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# MORTALITY OF *ANTICARSIA GEMMATALIS* (LEPIDOPTERA: NOCTUIDAE) CATERPILLARS POST EXPOSURE TO A COMMERCIAL NEEM (*AZADIRACHTA INDICA*, MELIACEAE) OIL FORMULATION

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## Abstract

The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), is the most important defoliator of soybean (*Glycine max* L. Merrill, Fabaceae) crops in Brazil. Early-instar caterpillars of this pest are the main target of control. The objective of this work was to evaluate the toxicity of a commercial product, Bioneem® [neem oil (*Azadirachta indica* A. Juss., Meliaceae)], sprayed on *A. gemmatalis* caterpillars. Bioneem® was sprayed on soybean plants and 3 h later specific branches were enclosed by organza bags in a greenhouse with third-instar *A. gemmatalis* caterpillars. Ten replications with 80 caterpillars were used per treatment. The mortalities of *A. gemmatalis* caterpillars (mean 3rd to 6th instars) was 3.17, 7.18, 5.00, 11.25, 16.74, and 18.15%, whereas the respective mortalities of the pupae obtained from treated the caterpillars were 20.57, 22.79, 29.64, 39.16, 51.50, and 59.17%. The viabilities until the pre-pupal stage were 76.26, 70.03, 65.36, 49.59, 31.76, and 22.68% with 2.5, 5, 10, 15, 20, and 25% of the neem oil, respectively. The larval stage of this pest (3rd instar to pre-pupa) lasted longer in the 20% neem oil treatment than at the other rates, but its longevity was not affected. *Anticarsia gemmatalis* pupae presented developmental interruptions and anomalies at all concentrations of neem oil. The commercial product Bioneem® (neem oil) can therefore be used as a suitable alternative to synthetic insecticides to control *A. gemmatalis*.

Key Words: alternative control, botanical insecticide, defoliator, soybean, velvetbean caterpillar

## Resumo

A lagarta-da-soja, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) é o principal desfolhador de culturas de soja (*Glycine max* L. Merrill, Fabaceae) no Brasil. Lagartas de estádios iniciais dessa praga são o principal alvo de controle. O objetivo deste trabalho foi avaliar a toxicidade de um produto comercial, Bioneem® [óleo de nim *(Azadirachta indica* A. Juss., Meliaceae)], para lagartas de *A. gemmatalis*. O Bioneem® foi pulverizado em plantas de soja e três horas após, ramos específicos foram fechados por sacos de organza em uma casa-de-vegetação com lagartas de terceiro estádio de *A. gemmatalis*. Dez repetições com 80 lagartas foram usadas por tratamento. A mortalidade de lagartas de *A. gemmatalis* (média do 3° ao 6° estádio) foi de 3,17; 7,18; 5,00; 11,25; 16,74 e 18,15%, enquanto que a mortalidade das pupas obtidas de lagartas tratadas foi de 20,57; 22,79; 29,64; 39,16; 51,50 e 59,17%. A viabilidade até o estágio de pré-pupa foi de 76,26; 70,03; 65,36; 49,59; 31,76 e 22,68% com 2,5; 5; 10; 15; 20 e 25% do óleo de nim, respectivamente. O estágio de larva desta praga (3° estádio a pré-pupa) durou mais tempo com o tratamento com 20% de óleo de nim do que nos outros, mas sua longevidade não foi afetada. Pupas de *A. gemmatalis* apresentaram interrupções de desenvolvimento e anomalias, com todas as concentrações de óleo de nim. O

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produto comercial BIONEEM® (óleo de nim) pode, portanto, ser utilizado como uma alternativa adequada para controlar *A. gemmatalis*.

Palavras Chave: controle alternativo, inseticida botânico, lagarta desfolhadora, lagarta-da-soja, nim, soja

The velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) is the primary soybean (*Glycine max* L. Merrill, Fabaceae) defoliator in Brazil (Panizzi et al. 2004; Sosa-Gomes 2004). This insect also damages crops of the peanut, *Arachis hypogaea* L.; alfafa, *Medicago sativa* L.; bean, *Phaseolus vulgaris* L.; pea, *Pisum sativum* L. (Fabaceae); rice, *Oryza sativa* L.; and wheat, *Triticum aestivum* L. (Poaceae) (Rahman et al. 2007). It is mainly controlled with synthetic insecticides (Guedes et al. 2012; Castro et al. 2013). However, the most preferred velvetbean caterpillar control methods are the use of transgenic crop plants expressing the *cry*/*Ac* gene from the gram-positive and soil-dwelling bacterium, *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Miklos et al. 2007; McPherson & Macrae 2009) together with biological controls involving parasitoids and predators of eggs, pupae and early-instar caterpillars (Ferreira et al. 2008; Tavares et al. 2011, 2012). Eggs oviposited on the undersides (abaxial surfaces) of the host plant leaves and *A. gemmatalis* pupae found beneath the soil surface add to the difficulty in controlling this pest, and therefore the early-instar caterpillars are the main target in the control of this pest (Pereira et al. 2010).

Early-instar *A. gemmatalis* caterpillars scrape the leaves while the later instars feed on the leaves and stems of the host plants (Lourenção et al. 2000). Natural insecticides, which are feeding inhibitors, are effective as a pest control method (Tavares et al. 2013a). Neem oil (*Azadirachta indica* A. Juss., Meliaceae) with strong insecticidal properties is an alternative to synthetic insecticides to control *A. gemmatalis*, especially for small-scale producers and in organic agriculture, where synthetic chemicals are prohibited (Mourão et al. 2004a, 2004b; Almeida et al. 2010). Neem oil is available in Africa, America, Asia and Europe, as well as in countries such as Brazil and India, where it originated (Isman 2008; Isman et al. 2011). Because synthetic insecticides can contaminate the environment, induce insecticide resistance, and cause secondary pest outbreaks, interest has grown in the use of natural products associated with transgenic plants and biological control (Tavares et al. 2009, 2010a). Neem plants contain substances possessing insecticidal properties against the defoliator caterpillars, as reported for the species of the Noctuidae family (Tavares et al. 2010b, 2011).

The objective of this work was to evaluate the toxicity of the commercial neem oil formulation, Bioneem®, sprayed on the soybean plants to control *A. gemmatalis* caterpillars.

## Materials and Methods

### Study Site

This research was conducted with a natural photoperiod, mean temperature of 28 ± 2 °C from Mar to Jun 2012 in a greenhouse of the Graduate Program of Genetic Improvement, Department of Crop Science (DFT), Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil (S 20° 45' × W 42° 51', 648 m asl). Weather data were obtained from a weather station near the greenhouse.

### Soybean Plants

Seeds of the 'UFV-16 (Capinópolis)' soybean cultivar (planting to harvest cycle of 111-115 days) were obtained from the DFT of UFV and the soybean plants were cultivated in 3 L plastic pots with ravine soil on benches, 1 m wide and 3.5 m long. Fertilization was carried out according to the technical recommendations of the Soil Fertility Commission of the Minas Gerais State and the plants in the vegetative stages $V_3$ or $V_4$ were used because they are the ones most damaged by insects in the field (Corrêa-Ferreira 2005).

### *Anticarsia gemmatalis*

The *A. gemmatalis* caterpillars were obtained from a rearing facility at the Laboratory of Biological Control of Insects (LCBI) of the Institute of Biotechnology Applied to Agriculture (BIOAGRO) of the UFV, where this insect is maintained on an artificial diet (Ferreira et al. 2008).

### Neem

BIONEEM® (common name: neem oil, azadirachtin, a.i.: 0.15 $mL.L^{-1}$, i.s.: 0.25%) was used.

### Experiment

The Bioneem® (neem oil) was applied on the soybean plants in the $V_3$ or $V_4$ (vegetative) stages with a $CO_2$ pressurized Jacto® sprayer (Pompéia

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city, São Paulo, Brazil) with a tip type fan couple $TJ_{11002}$vs, pressure of 2.5 bars, and a solution volume of 100 $L \cdot ha^{-1}$. The treatments included: $T_1$- control, soybean plants sprayed with distilled water; $T_2$- soybean plants sprayed with neem oil at 2.5%; $T_3$- soybean plants sprayed with neem oil at 5%; $T_4$- soybean plants sprayed with neem oil at 10%; $T_5$- soybean plants sprayed with neem oil at 15%; $T_6$- soybean plants sprayed with neem oil at 20%, and $T_7$- soybean plants sprayed with neem oil at 25%. Three hours after spraying with the neem oil, the soybean plants were removed to a greenhouse and their branches were enclosed with organza bags with 8 *A. gemmatalis* third-instar caterpillars per branch (Almeida et al. 2010). The experiment had 10 replications. Mortalities of the caterpillars (3rd to 6th instars) in the various treatments were evaluated each day following the spray application. Pupae obtained from treated caterpillars were transferred to plastic pots (500 mL) (10 pupae per pot) lined with cotton wool, and maintained until adult emergence. The mortality of these pupae was evaluated each day, and those that had become black were considered to have died. Viabilities of the immature stages from the 3rd instar until the pre-pupal stage were recorded. Also the durations (days) of the immature stages from the 3rd instar until the pre-pupal stage were also recorded. Deformities of *A. gemmatalis* pupae were evaluated and recorded after pupation.

### Statistics

The design was completely randomized. The data collected were subjected to analysis of variance (ANOVA) and the means were compared with the Tukey test at 5% probability with the statistical program SAEG version 9.1. (Supplier: UFV) (SAEG 2005). Data were transformed to $\sqrt{x + 0.5}$ whenever necessary.

## Results

### Caterpillar Mortality

The percent mortality of *A. gemmatalis* caterpillars (mean 3rd to 6th instar) fed on soybean leaves was found to be 3.17, 7.18, 5.00, 11.25, 16.74, and 18.15% with the neem oil (Bioneem®) at the concentrations of 2.5, 5, 10, 15, 20, and 25%, respectively (Table 1).

### Pupal Mortality

The percent mortality of *A. gemmatalis* pupae obtained from treated caterpillars was 20.57, 22.79, 29.64, 39.16, 51.50, and 59.17% after exposure to neem oil (BIONEEM®) at 2.5, 5, 10, 15, 20, and 25%, respectively (Table 1).

### Viability

The total viability—as measured by adult emergence—of all immature stages from the 3rd instar to pre-pupa of *A. gemmatalis* was the greatest (82.35%) at the smallest concentration of neem oil (2.5%) and progressively decreased with increasing concentrations of neem oil to only 22.68% adult emergence at the greatest concentration of neem oil (25%) (Table 1). However, the total viability in the 15% neem oil treatment was somewhat less than expected.

### Cumulative Mortality and Duration from the 3rd Instar to the Pre-Pupa

The cumulative mortality of *A. gemmatalis* 3rd to 6th instars increased with increasing concentrations of neem oil (BIONEEM®) applied to the soybean plant on which the fed (Fig. 1).

The duration (days) of the larval stage from the 3rd instar to the pre-pupa (Fig. 2) in most of the

Table 1. Concentration of neem (Conc. Neem) (%), percent mortality of caterpillars (Mort. Larvae) (mean 3rd to 6th instar) (%) and percent pupal mortality (Mort. Pupae) (%) obtained when 8 *Anticarsia gemmatalis* third-instars were allowed to feed and develop on treated soybean branch from treated caterpillars (%) and total viability (3rd instar to pre-pupa) (Mean ± SD) (%) of *Anticarsia gemmatalis* fed on soybean plants treated with neem oil (Bioneem®) or distilled water at Viçosa, Minas Gerais, Brazil.

| % Conc. Neem | % Mort. Larvae | % Mort. Pupae | % Total Viability |
|---|---|---|---|
| 0 | 0.00 ± 0.00 a | 17.65 ± 3.31 c | 82.35 ± 3.31 d |
| 2.5 | 3.17 ± 2.09 a | 20.57 ± 7.64 c | 76.26 ± 7.20 d |
| 5.0 | 7.18 ± 2.29 a | 22.79 ± 4.18 c | 70.03 ± 3.56 cd |
| 10.0 | 5.00 ± 3.33 a | 29.64 ± 3.61 bc | 65.36 ± 3.73 cd |
| 15.0 | 11.25 ± 5.08 ab | 39.16 ± 5.52 abc | 49.59 ± 4.83 bc |
| 20.0 | 16.74 ± 4.96 b | 51.50 ± 6.72 ab | 31.76 ± 6.11 ab |
| 25.0 | 18.15 ± 7.21 b | 59.17 ± 7.53 a | 22.68 ± 5.76 a |

Means followed by the same letter per column do not differ by the Tukey test at 5%.

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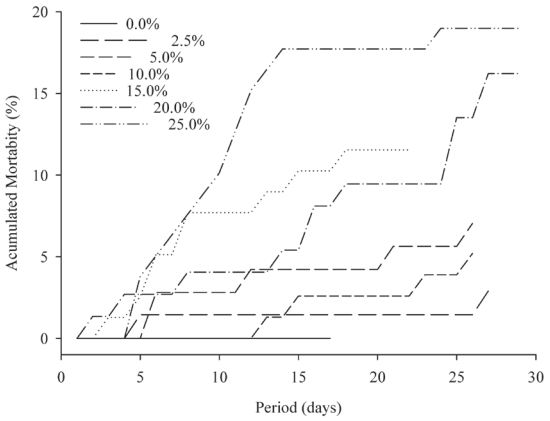



Fig. 1. Cumulative daily mortality of 3rd to 6th *Anticarsia gemmatalis* instars fed on soybean plants treated with neem oil (Bioneem®) or distilled water at Viçosa, Minas Gerais, Brazil. In each of 10 replicates, 8 *A. gemmatalis* 3rd instars were placed on a treated soybean branch and restricted to it by an organza bag. Mortality was recorded each day.

treatments was not greatly affected by the neem oil treatments. However, in the 20% Bioneem® treatment, the duration of the larval stage was substantially longer than in the control and in all of the other treatments.

Deformities

The *A. gemmatalis* pupae presented developmental interruptions and anomalies (Fig. 3) at all neem oil concentrations (BIONEEM®) to which they had been exposed as larvae feeding on treated soybean foliage.

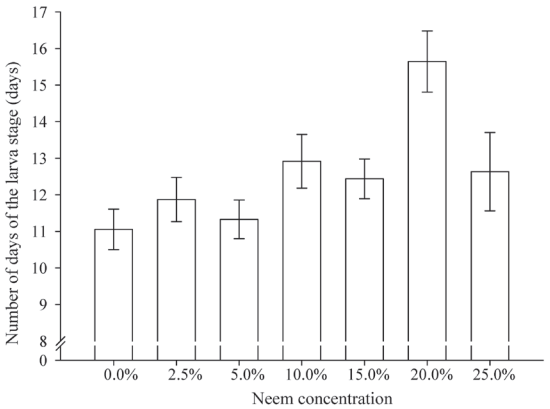



Fig. 2. Duration (days) of the larval stage (3rd instar to pre-pupa) of *Anticarsia gemmatalis* fed on soybean plants treated with neem oil (Bioneem®) or distilled water at Viçosa, Minas Gerais, Brazil.

Adult Emergence

The neem oil (BIONEEM®) reduced the emergence of *A. gemmatalis* adults even with the low mortality of caterpillars (3rd to 6th instar) and pupae obtained from treated caterpillars of this insect with lower concentrations of this oil.

## Discussion

The mortality of *A. gemmatalis* caterpillars due to the neem oil can be attributed to the impact of the azadirachtin, the main secondary metabolite of this plant, acting as a feeding inhibitor, by delaying development and growth, and by causing anomalies in the cells and physiology of the caterpillars (Koul et al. 2004a, 2004b; Petacci et al. 2012). The mortality of *A. gemmatalis* caterpillars on treated foliage can vary, because of variable the leaf consumption of treated plants (Almeida et al. 2010). Azadirachtin, an antifeedant, reduces the food consumption of insects (Bruce et al. 2004; Isman 2006; Silva et al. 2007), and it reduces insect damage to soybean crops. Thus the antifeedant property of azadirachtin increases its importance in Integrated Pest Management (IPM).

The mortality of *A. gemmatalis* pupae obtained from neem oil-treated caterpillars is important, because controlling the pupae of this insect within the soil is quite difficult. Therefore, the eggs and young *A. gemmatalis* caterpillars are more amenable to insecticidal exposure (Pereira et al. 2010; Tavares et al. 2013b). Commercial products based on neem, generally exhibit moderate toxicity (toxicological classification class III) and a moderate potential environmental hazard rating (class IV - a little hazardous to the environment). As these products are water soluble, they do not require mandatory preharvest intervals, i.e., periods before the crop can be harvested, according to regulations of the Ministry of Agriculture, Livestock and Supply (MAPA) of Brazil.

The reduction in the total viability from the 3rd instar to the pre-pupa of *A. gemmatalis* by exposure to Bioneem® is similar to that of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), and 80% of its caterpillars did not reach the pupa stage 24 h after the application of 0.5 ppm of azadirachtin suspension made from the mature leaves of *A. indica* grown in Brazil, dried and crushed in cold water at the ratio of 1:10 w.v$^{-1}$. The mortality of this insect was 100% with 1.0 ppm of this product, caused mainly by feeding inhibition following contact with the neem extract (Prates et al. 2003).

The highest cumulative mortality of 3rd to 6th instars and the longest duration of the period of the 3rd instar to the pre-pupa of *A. gemmatalis* were recorded with 20% concentration of Bioneem® sprayed on the soybean leaves.

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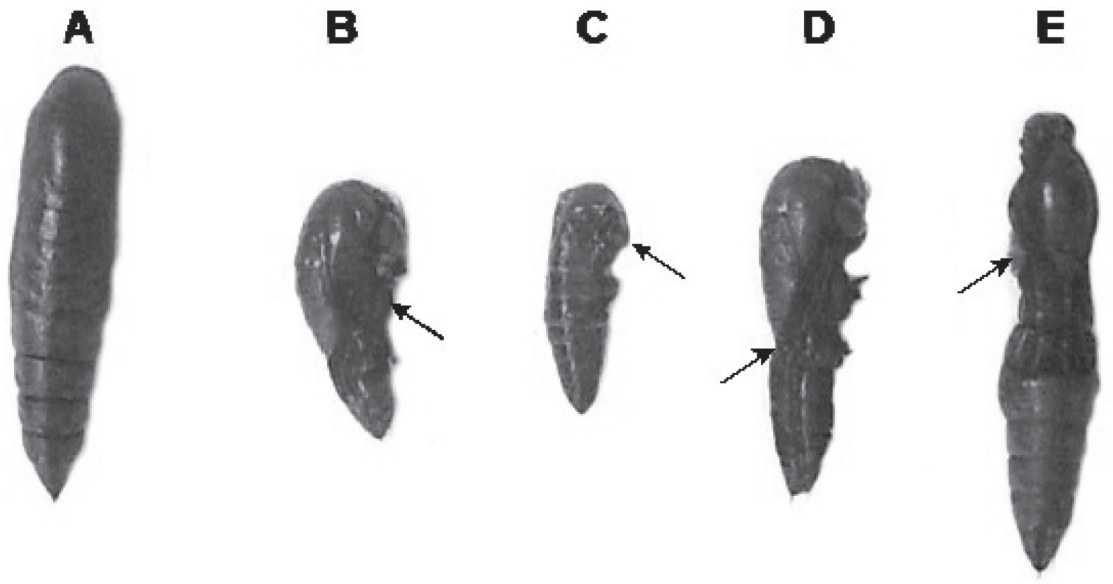



Fig. 3. *Anticarsia gemmatalis* pupae. A- normal. B to E- pupae with anomalies (arrows) that developed after the caterpillars were fed on soybean plants treated with neem oil (Bioneem®) at Viçosa, Minas Gerais, Brazil.

Similar effects were also reported for nymphs of the southern green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae) fed on leaves sprayed with different concentrations of 2 products [NeemAzal® T/S from Germany, containing 1% azadirachtin, and Neem Oil from USA, with fatty acid of the neem seeds and 25% potassium salt]. These 2 products, NeemAzal® T/S at 0.5% and Neem Oil at 2%, provided approximately 60% control of this pest in soybean crops (Durmusoglu et al. 2003).

Developmental anomalies of *A. gemmatalis* pupae from caterpillars fed with Bioneem® displayed abnormalities after the last larval molt. This is of great importance, because these abnormal insects exhibit a high mortality rate, and survivors are vulnerable to control by their natural enemies (Tavares et al. 2013c). The reduced the emergence of *A. gemmatalis* adults obtained from treated caterpillars with neem oil indicates that the commercial product Bioneem® obtained by cold pressing neem seeds, contains a low azadirachtin concentration, because 90.0% of this compound was retained as solid residue, named 'neem cake' (Silva et al. 2007). However, the control efficiency and the selectivity of neem formulations available in Brazil are variable because of the lack of mandatory standardization of azadirachtin levels (Brito et al. 2006).

The commercialized neem oil (Bioneem®) revealed the potential to reduce the populations of *A. gemmatalis*, particularly at a concentration of 25%. We suggest that Bioneem® can be used for soybean protection as a suitable alternative to synthetic insecticides in integrated management programs of *A. gemmatalis*.

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