Colegio de Postgraduados

Sublethal Effects on the Biological Development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) When Consuming Neem

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ABSTRACT

Objective: The Fall armyworm *S. frugiperda* was bred in laboratory and put under dose of watery extracts and oil of seed of *A. indica* with the aim of determining and to quantify the alterations in the biological development of larvae and pupae of caused by the consumption of *A. indica* and the doses in which the alterations happen.

Design/Methodology/Approach: Two experiments were development. In one eight doses were evaluated, with watery extract of seed of *A. indica* in 4.0, 1.0, 0.7, 0.4, 0.1, 0.07, 0.04 and 0.01%. In the second the doses of oil of *A. indica* were evaluated 0.16, 0.09, 0.02, 0.016, 0.009, 0.002, 0.0016 and 0.0009%. In both experiments the variables were quantified: duration of the stage of larvae and pupae, survival of the larval stage and pupae, survival of the larva stage and pupae and weight of pupae.

Results: The larvae prolonged their development in three days more than the witness when consuming watery extract of *A. indica*; They reduced its survival like la rvae and pupa in 18 and 27.5% respectively. In Addition, was reduced the weight of pupas. When consuming oil of *A. indica*, the larvae delayed their development from three to 15 and 19 days more than the witness.

Study limitations/Implications: The study was carried out under laboratory conditions and in a single cycle; it is desirable to repeat the study for more cycles and with different populations of fall armyworm.

Finding/Conclusions: The adverse effects in larvae were pronounced of three forms: 1) the duration of the larval stage altered when staying the larvae by more days in that stage without changing pupae. 2) the survival of the larval stage was reduced and 3) it decreased weight of pupa.

Keywords: Inhibition of growth, watery extracts, oil of *A. indica*, survival of larvae.

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INTRODUCTION

The most important pest insect for maize cultivation is the fall armyworm (*Spodoptera frugiperda* Smith) (Ngegba *et al.*, 2022). Various control methods are used to manage this pest, including chemical, rational, and neem (*Azadirachta indica*) (Silva *et al.*, 2015; Ewansiha *et al.*, 2023). Neem causes mortality in more than 400 evaluated pest insect species (Schmutterer and Rembold 1995; Ahissou *et al.*, 2022). Ingestion of this substance in insects results in inhibition of feeding and growth, as well as repellency, reduction in oviposition, sterilization, and changes in the neuroendocrine system, leading to suppression of ecdysis (Dorn 1996; Roel *et al.*, 2010; De Campos *et al.*, 2014; Pérez-Cogollo *et al.*, 2015; and Figueroa Gualteros *et al.*, 2019). Various studies have been conducted to understand the effects of neem on the physiological and behavioral alterations across multiple insect

species (Schmutterer and Rembold 1995; De Oliveira *et al.*, 2010; Hakeem *et al.*, 2018; Moonga *et al.*, 2018; Mwanauta *et al.*, 2021).

In larvae of *Trichoplusia ni* (Hübner) and *Spodoptera exigua* (Hübner), the developmental period and larval mortality were prolonged upon ingesting *A. indica* extracts (Prabhaker *et al.*, 1986), as well as growth inhibition in first-instar larvae of *S. frugiperda* (Jacobson *et al.*, 1984). In *S. frugiperda*, the efficacy of neem in causing mortality has been studied (Phambala *et al.*, 2020; Ahissou *et al.*, 2022; Ngegba *et al.*, 2022; Chan *et al.*, 2023) and general symptoms caused by the ingestion of *A. indica* have been documented (De Oliveira *et al.*, 2010). However, the effects of consumption and the alterations in the biology of larvae and pupae have not been determinated.

The present study was conducted to determine and quantify the alterations in the biological development of *S. frugiperda* larvae and pupae caused by the consumption of neem, as well as the levels of ingestion at which these alterations occur.

MATERIALS AND METHODS

Aqueous extracts and seed oil of *A. indica* were evaluated in an artificial diet against *S. frugiperda* at a temperature of 25±2 °C, relative humidity of 60±10%, in the Entomology Laboratory of the Maize Program at the International Maize and Wheat Improvement Center (CIMMYT) in El Batán, Texcoco, State of Mexico.

To conduct the experiments, it was necessary to have a population of corn earworm larvae for artificial infestation. The first-instar larvae of *S. frugiperda* were obtained from the mass rearing of insects at the Entomology Laboratory of CIMMYT.

The aqueous extracts were obtained from ground seeds of *A. indica* from maize plantations in the municipality of San Pedro Tututepec, on the Oaxaca Coast, Oaxaca. The *A. indica* oil, NEEM OIL EXTRACT® at 93% with a concentration of azadirachtin of 4,463 ppm, was recommended at a dose of 250 ml in 200 liters of water. To determine the doses evaluated in the experiments of this study, 2 preliminary bioassays were conducted with generalized ranges of the concentration of *A. indica*, evaluating the same variables presented here, until the doses to be evaluated in these treatments were determined.

Experiment with Aqueous Extract of *A. indica*

A laboratory experiment was conducted using eight doses of *A. indica* seed aqueous extract at concentrations of 4.0, 1.0, 0.7, 0.4, 0.1, 0.07, 0.04, and 0.01%. To prepare the different doses, 4.0 g of *A. indica* seed was mixed with 100 ml of water 24 hours before preparing the diet. The doses were kept in an amber bottle to allow for better extraction and preservation of water-soluble compounds. After 24 hours, the solid portion was separated from the liquid by filtering through a sieve. Once the artificial diet was prepared, the obtained liquid compound was added. This way, the indicated doses were prepared, along with a control to which only 100 ml of water was added.

Experiment with *A. indica* Oil

In the *A. indica* oil experiment, the following doses were applied: 0.154, 0.088, 0.022, 0.0154, 0.0088, 0.0022, 0.00154, and 0.00088 ml (equivalent to 0.16, 0.09, 0.02, 0.016,

0.009, 0.002, 0.0016, and 0.0009%). In each of the doses, the oil was measured with a 200μl Gilson pipette and mixed with water to obtain a volume of 100 ml for each treatment. The artificial diet described by Mihm (1983) was used for these tests, into which the aqueous extracts at 4.0, 1.0, 0.7, 0.4, 0.1, 0.07, 0.04, and 0.01% were incorporated during its preparation, resulting in approximately 600 g of diet for each treatment. The amount of water required for preparing the extract was included in the total amount of water normally used for preparing the artificial diet. In addition to the corresponding diet, a control diet was also prepared for each dose, to which only water was added. After preparation, the diets were poured into glass tubes with a capacity of 5 cm in height × 1.5 cm in diameter. Twenty-four hours after the preparation of the artificial diet with *A. indica* at different concentrations, first-instar *S. frugiperda* larvae were placed in the glass tubes. There were 100 tubes for each dose, with one newly hatched larva placed in each tube. Each tube was covered with sterilized cotton and kept in a rearing chamber at 25±2 °C and 60±10% relative humidity. The 100 larvae per dose were checked daily to quantify mortality and the duration of the larval stage until transformation to the pupal stage.

The pupae were weighed at 24 hours using an analytical scale with a precision of 0.001 g and were transferred to other tubes, where they remained until the emergence of adults. For each dose, the duration and survival of the larval and pupal stages, as well as the weight of the pupae, were evaluated. Larval survival is the percentage of larvae that progress to the pupal stage, and pupal survival is the percentage of pupae that emerge as adults. In the nine doses evaluated, eight concentrations of *A. indica* oil (0.154, 0.088, 0.022, 0.0154, 0.0088, 0.0022, 0.00154, 0.00088 ml made up to 100 ml with water) and a control without *A. indica* were included. The procedure and variables evaluated were the same as those used for the experiments with aqueous extracts of *A. indica* seeds.

For conducting these tests, the artificial diet described by Mihm (1983) was used, into which the aqueous extracts at 4.0, 1.0, 0.7, 0.4, 0.1, 0.07, 0.04, and 0.01% were incorporated during its preparation, resulting in approximately 600 g of diet for each treatment. The amount of water required for preparing the extract was included in the total amount of water normally used for preparing the artificial diet. In addition to the diet corresponding to each dose, a control diet was also prepared, to which only water was added. After preparation, the diets were poured into glass tubes with a capacity of 5 cm in height × 1.5 cm in diameter.

Twenty-four hours after preparing the artificial diet with *A. indica* at different concentrations, first-instar *S. frugiperda* larvae were placed in the glass tubes. One hundred tubes were prepared for each dose, with one newly hatched larva placed in each tube. Each tube was covered with sterilized cotton and kept in a rearing chamber at 25±2 °C and 60±10% relative humidity. The 100 larvae per dose were checked daily to quantify mortality and the duration of the larval stage until transformation to the pupal stage.

The pupae were weighed at 24 hours and transferred to other tubes, where they remained until the emergence of adults. For each dose, the duration and survival of the larval and pupal stages, as well as the weight of the pupae, were evaluated. Larval survival is the percentage of larvae that progress to the pupal stage, and pupal survival is the percentage of pupae that emerge as adults.

In the nine doses evaluated (eight concentrations of *A. indica* oil: 0.154, 0.088, 0.022, 0.0154, 0.0088, 0.0022, 0.00154, 0.00088 ml rated at 100 ml with water, and a control without *A. indica*), the procedure and variables evaluated were the same as those used for the experiments with aqueous extracts of *A. indica* seeds.

Statistical Analysis

The obtained data were subjected to an analysis of variance and analyzed using the non-parametric rank test (PROC RANKS) (SAS 2002). Each treatment had 5 repetitions, and each repetition consisted of a batch of 20 larvae. For both experiments in this stage, the following variables were evaluated: duration of the larval stage, duration of the pupal stage, larval and pupal survival, and pupal weight. These variables are suitable for describing the developmental stages of *S. frugiperda* (Figueroa Gualteros *et al.*, 2019; Phambala *et al.*, 2020).

RESULTS

Experiment with Aqueous Extract of *A. indica*

In all treatments, the larvae fed on the provided diet. The concentrations of *A. indica* aqueous extracts at 0.4, 0.7, 1, and 4% added to the artificial diet resulted in 100% mortality in *S. frugiperda* larvae. This analysis presents the sublethal effects of *A. indica* on the larval and pupal development of the insect.

In the experiment with aqueous extracts, for the variable of larval stage duration, all concentrations were significantly different from the control, except for the 0.01% concentration ($F_{4, 76}$=0.526; P<0.0621) (Figure 1). In contrast, there were no statistically significant differences between treatments for the variable of pupal stage duration in days ($F_{4, 76}$=7.407; P<0.023) (Figure 1).

Larval survival was statistically different among treatments (Figure 2). This significant difference occurred between the 0.04% concentration and the control ($F_{4, 76}$=1.9437; P <0.111). Pupal survival was significantly different among the various concentrations ($F_{4, 76}$=2.9903; P=0.024), with the 0.1% aqueous extract concentration showing the most pronounced effect, reducing survival by 27.5% compared to the control. Regarding pupal

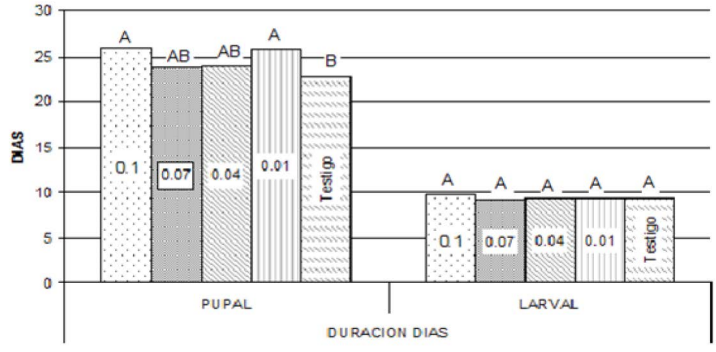

Figure 1. Duration in days of the larval and pupal stages, and pupal weight of *S. frugiperda*, reared on artificial diet mixed with aqueous extracts of *A. indica* seeds. Means followed by the same letter in the columns do not differ from each other, according to the rank test at P<0.05.

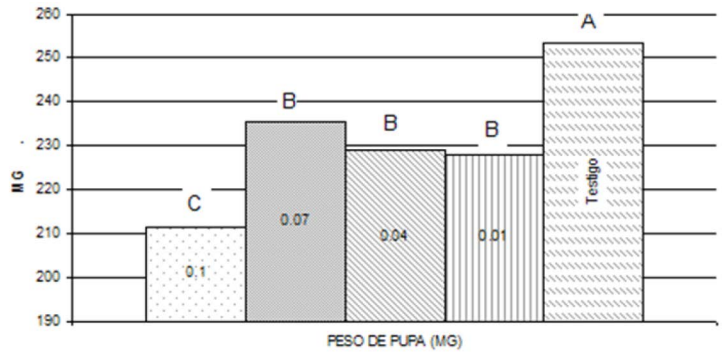

Figure 2. Pupal weight of *S. frugiperda*, reared on artificial diet mixed with aqueous extracts of *A. indica* seeds. Means followed by the same letter in the columns do not differ from each other, according to the rank test at P<0.05.

weight, there are statistical differences between the treatments and the control, with the 0.1% aqueous extract concentration standing out (Figure 2).

Experiment with *A. indica* Seed Oil

For the variable of larval stage duration, all treatments with different doses of *A. indica* oil were not significantly different from the control, except for the treatment with the 0.09 concentration of *A. indica* oil ($F_{7, 133}$=5.44; P=0.06317) (Figure 3). However, there were statistical differences among all treatments with different concentrations of *A. indica* oil and the control for the variable of pupal stage duration, except for the treatment with the 0.002 concentration, which was similar to the control ($F_{7, 133}$=7.538; P=0.04287) (Figure 3).

Larval survival was significantly different among the treatments with the concentrations of 0.02, 0.009, and 0.0016% compared to the control ($F_{7, 133}$=13.018; P=0.03128) (Figure 4). For pupal survival, statistical differences were observed among the treatments with concentrations of 0.16, 0.09, and 0.009, while the other treatments were similar to the control ($F_{7, 133}$=12.1411; P=0.02872) (Figure 4). In the variable of pupal weight, all treatments with different concentrations of *A. indica* oil were statistically different from the control ($F_{7, 133}$=35.2; P<0.01947) (Figure 5).

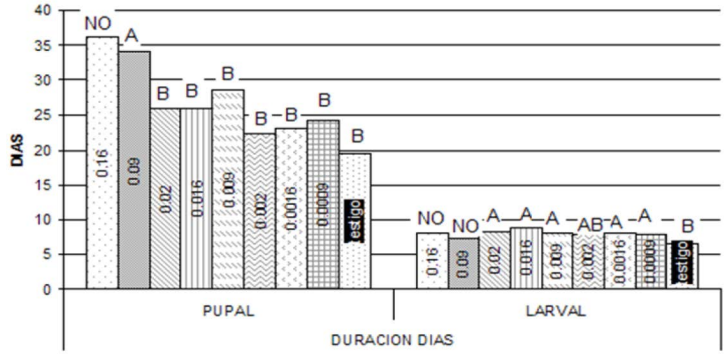

Figure 3. Duration of the larval and pupal stages of *S. frugiperda*, fed on artificial diet with *A. indica* oil. Means followed by the same letter in the columns do not differ from each other according to the rank test at P<0.05.

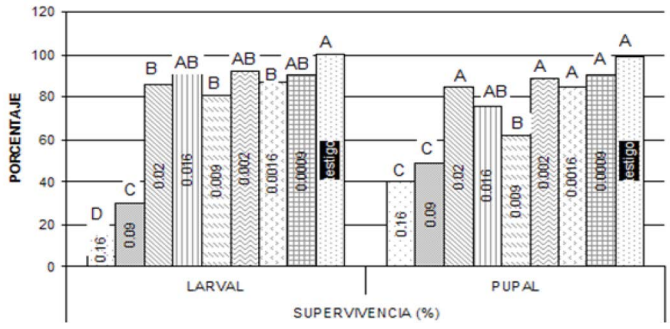

Figure 4. Survival of the larval and pupal stages of *S. frugiperda*, fed on artificial diet with *A. indica* oil. Means followed by the same letter in the columns do not differ from each other according to the rank test at P<0.05.

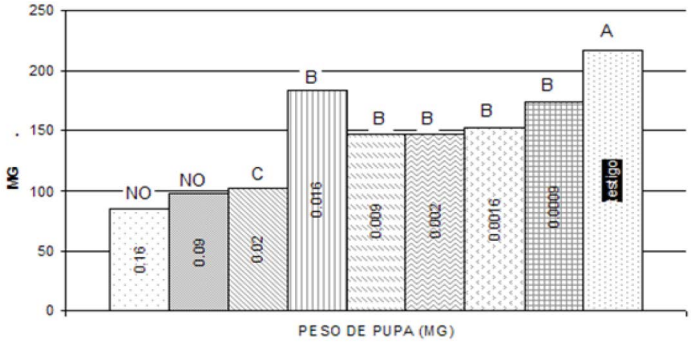

Figure 5. Pupal weight of *S. frugiperda*, fed on artificial diet with *A. indica* oil. Means followed by the same letter in the columns do not differ from each other according to the rank test at P<0.05.

DISCUSSION

Experiment with Aqueous Extracts of *A. indica*

When *S. frugiperda* larvae consumed diet with aqueous extracts of *A. indica* at the concentrations of 0.4, 0.7, 1, and 4%, there was 100% mortality. This effect is significant as it can reduce the number of individuals and/or the population of the pest due to ingestion poisoning, which is consistent with observations made in *Spodoptera littoralis* (Pineda *et al.*, 2004a; Pineda *et al.*, 2006b; Schneider *et al.*, 2004).

Larvae fed with 0.1% and 0.01% aqueous extract of *A. indica* mixed in artificial diet only showed sublethal effects, lasting 3.1 and 3.0 days, respectively, longer than the control in the larval stage (see Figure 1). This effect is likely related to the neurotoxic action of natural insecticides, which causes paralysis in intoxicated insects; these insects stop feeding and grow slowly (Pineda *et al.*, 2006).

Survival at the different concentrations of aqueous extracts of *A. indica* mixed with artificial diet was similar to the control, except at the 0.04% concentration, which recorded a 16.6% lower survival rate of the larval stage compared to the control larvae (Figure 2). Similar results were obtained by Figueroa Gualteros *et al.* (2019) and Duarte *et al.* (2019). Pupal survival was different among all treatments fed on artificial diet with various concentrations of aqueous extracts of *A. indica*, with the most notable effect at the 0.1% concentration, which reduced survival by 27.5% compared to the control. These data are consistent with Prabhaker *et al.* (1986), who found that incorporating *A. indica* seed extracts

into artificial diet at concentrations of 0.02%, 0.2%, and 2.0% prolonged development and induced mortality in all larval stages of *Spodoptera exigua* (Hubner), preventing pupation. Regarding pupal weight, all concentrations differed from the treatment with artificial diet without aqueous extract of *A. indica*, which had the highest pupal weight. The 0.1% aqueous extract concentration was significantly the lowest, reducing the average pupal weight by 18.55% compared to the control (Figure 3). This finding is consistent with the data observed by Prabhaker *et al.* (1986) in *Spodoptera exigua* (Hübner) larvae, who noted that reduced feeding led to a decrease in pupal weight (PP) compared to control larvae.

Experiment with *A. indica* Seed Oil

When analyzing the duration of the larval stage, treatments with the concentrations 0.09, 0.009, and 0.0009 of *A. indica* oil mixed in artificial diet had 14.6, 9.2, and 15 days longer duration than the control. This effect is caused by intoxication or inhibition of feeding on the artificial diet containing substances unpleasant to the insect; reactions observed in intoxicated insects, which stop feeding and grow slowly (Pineda *et al.*, 2006).

Pupal duration between the control and the different treatments varied, except in the 0.002% treatment, which was the same. The 0.016% concentration stood out with 2.27 days longer duration, possibly due to interference with the neuroendocrine system by affecting ecdysone and juvenile hormone synthesis (Schmutterer, 1988).

Larval survival was reduced by 95% at the 0.16% concentration and by 70% at the 0.09% concentration of *A. indica* seed oil (Figure 5) compared to the larval survival in the control. Pupal survival showed the most significant reductions at the 0.16% and 0.09% concentrations, with reductions of 59% and 50.3%, respectively, also caused by insect intoxication. This toxic effect was demonstrated in the study conducted by Chan *et al.* (2023). In contrast, pupal weight decreased in all *A. indica* oil treatments compared to the pupal weight in the control, with the 0.16% and 0.09% oil treatments showing the greatest reductions at 60.9% and 55.17%, respectively. This could be a consequence of the effects of consuming artificial diet with *A. indica*, as reported by various researchers in other insect species (Gaaboub and Hayes 1984; Koul *et al.*, 1990; Dorn, 1996; Mitchell *et al.*, 2004; García *et al.*, 2006; Mwanauta *et al.*, 2021).

The results of both experiments (with aqueous extract and with seed oil) demonstrated that the best effects were achieved when using aqueous extracts rather than seed oil of *A. indica*. This may be because aqueous extracts contain other secondary compounds in addition to azadirachtin (Opender, 2004), which are removed during the oil extraction process and discarded with the rest of the seed, or perhaps because the aqueous extract can penetrate or be ingested more easily into the insect's body than the oil.

CONCLUSIONS

Sublethal effects on *S. frugiperda* larvae fed artificial diet mixed with different concentrations of aqueous extracts or *A. indica* oil were observed in the following ways: the duration of the larval stage was altered as larvae remained in this phase for more days. Survival of the larval stage was reduced because it prevented all larvae from pupating; in other words, a portion of the insect population was killed. The 0.4% concentration of *A.*

indica aqueous extract and higher concentrations in artificial diet caused 100% mortality in *S. frugiperda* larvae. It was found that the 0.1% treatment extended the duration of the larval stage by 3.2 days, reduced larval survival by 18%, and pupal survival by 27.5% compared to the control. There was an increase in the duration of the larval stage of up to 86.6% and 75.3% when *S. frugiperda* was fed artificial diet with *A. indica* oil at 0.16% and 0.09% concentrations, and 21.2% and 12.1% in the duration of the pupal stage for the same treatments. Larvae of *S. frugiperda* fed with artificial diet mixed with the highest concentrations of *A. indica* oil showed a longer duration of the larval stage and recorded lower weights, approximately 61% less than the control. The 1.0% and 0.4% aqueous extract treatments caused pupae to start halting their growth in the 5th and 6th instars, decreasing on average by 14% to 35%. The 0.5% *A. indica* oil treatment recorded 40% of pupae and 45% of larvae in the 6th instar, and the 0.16% treatment had 58% of pupae and 23% in pre-pupa.

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