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Effect of *Azadirachta indica* Extracts on Oriental Leafworm, *Spodoptera litura* (Lepidoptera: Noctuidae)

Nirupa Gadi*

Boston University College of Arts & Sciences, 725 Commonwealth Avenue, Boston, MA 02215, Email: nirupa@bu.edu

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Author's Summary: Research was conducted to determine if crude extracts from the tropical neem tree could control the Oriental leafworm caterpillar, a significant pest worldwide. While synthetic chemical insecticides can provide effective control of this pest, they leave residues on the plant that may be objectionable for some farmers, such as organic growers. However, organic production often allows chemicals derived from plants to be used. Neem extracts are allowed in organic production systems and have the potential to be effective alternatives to synthetic insecticides. This study investigated the effect of biologically based neem extracts on the Oriental leafworm, a generalist foliage-feeding caterpillar that damages a wide variety of crops. The results indicated that neem extracts was able to kill eggs and larvae, pupae and adults.

Abstract

The tropical neem tree, *Azadirachta indica*, has many medicinal and pesticidal properties. Virtually every part of the neem tree has been used by indigenous cultures for medicine or pest control for over 2,000 years. Neem extracts are used to treat various infections and to repel insects. In this study, the efficacy of *A. indica* extracts was evaluated against eggs and second instar larvae of the tropical armyworm *Spodoptera litura* (also known as the Oriental leafworm). We found that *A. indica* extracts killed 98.4% of eggs and 100% of larvae, pupae, and adults of *S. litura* exposed to extracts of neem leaves. Because *A. indica* extracts are not harmful to plants or humans, these chemicals can be used to control insects in gardens and around dwellings.

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*Email: nirupa@bu.edu

Introduction

Neem, *Azadirachta indica*, is a fast growing evergreen tree common in dry tropical regions [1]. Azadirachtin, a product derived from *A. indica*, is used in many parts of the world in applications ranging from the treatment of stomach ulcers to cosmetics [2]. Neem extracts are toxic to insects but not to humans or plants [3], and even body soap and toothpaste can be found that incorporate material from neem leaves [4]. Neem leaves are also used to treat leprosy, eye disorders, intestinal worms, skin ulcers, cardiovascular disease, diabetes, fever, gingivitis, and liver problems [5]. Neem extracts, which can be made from the entire plant, repel insects without damaging the crop itself [2].

Amid growing concerns about the toxic residue that synthetic chemical insecticides can leave on plants [6], many farmers have started to use neem extracts as a naturally occurring pesticide with low toxicity on humans [7]. These extracts have various biological effects on insects, pathogens, plant parasitic nematodes, and arachnids [3], and have also been reported to be toxic to all insect life-stages [8,9], causing either mortality or physical abnormalities [10] (Fig. 1). A number of commercial formulations of neem extracts are available.

Oriental leafworm, *Spodoptera litura,* is one of the most damaging insect pests of agricultural crops and is a generalist defoliator [11], feeding on over 120 plant species, including many cash, food, and ornamental crops [12]. Damage is due to complete or partial defoliation by larvae, which can be extensive [13] (Fig. 2). While Oriental leafworm larvae are primarily leaf feeders, they also act as cutworms, severing stems of young plants. Extensive feeding by *S. litura* on seedlings or young plants stunts their development, leading to small or late-maturing fruit [14].

Although many broad spectrum synthetic insecticides are available to control *S. litura*, the persistent nature of some of these chemicals limits their use near houses or in gardens. In addition, many populations of *S. litura* have developed resistance to some

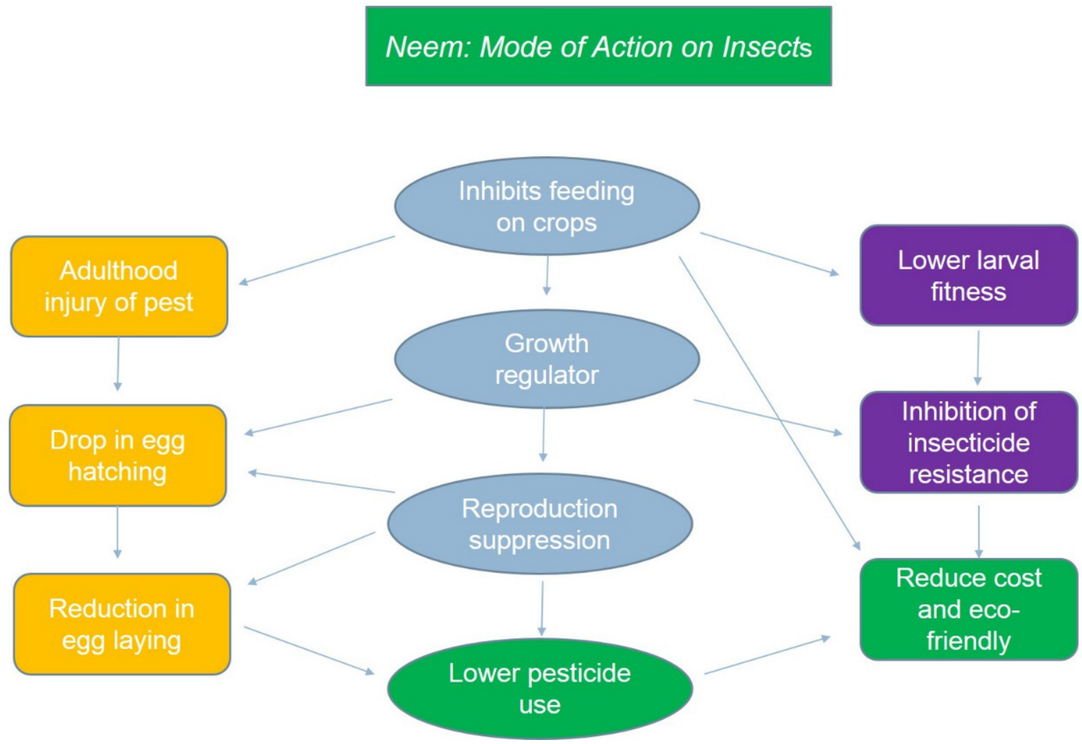

Figure 1. Neem has multiple advantages as an insecticide.

insecticides due to extensive exposure [15,16]. The evolution of resistance to insecticides is relatively swift in *Spodoptera* species due to their generalist feeding habits and short generation time of about one month [17]. Recent work has suggested that ecologically based insect pest management strategies can provide more environmentally sensitive solutions to problems caused by this pest.

A female moth lays batches of up to 300 eggs on the bottom of the leaves two to three days after mating. The eggs are whitish-yellow egg mass covered with hair scales and hatch in about 4 days [13]. Larvae pass complete six instars in roughly 13 days. The pupa develop in about 6-7 days while adults can live up to 10 days. The average life span from egg to adult is about 30 days (Fig. 3).

Figure 2. Leaf damaged by the caterpillar.

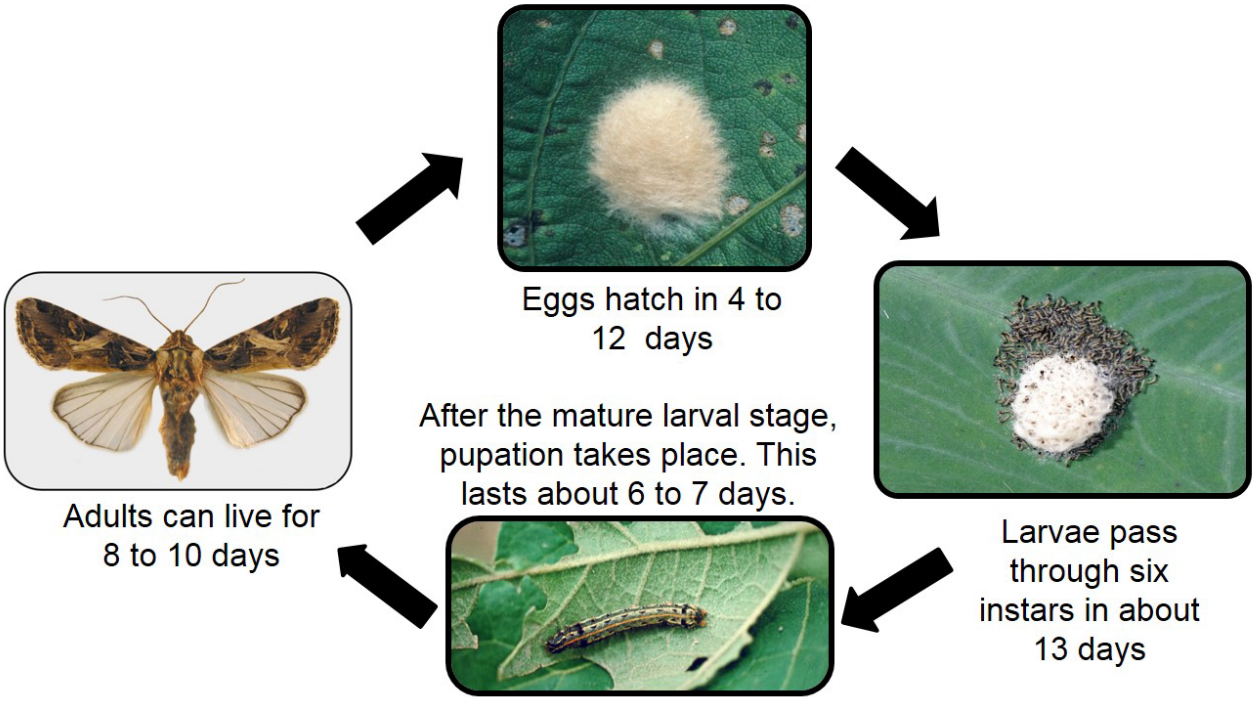

Figure 3. Life cycle of the Oriental leafworm (copyright: A.C. Hodges, IFAS, University of Florida).

Here, we examined the efficacy of crude extracts of *A. indica* leaves against various life stages of *S. litura*. Such information can be useful for both home gardeners and commercial growers.

Materials and Methods

Insects: Five hundred egg masses of *S. litura* were collected from eggplant *Solanum melongena* L. (Solanaceae) fields and taken to the laboratory for mass rearing. Fifty batches of egg masses were placed in each collapsible cage (12 × 10 × 10 cm) where the emerging larvae fed on eggplant foliage and fruits. The laboratory conditions were maintained at 23 ± 2 °C, 70–80% relative humidity and a 16:8 h L:D photoperiod.

Leaf extract from A. indica: Fresh *A. indica* leaves from a tree in Guam (USA) were collected a day before the start of the experiment and were soaked in tap water for 48 hours (1 kg leaves: 5 L water). The leaves were then ground into the water using a mortar and pestle to make the crude extracts that were used for the laboratory experiments.

Effect of extract on S. litura eggs: Twenty *S. litura* eggs were placed in a petri dish (90 mm) on filter paper, and *A. indica* extract (2 mL) was then sprayed directly on the eggs using a 30 mL Boston glass bottle with a mist sprayer. Five replicates were used along with five control

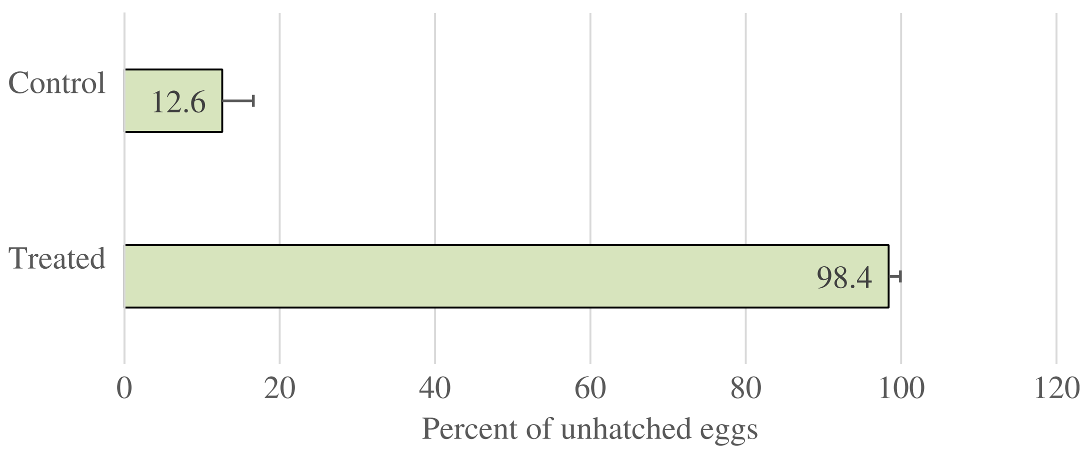

Figure 4. Mean ± SE percent of *Spodoptera litura* eggs that failed to hatch. Counts were based on 20 eggs per treatment, each treatment repeated five times. There are significant differences between the control and the treated groups (P = 0.05; Paired t-test).

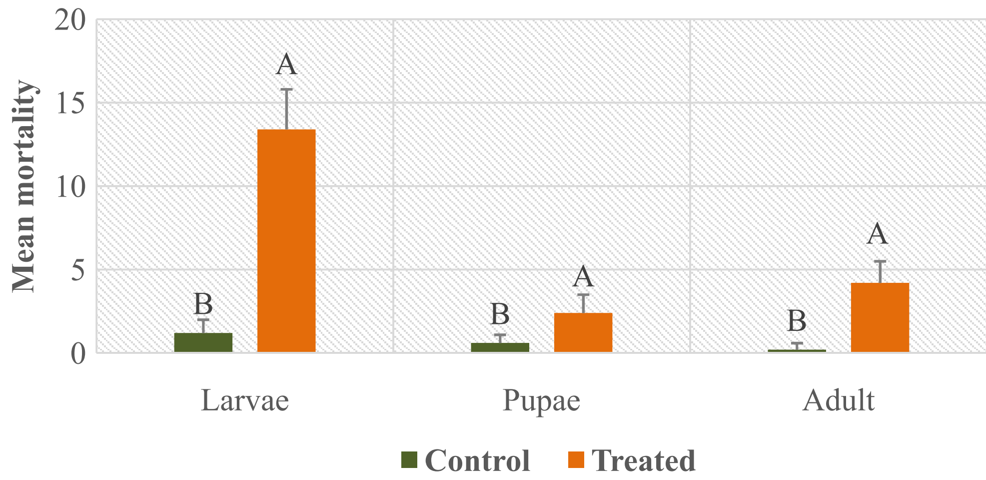

Figure 5. Mean ± SE mortality of different life stages of *Spodoptera litura*. Mortality was out of 20 larvae per treatment, each treatment repeated five times. Different letters on bars indicate significant differences (P = 0.05; Paired t-test).

replicates that were sprayed with 2 mL of water, for a total of 100 eggs exposed to either the neem extract or to the water control.

Effect of extract on S. litura second instar larvae. Twenty second-instar larvae of *S. litura* were placed in each of 5 petri dishes (90 mm) with cut eggplant leaves (5 cm diameter), and 2 mL of *A. indica* extract were then sprayed directly on the larvae. A treatment without the spray served as the control. Each treatment and control were replicated 5 times.

Observations: After 32 hours from the time of treatment to hatching in the treated and control groups, the unhatched eggs were counted. Dead larvae, pupae, and adults were also counted in the treatments and controls each day and expressed as mean mortality.

Statistical Analysis: Analyses were conducted using SAS version 9.3 [18]. The data represent two sorts of effects. The first data set refers to numbers of individuals that died in the stage that was treated (eggs and second instar larvae). The second data set represents the number of individuals that died from delayed effects, in the stages that followed the treated stage. The number of unhatched eggs and the mortality of insects at different stages were analyzed using a paired t-test.

Results

We found a significant difference ($P = 0.05$; paired t-test) in the number of eggs that hatched between the treatment with *A. indica* and the control (Fig. 4), with only 1.6% of eggs surviving neem exposure and successfully hatching, versus 87.4% hatch of eggs in the control group (exposed to water).

We also found a significant difference ($P = 0.05$) in the mortality of subsequent life stages following treatment of insects as second instar larvae between the treatment and control (Fig. 5). Second instar larvae treated with *A. indica* extracts had subsequent mortality rates of 67.0, 12.0, and 21.0% for larvae, pupae and adults, respectively. In the control, only 6.0, 3.0, and 1.0% of insects died as larvae, pupae, or adults during the study period, respectively.

Discussion

Limonoids are phytochemicals that are derived from limonin, belonging to a group of tetranortriterpenoids that exhibit a range of biological activities like insecticidal, insect antifeedant, and growth regulating activity for insects. Antibacterial, antifungal, antimalarial, anticancer, antiviral, and a number of other pharmacological activities have been shown in humans [19]. According to Srivastava [3], of the 350 known limonoids, 150 are found in *A. indica*, and many of these have insecticidal properties. The insecticidal effect of *A. indica* extracts has been reported for over 350 species of arthropods. Azadirachtin, a limonoid derived from *A. indica*, is a strong antifeedant and interrupts growth and reproduction for many insect species. However, its biochemical effects at the cellular level are not yet known [3].

Our study found that an *A. indica* extract affected eggs, larvae, pupae, and adults, causing direct mortality in treated stages and some additional mortality in subsequent life stages for individuals that did not die in the treated stage. Although pupae and adults were not treated, these are survivors of treatment as second instars still showed subsequent levels of mortality in later stages. These lingering effects are likely very different in rate and perhaps mechanistically from what would have occurred if directly treated those stages.

Although *A. indica* extracts are effective against many insect species [3,20], our study demonstrates the simplicity of using neem tree extracts against *S. litura*, a polyphagous, worldwide pest. Tests in India found that among various *A. indica* derivatives tested against *S. litura*, 5% extracts from *A. indica* seed kernels caused the highest larval mortality at 40%, and a subsequent IGR (insect growth regulator) effect on insects was observed for both the 5% seed kernel extract and the 3% oil (by pressing it out of the seeds) [21]. Koul *et al.* [22] found that salannin and nimbinene (*A. indica* constituents) had no toxic effects effects on *S. litura* larvae and determined that the observed antifeeding effects were the result of deterrents on chemoreceptors. Azadirachtin also directly and indirectly inhibits the secretion of trypsin in the gut, [22] and resulting disrupting tissues would function atypically and could induce disruption in enzyme secretion in the insects [23].

At higher concentrations, *A. indica* derivatives also reduce the survival of non-target organisms, including beneficial insects. Khattak and Rashid [24] determined that *A. indica* oil and seed water extracts at high concentrations had a slight effect on the biology of the parasitoid wasp *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae). Low concentrations, therefore, would be more compatible with biological control programs than higher concentrations. As noted by Mader and Adamson [25], while other pesticides approved for organic agriculture can cause significant harm to pollinators, neem derivatives have a low toxicity to honey bees. Direct contact with *A. indica* had no noticeable effect on worker honey bees, even at rates considerably above normal field applications. Even so, Riedle *et al.* [26] advised spraying neem extracts early in the morning, late in the evening or at night to lessen contact with adult bees. The derivatives from *A. indica* are considered to be non-toxic to humans.

While *A. indica* derivatives do not have a quick mortality effect, their safety for most non-target organisms makes such extracts more suitable for use than traditional broad spectrum synthetic pesticides. Further studies are needed to examine the influence of neem extracts on the feeding physiology, life table parameters, oviposition, and mating behavior of *S. litura*.

Conclusions

There is no evidence of insects developing resistance to *A. indica* derivatives, most likely because multiple toxins are present in such extracts. Therefore, *A. indica* extracts likely can be used indefinitely in integrated pest management programs without provoking resistance. In developing countries, if permitted by local laws governing pesticides, neem extracts, which are simple to prepare, can be used by home owners and farmers to replace more toxic insecticides in their gardens and crop fields.

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