest due to resistance to the active ingredient. Additional disadvantages of the use of pesticides in developing countries are the high costs of these chemicals as well as their inappropriate use due to a lack of awareness of the risks involved.

The tropics offer a wide range of plants with insecticidal properties. Some of the active chemical compounds are promising in their use against pests and can be often extracted by simple means so that small scale farmers ideally could become independent from chemical pesticides in the future. Since these resources disappear rapidly, research is urgently needed to screen the plants of

the tropical rain forests for their potential as pesticides. Plant-derived insecticides can be extracted e. g. from the Neem tree (*Azadirachta indica*), the Persian Lilac or Chinaberry tree or White Cedar (*Melia azedarach*), *Crotalaria spp.*, lemon grass or citronella (*Cymbopogon citratus*), pyrethrum (*Tanacetum cinerariaefolium*) and derris (*Derris elliptica*) (Prakash & Rao 1996). Plant-derived insecticides, particularly Neem products are promising and effective elements of Intergrated Pest Management (IPM) in agriculture in Papua New Guinea (Saucke 1994).

The Neem tree *A. indica* (Meliaceae) originated from the Indian sub-continent, and it is now distributed throughout the drier tropics and subtropics. In Papua New Guinea the Neem tree was introduced to the Port Moresby area and in the Markham

Valley as a shade tree along road sides (Schmutterer, 1995). Extracts of various parts of the Neem tree have been used traditionally for medicinal purposes due to their anti-malarial, analgesic and anti-pyretic properties (Ketkar & Ketkar 1995; Kak & Kavathekar 1996). Furthermore, Neem products exhibit insecticidal properties amongst others against Homoptera, Orthoptera, Lepidoptera, Diptera and Coleoptera (Schmutterer & Singh 1995). The major compound found in Neem is the tetranortriterpenoid azadirachtin A but Neem also contains other biologically active terpenoids (Kraus 1995) which affect the insect's feeding behavior, growth, metamorphosis, reproduction, oviposition, activity and fitness (Schmutterer & Rembold 1995).

The origin of the Persian Lilac *M. azedarach* (Meliaceae) is northwestern India and now it can be found in many warmer regions of the world. The tree is used for firewood and timber production, as a shade tree and as a medicinal plant with antihelminthic and antiseptic properties. Additionally the leaves and fruits of *M. azedarach* are used as an insecticide. The range of the insects affected by *Melia* extracts are amongst others from the orders Orthoptera, Isoptera, Thysanoptera, Hemiptera, Coleoptera, Lepidoptera and Diptera. The active compounds of the *M. azedarach* are triterpenoids such as meliantriol, melianone and melianol which act as antifeedant, disturb growth and metamorphosis and cause high mortality (Ascher *et al.* 1995)

*Eurema blanda* (Lepidoptera: Pieridae) is abundant in areas of secondary vegetation, such as old food gardens, areas of regrowth along roadside verges and open areas of low growing shrubs at an altitude ranging from sea level up to 1,600 m. a. s. l. (Parsons 1991). The females lay their eggs in large patches of over 100 on the underside of young leaves of their larval food plant *Albizia falcataria* (Mimosaceae). The monophagous caterpillars can completely defoliate the host and are therefore considered as a pest of minor

economic importance. The species was chosen due to the fact that it is a simple and convenient model organism that occurs around Bulolo in a relatively high abundance throughout the year. *Albizia* is a fast coping, nitrogen fixing species used in agroforestry as a shade tree and for the production of green manure, the timber is used as fuelwood and sawn timber. As a pioneer species it can be found in areas affected by landslides, in secondary or disturbed forests and along creek or river banks. The natural distribution includes Papua New Guinea and other parts of Oceania (Sosef *et al.* 1998).

The aim of this study was to compare the insecticidal effects of aqueous seed extracts obtained from Neem and *Me. azedarach* grown in Papua New Guinea with the efficacy of a commercial Neem product as a standard. A very simple extraction method was chosen so that this can be also used by small-scale farmers in Papua New Guinea.

## MATERIALS AND METHODS

### *Study Site*

The trials were carried out in the agroforestry garden of the Bulolo University College, Bulolo, Morobe Province, Papua New Guinea, located 7° south, 147° east, having a mean annual temperature of 24.3°C, a mean annual rainfall of 1513 mm and heavy clay soils (McAlpine *et al.* 1990).

### *Seed and Plant Material*

Neem (*A. indica*) and *Melia azedarach* fruits were collected from trees in Bulolo. After collection the fruits were pulped and the seeds (including endocarp) allowed to dry at room temperature for several weeks before use. *A. falcataria* (Mimosaceae) seeds were obtained from the National Tree Seed Centre, Bulolo and sown in October 1994 and February 1996, respectively.

*Preparation and Application of the Natural Insecticides*

Dried *Melia* and Neem seeds were ground, the required amount was weighed and soaked in a defined volume of water for 10 to 14 hours and finally strained to obtain the spray solution (Hellpap & Dreyer 1995). Neem Azal-T/S (Trifolio-M, Lahnau, Germany) containing 1% azadirachtin A was diluted with water to produce the desired concentration (v/v) of the insecticide.

Each day the undersides of the leaves of the host trees were checked for newly laid eggs. Thus, the eggs were treated within the first day after oviposition with approximately 2 ml of the respective natural insecticide per egg patch by the use of a hand sprayer. For control treatments the egg patches were sprayed with 2 ml of water. Certain *Albizia* trees were sprayed with water only, others with one particular insecticide in order to avoid systemic effects. The applications were carried out during late afternoons to avoid the

degradation of the active ingredients by UV and heat.

The number of live eggs and larvae was assessed before and every 24 hours after spraying until a mortality rate of 100% was reached. The assessment was carried out only if there was no precipitation for at least 12 hours after spraying. The day of the treatment was defined as day 0.

**RESULTS**

Figure 1 shows the survival rate of control *E. blanda* eggs and larvae. During the first days there was no decline of the survival rate of the control individuals but from day 2 onward there was a slight decrease in the number of individuals from approximately 95% to 65% on day 14 after the treatment.

The survival rate of *E. blanda* eggs and larvae treated with 2 ml Neem Azal-T/S is shown in Figure 1. The treatment with Neem Azal-T/S at a

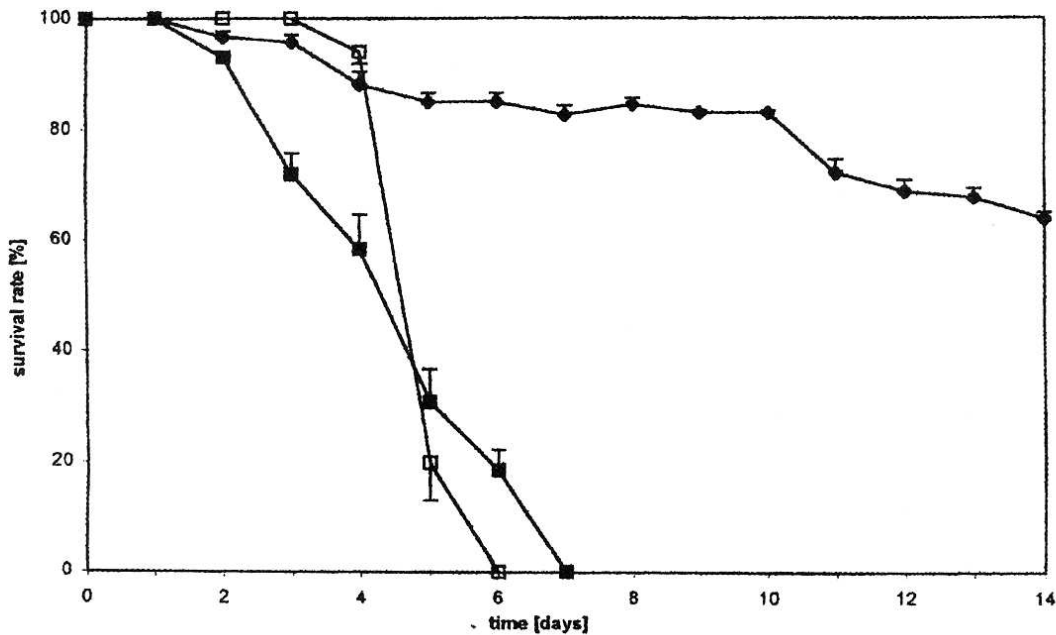


Figure 1. Survival rate of *E. blanda* larvae after treatment of eggs with 2 ml Neem Azal-T/S (3 ml/l) (—■—), 2 ml Neem Azal-T/S (5 ml/l) (—□—) and 2 ml water (—◆—). Each data point represents the mean of three to five determinations. Bars indicate the standard error of the mean ( $\pm$ S.E.M.).

concentration of 3 ml/l resulted in a steady decline of the survival rate from approximately 90% at day 2 to 100% mortality at day 7 after the treatment. The survival rate of eggs treated with a concentration of 5 ml/l Neem Azal-T/S remained 100% until day 3 and then decreased to 0% on day 6.

The survival rate of individuals treated with 2 ml aqueous *M. azedarach* seed extracts (50 g/l) is shown in Figure 2. From day 1 onward there was a constant decrease of the survival rate to day 14 from approximately 90% to 0% (100% mortality). The treatment of *E. blanda* eggs with 2 ml aqueous Neem seed extracts (50 g/l), also indicated in Figure 2, caused a slight decline of the survival rate from 100% on day 1 to approximately 85% on day 4 and then dropped to 100% mortality within the following two days.

The comparison of the results of the various treatments with the control using the Student's test showed significant lower survival rates from day 5 after the insecticide treatments and onward, according to Table 1. Contrasting the treatment with aqueous Neem and *Melia* seed extracts very significant lower survival rates were the result of the treatment with Neem seed extracts from day 6 on. No significant difference was found for the comparison of the results of aqueous Neem seed extracts with the results of the treatment with both concentrations of Neem Azal-T/S. The treatments with Neem Azal-T/S at a concentration of 3 ml/l and 5 ml/l caused a significant higher mortality (< 1%) from day 6 and day 7 respectively than the treatment with *Melia* seed extracts. The comparison of the treatments with the two concentrations of Neem Azal-T/S showed significant higher mortality rates after the treatment with 3 ml/l on day 3 and 4 and a significant lower mortality on day 6.

#### DISCUSSION

A mortality of 100% of the *E. blanda* larvae was gained when the eggs were treated with extracts of 50 g/l *M. azedarach* seeds as shown in Figure 2.

According to Ascher *et al.* 1995), spray applications of 40 ml/m<sup>2</sup> of 3% aqueous extracts of *M. azedarach* leaves and fruits resulted in 93% and 100% mortality respectively, of first and third instar nymphs of *Locusta migratoria*. Furthermore, toxic effects on the Pieridae *Pieris rapae* and *P. brassicae* were described after the treatment with *M. azedarach* leaf extracts in higher concentrations (Ascher *et al.* 1995). In our study the treatment with extracts of 50 g/l Neem seeds resulted in 100% mortality after 6 days as shown in Figure 2. Similar experiments showed a reduction of feeding, high mortality, disturbance of growth and metamorphosis and other effects on *P. rapae* and *P. brassicae* after the treatment with Neem products. Generally, azadirachtin A causes a substantial mortality in early lepidopteran instars (Isman 1995).

Regarding the larval mortality the results clearly indicate that the Neem seed extracts were as effective as Neem Azal-T/S at a concentration of 3 ml/l and 5 ml/l, respectively. The treatment with *Melia* seed extracts showed the weakest effects when contrasted with the other treatments since it took 7 to 8 days more to reach 100% mortality of the larvae. This might be due to the different chemical composition of the *M. azedarach* seeds, the different mode of action of the biologically active ingredients or the relatively smaller size of the *Melia* seed kernels.

The apparently higher efficacy of Neem Azal-T/S at a concentration of 3 ml/l on day 3 and 4 than the concentration of 5 ml/l might have been the result of a high-dose-effect.

The control treatment of *E. blanda* eggs with water showed a slight decline in the larval survival rate to approximately 65% on day 14. This was due to parasitization and predation of the emerged larvae that were eaten by spiders and ants. This is supported by Parsons (1991), who observed that *E. blanda* caterpillars were an attractive source of food for ants and beetles.

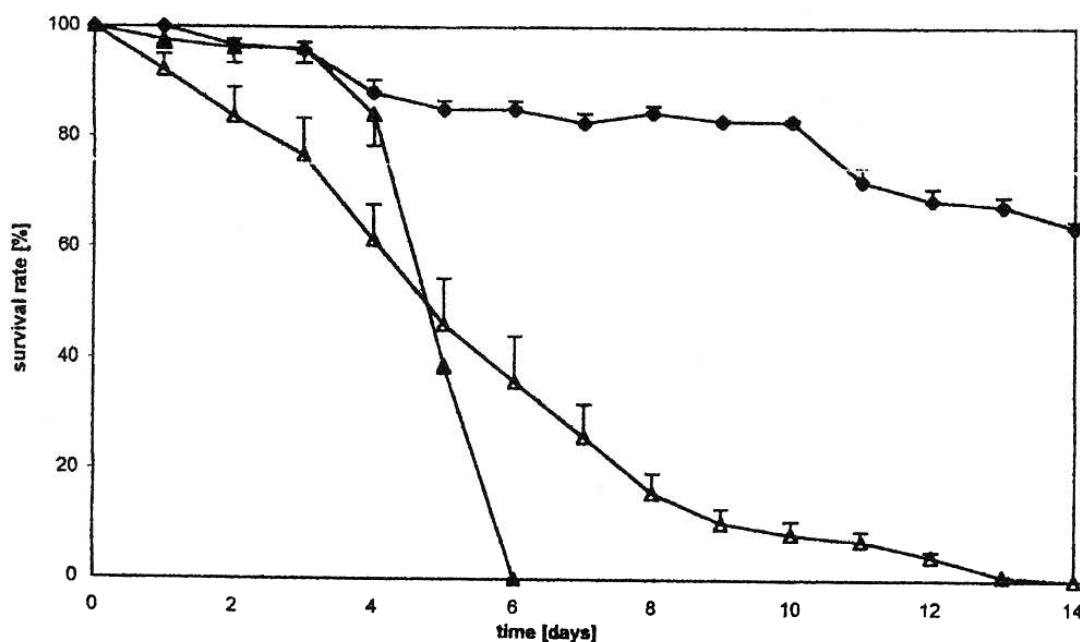


Figure 2. Survival rate of *E. blanda* larvae after treatment of eggs with 2 ml aqueous Neem seed extracts (50 g/l) (—▲—), 2 ml *Melia azedarach* seed extracts (50 g/l) (—△—) and with 2 ml water (—◆—). Each data point represents the mean of two to four determinations. Bars indicate the standard error of the mean ( $\pm$ S.E.M.).

According to our observation, the egg stage lasted between four and five days. Regardless of the kind of treatment, in all cases almost all eggs hatched into larvae i. e. no egg mortality was observed. From this it can be concluded that the compounds contained in the used Neem and *Melia* products did not act as an ovicide. This was also supported by transfer experiments of treated eggs on untreated leaves as well as the respective controls (unpublished data). Apparently the active ingredients persisted until the caterpillars hatched and then acted as contact or stomach poison when the larvae ate the contaminated remains of their eggs or started feeding upon the treated leaves.

Due to the high precipitation in Papua New Guinea the yield of Neem fruits is much lower than the yield in drier countries (Schmutterer 1995) but apparently the seeds show quite high biological activity (J. Breithaupt, *pers.* communication). A relatively high activity was indicated by this study. On the other hand *M. azedarach* bears much

higher yields in Papua New Guinea than Neem but is, according to this study, less effective. Further disadvantages for the use in small-scale agriculture are the hard endocarp of *Melia* which makes the seeds more difficult to crush and the toxicity of some *M. azedarach* compounds for human beings. Hence, the results of this study clearly show the potential of local Neem and *Melia* products for the control of insect pests in agroforestry systems.

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Table 1. Results of the T-test showing very significant ( $\leq 1\%$ ), significant ( $\leq 5\%$ ) or non-significant (-) differences in the survival rate of *E. blanda* eggs and larvae after the various treatments.

Treatment versus treatment	Days after treatment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control vs Neem 50 g/l	-	-	-	-	<1%	«1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Control vs Melia 50 g/l	-	-	-	-	<5%	<5%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Control vs N/Azal 3 ml/l	-	-	-	-	<5%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Control vs N/Azal 5 ml/l	-	-	-	-	«1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Neem 50 g/l vs Melia 50 g/l	-	-	-	-	-	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Neem 50 g/l vs N/Azal 3 ml/l	-	-	-	-	-	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Neem 50 g/l vs N/Azal 5 ml/l	-	-	-	-	-	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Melia 50 g/l vs N/Azal 3 ml/l	-	-	-	-	-	-	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Melia 50 g/l vs N/Azal 5 ml/l	-	-	-	-	-	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
N/Azal 3 ml/l vs N/Azal 5 ml/l	-	-	<1%	<1%	-	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%