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RESEARCH ARTICLE

Sublethal influences of *Azadirachta indica* on the developmental stages of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

This research investigated the sublethal and antifeedant effects of three different neem-based formulations on the development of the fall armyworm, *Spodoptera frugiperda*, a major pest of maize. The study was conducted under controlled laboratory conditions to ensure consistency and reliability of results. The tested formulations included a commercially available neem product, neem oil and neem seed kernel extract (NSKE). Second instar larvae of *S. frugiperda* were exposed to sublethal concentrations of each formulation and various biological and developmental parameters were closely monitored. These included larval and pupal durations, pupal weight, adult emergence rate, adult body size and adult longevity. The findings revealed that all three neem-based treatments significantly influenced these developmental traits compared to the control. Among the treatments, the commercial neem formulation had the most pronounced effects, notably prolonging both larval and pupal stages while simultaneously reducing pupal weight, adult emergence, adult size and lifespan. These disruptions in development suggest that neem-based products, particularly commercial formulations, have considerable potential as environmentally friendly tools in integrated pest management (IPM) programs. Their use could help to suppress *S. frugiperda* populations by impairing their development and reproductive capacity, offering a sustainable alternative to conventional chemical insecticides in maize cultivation.

Keywords: developmental stages, neem-based formulations, *Spodoptera frugiperda*; sublethal effects

Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera; Noctuidae), is a polyphagous and noxious pest native to the tropical and subtropical regions of America. It is a major pest of maize crops but also affects approximately 353 plant species, including rice, sorghum, cotton, alfalfa, millet, peanuts and other cultivated and wild plant species (1–6). The name "fall armyworm" was derived from its damage type, in which the infestation sometimes resembles an army as it moves through large agricultural fields. Maize (*Zea mays* L.), a member of the Poaceae family, is one of the worlds' most significant cereal crop species, contributing to food security in the majority of poor countries. After rice and wheat, maize is becoming Indias' third most significant crop. *Spodoptera frugiperda* attacks all stages of the plant, from seedling until tasselling and causes defoliation, killing the young plant, resulting in grain damage and subsequently reducing the quantity and quality of yield (7). The excessive use of synthetic insecticides has caused resistance in *S. frugiperda* in America and Africa. No reports of resistance have been recorded in India. This pest in the United States is resistant to 29 insecticides with different modes of action. Plant-based insecticides are designed to act on specific biochemical sites or physiological processes of the target pest (8, 9). Environmentally friendly pesticides are known for their biodegradability and generally low toxicity to non-target plants and animals because of their botanical origin. As a result, such pesticides can be used in integrated pest control programmes (10). Numerous studies have been conducted in search of plant sources of safe pesticides as a result of the increased advocacy for the use of safer insecticides (11). For instance, azadirachtin, a significant insect repellent, is derived from the Indian neem tree (10). Neem or Indian lilac (*Azadirachta indica* A. Juss.) belongs to the family Meliaceae, which has been known for thousands of years for its antiseptic properties. It is an indigenous plant of the Indian subcontinent and Southeast Asia (12–16). There are more than 100 biologically active chemicals found in this plant, such as azadirachtin and terpenoids, which have antifeedant, insecticidal and repellent properties (14, 17). These properties are effective in reducing the population of insects (18, 19). Azadirachtin, a steroid-like tetranortriterpenoid, is the primary active compound responsible for a wide range of bioactivities in many insect pests (10, 18, 20). This compound interferes with the process of chemoreception by blocking chemoreceptors (21). This compound inhibits mitosis and induces detrimental effects on insect tissues such as epithelial cells of the gut, muscles, fat body and flight muscles (22, 23). Azadirachtin is usually associated with a marked antifeedant activity and even behavioural avoidance in a large number of insect species, including hemipterans, lepidopterans, orthopterans, coleopterans and dipterans (23–27). Therefore, considering the seriousness of this pest and its ability to develop resistance to many commonly used insecticides. In light of the above discussion, it was planned to evaluate the influence of the sublethal effect of *A. indica* on the developmental stages of *S. frugiperda*. Considering the economic significance of *S. frugiperda* and its known ability to develop resistance against several conventional insecticides, the present study was undertaken to evaluate the sublethal impacts of *A. indica* on its developmental parameters.

Materials and Methods

Raising of food crops and test insect rearing

The sowing of food crops was performed as per the recommendation of the package of practices for the cultivation of kharif crops. The crop was checked for any insect pest infestation without any insecticide application. Fresh leaves from thirty-day-old crops were used as food for the test insects. The *S. frugiperda* population was collected from maize fields in perforated polythene bags and brought to the Insect Physiology Laboratory of the Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India. The culture was maintained on the maize leaves in a biochemical oxygen demand (BOD) incubator at a temperature of 26 ± 1 °C and a relative humidity of 65 ± 5 %.

Determination of sublethal influences of *Azadirachta indica* on larvae, pupae and adults of *Spodoptera frugiperda*

The sublethal influences of three neem-based formulations (i.e., commercial neem formulations (0.15 %): Neem Kavach, neem oil: B natural cold pressed neem oil concentrate and neem seed kernel extract: homemade) on various development stages of *S. frugiperda* were investigated at a persistent temperature of 26 ± 1 °C and relative humidity of 65 ± 5 % in a BOD incubator. Second-instar larvae of *S. frugiperda* were treated with the three neem-based formulations with the previously determined LC_{30} and LC_{50} levels. The test insect larvae (10/jar) were exposed for 72 hr. After that, they were provided with fresh leaves of maize plants. There were 5 replications, each comprising 10 larvae. Observations were made in the treatment and control lots (no treatment was given), viz. larval duration, pupal duration, pupal weight, pupal size, adult emergence, adult size and adult longevity. The time from hatching to the formation of the prepupa was considered as the larval period. The leaves were changed every 24 hr until the larvae entered the prepupal stage, at which point the larvae stopped feeding. After the larval stage, the formation of pupae occurs. The collected pupae were transferred to separate glass jars covered with muslin cloth, tightened with rubber bands and observed daily until adult emergence. The pupal period was recorded from the date of formation of the pupae to the date of emergence of the adults. Then, the pupal weight was measured with an electronic weighing balance. The pupal size was measured with the help of a stereo zoom microscope. Adult emergence from the pupae was recorded daily. The percentage of adult emergence was calculated from the number of adults out of the total number of pupae. The size of the surviving adults was measured with the help of a portable microscope. The interval between the emergence of the adults and their death was taken as the adult longevity.

Statistical analysis

The data were analysed using the statistical software package CPCS1 and SPSS 16.0 statistical software using a completely randomised design (CRD), factorial design, one-way ANOVA and t-test (28).

Results

The second-instar larvae of *S. frugiperda* were treated with sublethal concentrations (LC_{30} and LC_{50}) of the commercial neem formulation (0.15 %), i.e., 0.000169 % and 0.000288 %, neem oil (0.045 % and 0.238 %) and NSKE (0.695 % and 1.348 %), respectively (Table 1 & 2). The influences of these concentrations on larval duration, pupal duration, pupal weight, pupal size, adult emergence, adult size and adult longevity were recorded under laboratory conditions (Fig. 1). Compared with the treated lots, the untreated lot had the significantly shortest larval duration of 22.40 days. Compared with those of neem oil (23.20 and 30.80 days) and NSKE (22.80 and 30.0 days), the sublethal concentrations (LC_{30} and LC_{50}) of commercial neem formulations were more effective, as they increased larval duration (24.20 and 37.0 days). The pupal duration in the untreated lot was 6.60 days, which was significantly lower than that in the treated lots. The pupal duration of *S. frugiperda* also significantly increased in response to all the neem-based formulations, but a greater effect was noted for the commercial

Table 1. Comparison of subethal effects of neem-based formulations (LC_{30}) on the development stages of *Spodoptera frugiperda*

Formulation (LC_{30})	Larval duration* (days)	Pupal duration* (days)	Pupal weight* (g)	Pupal size* (mm)		Adult emergence* (%)	Adult size* (mm)				Adult longevity* (days)	
							Male		Female		Male	Female
				Length	Breadth		Length	Breadth	Length	Breadth		
Commercial neem formulation (0.15 %)	24.20 ± 1.85^{a}	8.40 ± 0.92^{a}	0.142 ± 0.011^{c}	7.960 ± 0.167^{b}	2.309 ± 0.109^{b}	62.0 (51.95)^{b}	9.343 ± 0.257^{b2}	4.670 ± 0.103^{b2}	8.699 ± 0.066^{b1}	3.993 ± 0.077^{b1}	4.80 ± 0.58^{b}	5.00 ± 0.54^{b}
Neem oil	23.20 ± 2.57^{b}	7.80 ± 0.58^{b}	0.179 ± 0.016^{b}	8.001 ± 0.172^{ab}	2.773 ± 0.067^{a}	70.0 (56.89)^{a}	9.574 ± 0.133^{a1}	4.834 ± 0.081^{a1}	8.808 ± 0.100^{ab2}	4.164 ± 0.065^{ab2}	5.00 ± 0.31^{b}	6.20 ± 0.20^{a}
NSKE 5 %	22.8 ± 1.24^{b}	6.80 ± 0.80^{c}	0.183 ± 0.015^{a}	8.174 ± 0.215^{a}	2.796 ± 0.034^{a}	72.0 (58.21)^{a}	9.651 ± 0.345^{a2}	4.951 0.275^{a2}	8.935 ± 0.266^{a1}	4.264 ± 0.050^{a1}	6.00 ± 0.31^{a}	6.40 ± 0.24^{a}
Untreated (0.00)	22.40 ± 0.81^{b}	6.60 ± 0.60^{b}	0.204 ± 0.013^{a}	8.467 ± 0.049^{a}	3.909 ± 1.261^{a}	92.00 (75.22)^{a}	14.081 ± 0.346^{a1}	6.127 ± 0.183^{a1}	13.598 ± 0.240^{a1}	5.996 ± 0.222^{a2}	8.60 ± 0.24^{a}	9.00 ± 0.31^{a}

*Mean ± SE, based on five replications each comprising 10 larvae. The figures in parentheses are arc sine transformations. Values with different alphabetic superscripts are significantly different (p<0.05) with respect to different columns. Values with different letters are significantly different (p<0.05) with respect to different rows.

Table 2. Comparison of sublethal effects of neem-based formulations (LC_{50}) on the development stages of *Spodoptera frugiperda*

Formulation (LC_{50})	Larval duration* (days)	Pupal duration* (days)	Pupal weight* (g)	Pupal size* (mm)		Adult emergence* (%)	Adult size* (mm)				Adult longevity* (days)	
							Male		Female			
				Length	Breadth		Length	Breadth	Length	Breadth	Male	Female
Commercial neem formulation (0.15 %)	37.00 ± 1.92^{a}	9.80 ± 0.37^{a}	0.138 ± 0.012^{b}	7.280 ± 0.063^{b}	2.256 ± 0.081^{b}	46.0 (42.67)^{b}	8.804 ± 0.484^{c2}	3.956 ± 0.157^{c2}	8.426 ± 0.085^{b1}	3.782 ± 0.269^{b1}	2.40 ± 0.50^{b}	3.60 ± 0.48^{b}
Neem oil	30.80 ± 2.63^{b}	8.80 ± 0.58^{b}	0.165 ± 0.017^{a}	7.478 ± 0.287^{ab}	2.278 ± 0.076^{b}	54.0 (47.28)^{a}	9.006 ± 0.192^{b2}	4.106 ± 0.228^{b2}	8.655 ± 0.066^{ab1}	3.924 ± 0.196^{ab1}	2.80 ± 0.58^{b}	4.20 ± 0.48^{b}
NSKE 5 %	30.00 ± 3.56^{b}	7.80 ± 0.52^{c}	0.169 ± 0.013^{a}	7.693 ± 0.200^{a}	2.372 ± 0.053^{a}	58.0 (49.64)^{a}	9.297 ± 0.177^{a2}	4.444 ± 0.161^{a2}	8.829 ± 0.412^{a1}	4.064 ± 0.077^{a1}	3.40 ± 0.24^{a}	4.60 ± 0.60^{a}
Untreated (0.00)	22.40 ± 0.81^{b}	6.60 ± 0.60^{b}	0.204 ± 0.013^{a}	8.467 ± 0.049^{a}	3.909 ± 1.261^{a}	92.00 (75.22)^{a}	14.081 ± 0.346^{a1}	6.127 ± 0.183^{a1}	13.598 ± 0.240^{a1}	5.996 ± 0.222^{a2}	8.60 ± 0.24^{a}	9.00 ± 0.31^{a}

*Mean ± SE, based on five replications each comprising 10 larvae. The figures in parentheses are arc sine transformations. Values with different alphabetic superscripts are significantly different (p<0.05) with respect to different columns. Values with different letters are significantly different (p<0.05) with respect to different rows.

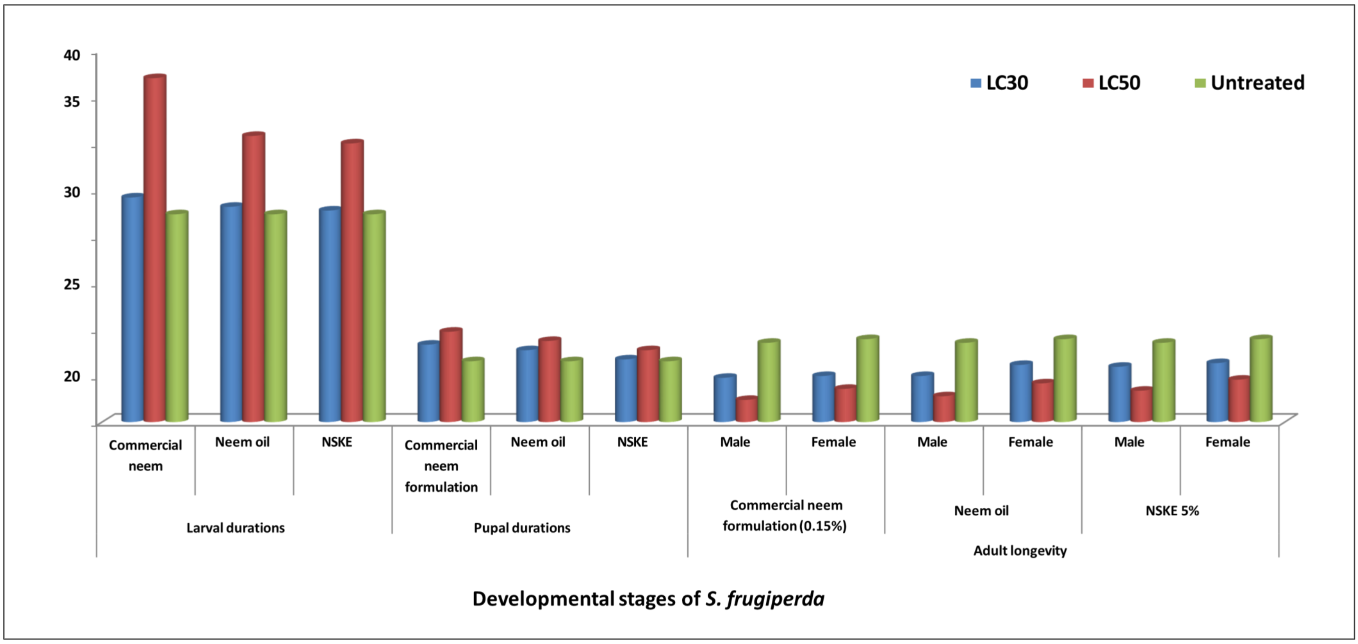

Fig. 1. Sub-lethal influence of neem-based formulations on larval duration, pupal duration and adult longevity of *S. frugiperda*.

neem formulation (8.40 and 9.80 days) than for the neem oil (7.80 and 8.80 days) and NSKE (6.80 and 7.80) formulations.

The pupal weight of *S. frugiperda* significantly differed between the untreated lot (0.204 g) and the lots treated with the LC_{30} and LC_{50}(Fig. 2). Treatment with the commercial neem formulation at sublethal concentrations (LC_{30} and LC_{50}) was more effective, as it led to a decrease in pupa weight (0.142 g and 0.138 g) compared with neem oil (0.179 g and 0.165 g) and NSKE (0.183 g and 0.169 g) (Fig. 3 & 4). In the overall comparison of all treatments, the pupal weight was greatest in the control group, followed by those in the LC_{30} and LC_{50}groups (0.15 %). Further, a decrease in the pupal length of *S. frugiperda* was reported for all the treatments at different concentrations (LC_{30} and LC_{50}) compared to the control. The commercial neem formulation was highly effective, as it decreased pupal length (7.960 and 7.280 mm) more than neem oil (8.001 and 7.478 mm) and NSKE (8.174 and 7.693 mm), which were significantly different from those of the untreated lot (8.467 mm) (Fig. 5). Similarly, a decrease in the breadth of *S. frugiperda* was also reported in all treatments (LC_{30} and LC_{50}), but the decrease was greatest in the commercial neem formulation (2.309 and 2.256 mm) compared to that in the neem oil (2.773 and 2.278 mm) and NSKE (2.796 and 2.373 mm) compared to that in the control lot (3.909 mm).

The adult emergence rate of *S. frugiperda* was significantly lower in the LC_{30} and LC_{50}treatment groups than in the untreated group (92.0 %) (Fig. 6). In the overall comparison of all treatments, adult emergence was lowest in the commercial neem formulation (62.0 and 46.0 %), followed by the neem oil (70.0 and 54.0 %) and NSKE (72.0 and 58.0 %). Adult size, i.e., male and female length and width, of *S. frugiperda* was greater in the untreated lot (14.081 and 6.127 mm) than in the lot treated with sublethal concentrations (LC_{30} and LC_{50}) of the commercial neem formulation, neem oil and NSKE. In the overall comparison of all treatments, adult emergence was lowest in the commercial neem formulation group, with a maximum male adult length of 11.804 mm and a maximum width of 4.336 mm for the commercial neem formulation group (Fig. 7). Similarly, the mean adult male length and width were 12.023 mm and 4.870 mm, respectively, for the commercial neem formulation at the LC_{30}. The adult longevity of males and females was greater in the untreated lot than in the treated lot for all formulations (LC_{30} and LC_{50}). The mean male adult longevity was 8.60 days and the female adult longevity was 9.0 days in an untreated lot, which was significantly different from the sublethal concentrations of the commercial neem formulation, neem oil and NSKE. In the overall

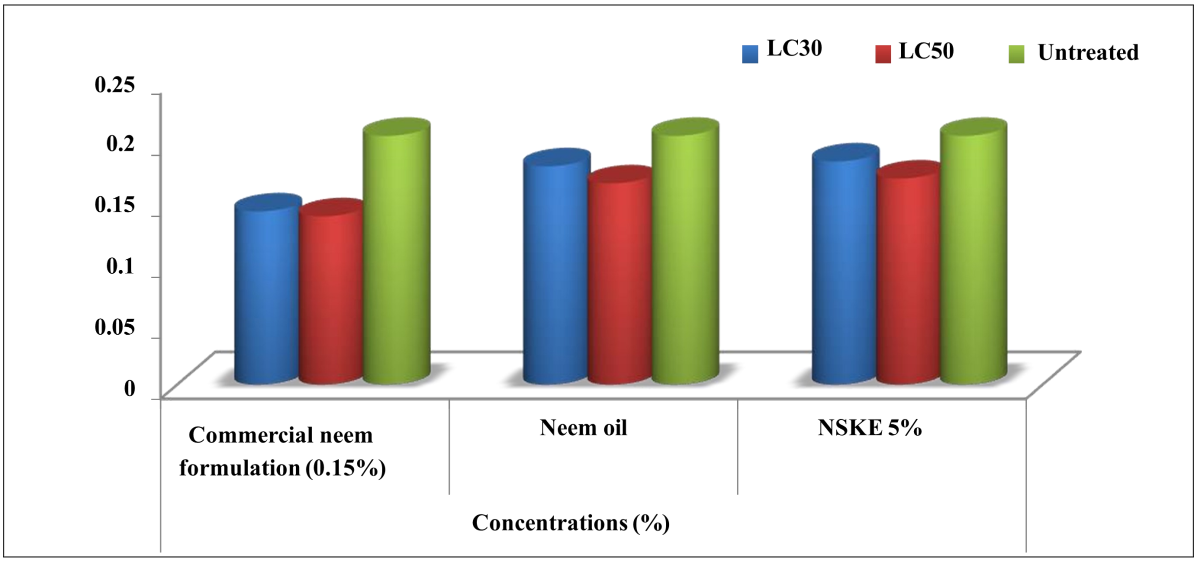

Fig. 2. Sub-lethal influence of neem-based formulations on pupal weight (mm) of *S. frugiperda.*

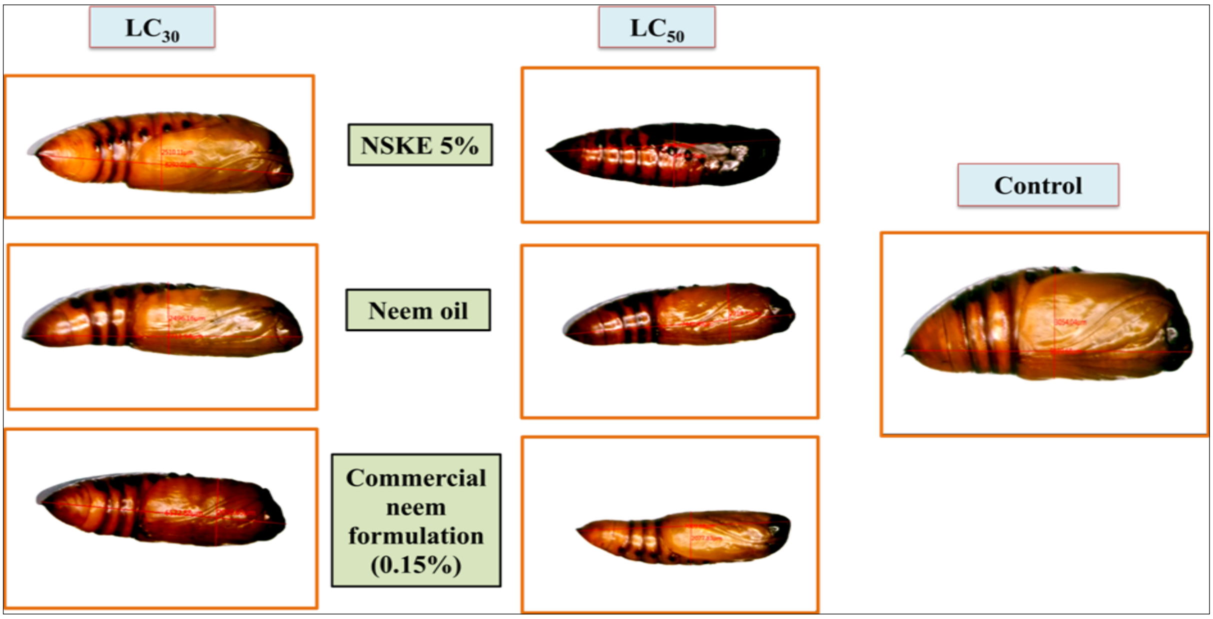

Fig. 3. Morphometry of *S. frugiperda* pupae under stereozoom microscope.

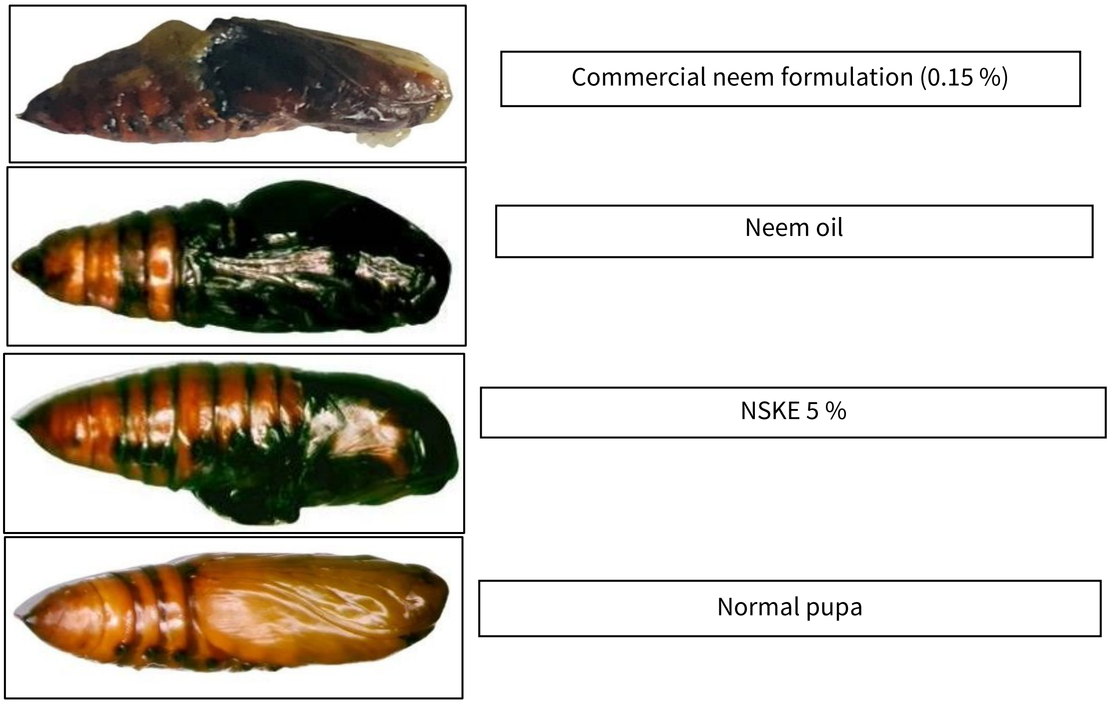

Fig. 4. Sub-lethal influences of different neem-based formulations on pupae of *S. frugiperda*.

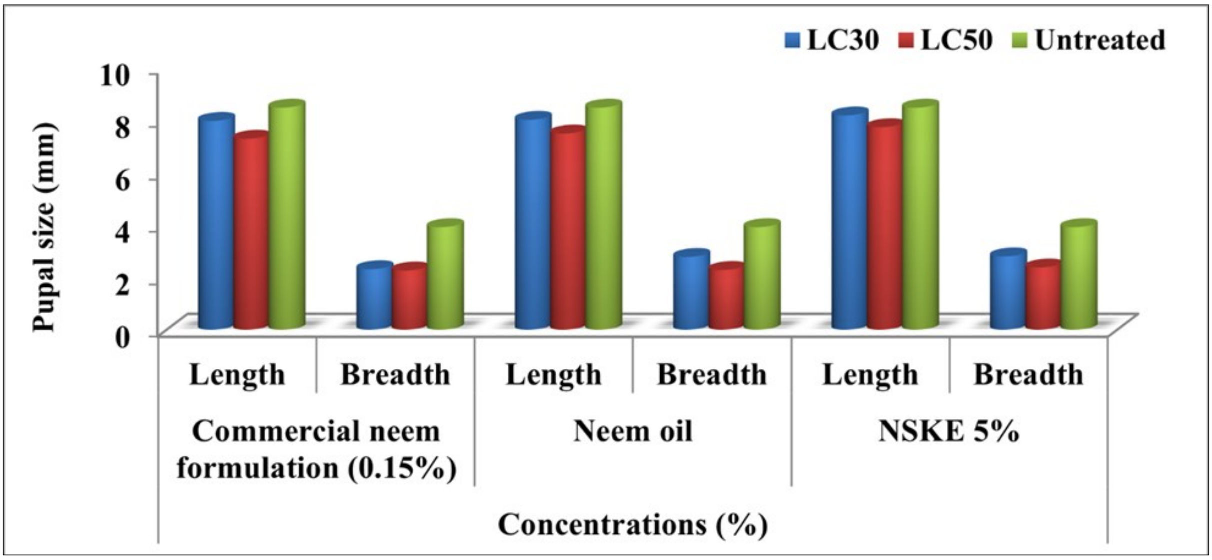

Fig. 5. Sub-lethal influence of neem-based formulations on pupal size of *S. frugiperda*.

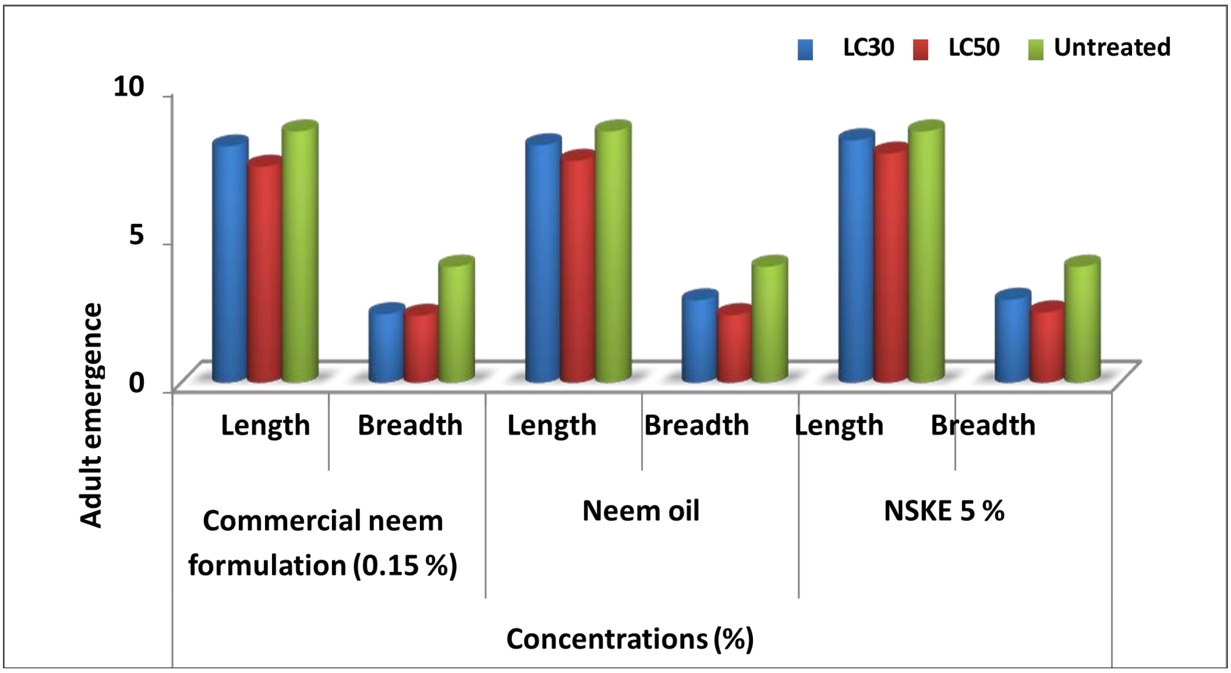

Fig. 6. Sub-lethal influence of neem-based formulations on the adult emergence of *S. frugiperda*.

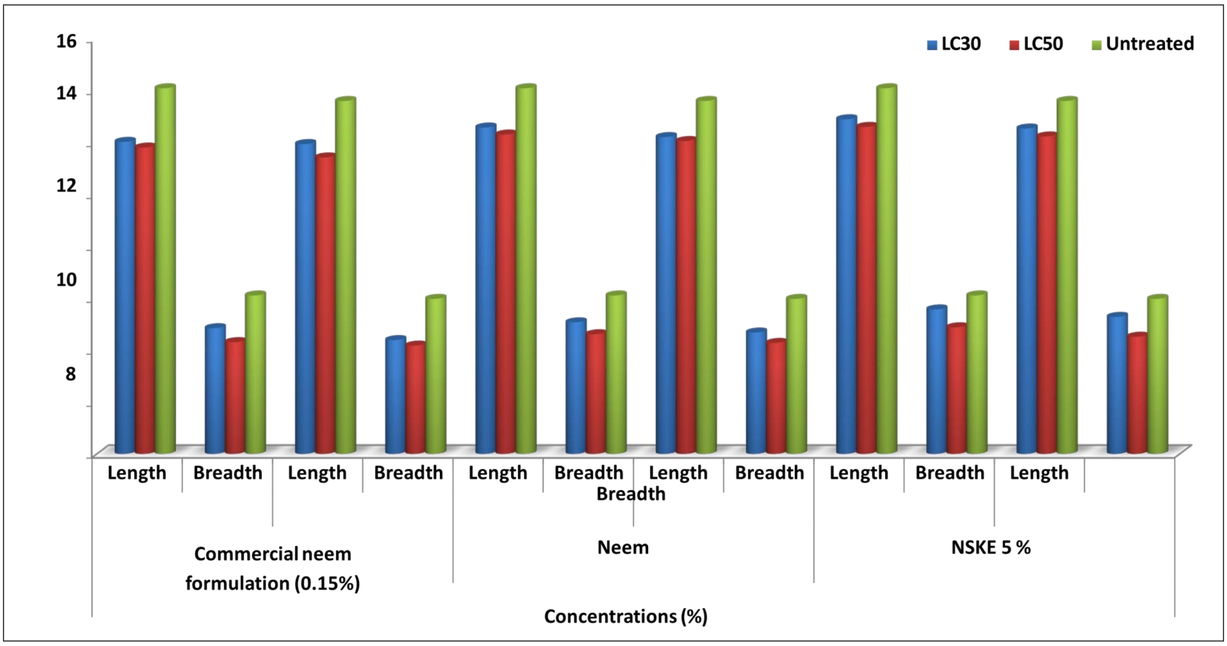

Fig. 7. Sub-lethal influence of neem-based formulations on adult size of the *S. frugiperda*.

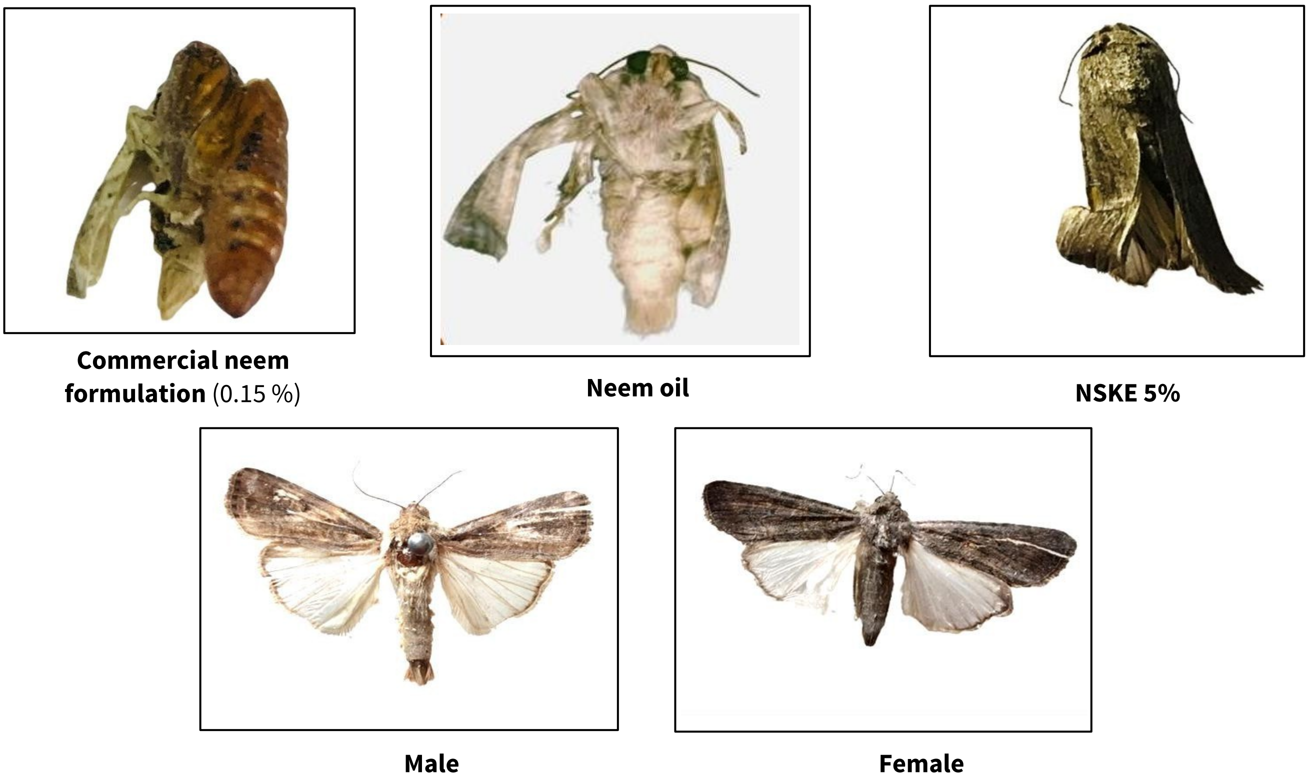

Fig. 8. Sub-lethal influences of different neem-based formulations on adults of *S. frugiperda*.

comparison of all treatments, the maximum decline in adult longevity of males and females was recorded for the commercial neem formulation (Fig. 8). The mean male adult longevity was 2.40 days and the mean female longevity was 3.60 days for the commercial neem formulation with the LC_{50}(Table 2). Similarly, the mean adult longevity of males and females was recorded at 4.80 days and 5.0 days, respectively, for the LC_{30} concentration of the commercial neem formulation (Table 1).

The developmental events of larvae, pupae and adults that were found to be malformed after second instar larvae were treated with *A. indica* (LC_{50}) could be grouped into three major categories, i.e., malformed larvae, malformed pupae and malformed adults. Certain malformations were also observed in the pupal stage, i.e., deformed pupae darkened, reduced in size and died before adult emergence. Larval mortality usually occurs within the first few days of exposure. The few larvae that survived the treatment further developed into larval pupal intermediates, which could be the result of incomplete moulting.

Discussion

Research has reported that prolongation of the larval instars of *Spodoptera littoralis* when fed an artificial diet supplemented with azadirachtin (from 0.01 ppm to 1 ppm wt/v) for two days (29). Research indicates that a significant increase in larval duration, i.e., 21.3 days, in *Helicoverpa armigera* after exposure to azadirachtin via an artificial diet (30). Research has evaluated the effect of different commercial neem formulations on the duration of *H. armigera* larval growth and reported an increase (18.69 days) in these parameters due to these formulations (31). Research has also reported a similar increase in the larval period of *H. armigera* after exposure to neemazal (15 and 20 mg/L) (32). Research has reported significant prolongation (18.5 days) in larval duration in comparison to 14.8 days in the control group in the case of the cabbage moth *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae) after treatment with Neem EC (1 % azadirachtin) (33). Research reported that this prolongation in the larval period might be due to the effect of neem formulations on larval feeding, resulting in a reduction in the rate of nutrient accumulation for ecdysis (34).

Research has demonstrated that 0.006 % and 0.05 % concentrations of neem oil prolonged the larval duration of *S. frugiperda* to 21.89 days and 23.47 days, respectively (16). Likewise, Research also noted an increase in the duration of larval growth at the highest concentrations (10 mL) of neem oil (35). Research recorded the sublethal influences on the larval duration of tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), when fed on neem oil-treated cauliflower leaves and observed that the larval period, i.e., 18.13, 17.23, 16.40 and 15.47 days, was prolonged due to neem oil treatment in comparison to 14.23 days in the control (36).

An increase in the larval duration of the diamondback moth *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) when treated with the LC_{50} (1.79 %) formulation of *Melia azedarach* extract, i.e., 7.0 -7.8 days, in comparison to the control (6.3-6.5) days (37). Research also reported an increase in larval duration, i.e., 23.8 and 60.3 days of *S. littoralis* and *A. ipsilon*, respectively, at higher concentrations, i.e., 50 ppm *M. azedarach* extract (38). Prolonged larval durations, i.e., 14.02 and 11.30 days, of the spotted bollworm *Earias vittella* (Fabricius) (Lepidoptera: Noctuidae) were recorded after treatment with lethal and sublethal concentrations, i.e., 5.50 and 1.59 %, respectively, of methanol-based extracts of *A. indica* (39). An increase in larval development time may be due to a delay in metamorphosis (40). Water extracts from different parts of neem delayed the larval development of *H. armigera* larvae (34).

The prolongation of pupal duration in *H. armigera* was recorded upon treatment with azadirachtin (8.8 µg a.i./mL) (30). Research has reported a non-significant increase in pupal duration in the case of *S. littoralis* when it was exposed to an artificial diet supplemented with azadirachtin (0.05 and 0.1 ppm) (29). However, the observations of the present study are significant. Research reported that, the mean pupal duration of *S. litura* was 6.80 days in the control group, which was significantly greater than that in the 2500 ppm neem oil treatment group (9.23 days) (36). These results are also consistent with the observations of previous studies which reported an increase in the pupal duration of 12.3 days in *S. frugiperda* exposed to 15000 ppm *A. indica* oil (15). Research reported that 0.006 and 0.05 % concentrations of neem oil significantly prolonged the pupal duration, i.e., 11.08 days and 11.67 days, respectively, in the case of *S. frugiperda* (16). The longevity of the pupal stage of *S. littoralis* increased significantly from 10.3 days in the control to 19.0 days when food was treated with 100 ppm neem oil (41).

Similarly, research also reported similar results to those of the present study, i.e., increases in the duration of pupae, 10.0 and 19.0 days, of *S. littoralis* and *A. ipsilon*, respectively, at higher concentrations (25 ppm) of *M. azedarach* extract (38). Prolonged pupal durations, (15.61 and 12.51 days) were recorded for the spotted bollworm *E. vittella* when it was treated with lethal and sublethal concentrations, 5.50 and 1.59 % methanol-based extracts, respectively, of *A. indica* in comparison to the control (9.42 days) (39). A non-significant increase in the pupal duration (3.4–3.6 days) of *P. xylostella* when treated with the LC_{50} (1.79 %) formulation of *M. azedarach* extract in comparison to the control (37). Similarly, research observed a significant increase in the duration of *H. armigera* pupae when larvae were treated with 2.5, 5.0 and 10 % concentrations of neem seed extract, neem leaf extract and Nimbecidine, respectively, in comparison to the control.

Research has demonstrated that azadirachtin (8.8 µg a.i./mL) exposure led to a significant decrease in pupal weight (205.1 mg) of *H. armigera* in comparison to 298.3 mg in the control (42). Research also noticed a reduction in pupal weight before exposure to azadirachtin (33, 66 and 132 ppm) in first- and second-instar larvae of *H. armigera* (42). Similarly, research has also found a decrease in pupal weight of 191.6 mg in *H. armigera* at the highest concentration (200 ppm) of azadirachtin (43). A significant decrease in the pupal weight (0.28 g) of *H. armigera* was recorded, following feeding on an artificial diet supplemented with a commercial neem-based formulation (0.16 mL) for 72 hr (31). Research observed a significant decrease in pupal weight, i.e., 260.6, 266.5 and 257.3 mg, in *H. armigera* before treatment with 15, 100 and 25 mg/L commercial neem-based formulations, respectively, compared to the control (278.6 mg) (44). In the overall comparison of all treatments, the pupal weight was greater in the control group than in the groups treated with neem oil at the LC_{30} and LC_{50} concentrations. Research has indicated that a decrease in pupal weight (0.191 and 0.156 g) occurred when the third-instar larval stage of *S. frugiperda* was treated with neem oil (0.006 and 0.05 %, respectively) (16). Similarly, research has also reported significant decreases in pupal weight, (182.7, 178.6 and 151.4 mg) of *S. frugiperda* when they were exposed to neem oil (5000, 10000 and 15000 ppm) via an artificial diet (15). Research has reported the effect of neem oil on *S. litura*. Compared with those in the control group, the weights of the pupae in the 30.43, 28.93, 27.33, 25.97 and 24.80 mg neem oil diet groups were 2500, 2000, 1500, 1000 and 500 ppm, respectively. The different concentrations of *M. azedarach* extract led to a decrease in the mean weight of pupae in the case of *S. littoralis* and *A. ipsilon* (38). Similarly, research has also reported a significant decrease in the pupal weight of *E. vittella* after treatment with lethal or sublethal concentrations of a methanolic extract of neem seeds.

A decrease in the adult emergence rate from young pupae (28.3 and 17.0 %) and old pupae (72.7 and 55.7 %) of the sugarcane borer *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) was found when treated with commercial neem formulations (1.0 and 2.0 %, respectively) (39). The adult emergence rates, i.e., 53.7, 56.6 and 59.6 % from the pupal stage, significantly decreased when sixth instar *H. armigera* larvae were fed commercial neem-based formulations–15, 25 and 100 mg/L treated food, respectively (44). Research has demonstrated similar findings in which a significant decrease in the adult emergence rate (13 and 14 adults) of *S. litura* in response to high doses of neem oil (2000 and 2500 ppm) was recorded in comparison to that in the control (30 adults) (39). Research has recorded that the adult emergence percentage of *S. littoralis* was reduced to 12.29 % at the fourth-instar larval stage when it was pretreated with neem oil in comparison to that of the control (84 %) (45, 46). A decrease in adult emergence (8–23 %) of *P. xylostella* before exposure to the LC_{50} formulation of *M. azedarach* compared to the control (91–95.5 %) (37). The topical treatment of *Rhynchophorus ferrugineus* with neem extract in the prepupal stage led to a reduction in the adult emergence rate (47). A significant reduction in *H. armigera* adult emergence after treatment with 2.5, 2.5 and 10 % concentrations of neem seed extract and neem leaf extract in comparison to the control (34).

A significant decline in adult emergence was found when *H. armigera* larvae of this pest were exposed to azadirachtin (8.8 µg a.i./mL). The exposure of *S. frugiperda* to neem oil could lead to a significant decrease in the wing length of adults, resulting in a smaller adult size than that of the control (15, 30). A longer pupal period and a reduction in pupal weight are associated with shorter wing length. Prepupal *R. ferrugineus* was treated with different doses of neem extract (50, 100 or 500 ppm) via topical treatment. This resulted in a reduction in adult size, which coincides with the observations of the present study (47).

Research reported a decrease in the longevity of *S. littoralis* adults, i.e., 11.6, 10.0 and 10.0 days, when exposed orally to 1, 10 and 100 ppm concentrations of azadirachtin, respectively, compared to the control (12.3 days) (48). Similar results were also reported previously for *H. armigera* adults upon treatment with azadirachtin (8.8 µg a.i./mL) (30). Research reported a significant decrease in the longevity of male (8.2 days) and female (9.1 days). *H. armigera* adults fed artificial diets supplemented with different commercial neem formulations (2.0 and 0.16 mL, respectively) compared with that of control *H. armigera* adults (10.2 and 11.2 days, respectively) (31). Research has demonstrated the effects of neem oil on adult longevity in an African pink stem borer, *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) male adult (6.8 days) and female adult (6.9 days) and found that adult longevity decreased upon treatment with 0.15 mL of neem oil (49). Similarly, a decrease in the longevity of adult *S. frugiperda* was recorded when they were fed a diet supplemented with different concentrations of neem oil. The longevity of adult *S. frugiperda* could decrease if the larvae were fed corn leaves treated with 10 mL of neem oil (35). A shorter longevity can have an impact in the field because it reduces the time needed to find a partner. Research reported a decrease in adult longevity (5.55–7.80 days) in *P. xylostella* in response to the LC_{50} formulation of *M. azedarach* compared to the control (8.90–9.50 days) (37). Similarly research has also reported a decrease in adult longevity of 12.0, 7.2 and 3.6 days in males and 11.6, 10.0 and 6.6 days in females of *S. littoralis* and 9.2, 6.4 and 5.2 days in males and 14.0, 6.2 and 7.0 days in females of *A. ipsilon* at relatively high concentrations (10, 15 and 25 ppm) of *M. azedarach* extract, respectively. Effect of sublethal concentrations, i.e., 1.4, 3.28 and 25.3 mL of neem, led to a significant decrease in the adult longevity of the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) (50). A significantly shorter mean adult longevity of *H. armigera* (1.83, 1.97, 3.07 and 3.42 days) was recorded in the different neem extract treatments than in the control (7.98 days) (34).

Moreover, malformations such as reduced body size, twisted antennae and wings that prevent normal flight and mating were also observed in *S. frugiperda* adults when larvae were pretreated with different neem-based formulations. Similar observations were also found in *S. littoralis* (51). Morphological alterations were found in prepupae and adults of *D. saccharalis* after treatment with neem extract (45). They also observed pupa-adult intermediate growth in the presence of relatively high concentrations of neem extract, which is consistent with the results of the present study. Intermediate larval pupae were observed before treatment with azadirachtin to the last instar of the larvae of *S. litura*, the lawn armywork moth (*Spodoptera Mauritia*) (Boisduval) (Lepidoptera: Noctuidae), the Mediterranean flour moth, (*Ephestia kuehniella*) (Zeller) (Lepidoptera: Pyralidae), the tobacco hornworm (*Manduca sexta*) (Linnaeus) (Lepidoptera: Sphingidae) and *S. littoralis* (52, 53). In *H. armigera* larvae, the treatment of successive stages of development and hence ultimately led to *H. armigera* death. Larval-pupal intermediate formation, i.e., 27.12 and 2.38 % for second and fourth instar larvae of *S. littoralis*, respectively, when pretreated with neem in comparison to the control, was also noticed, which did not show any deformations in the larvae (46). Azadirachtin (200 and 500 ng insect^{-1}) treatment could lead to abnormalities in moults, the formation of pupa-adult intermediates and the inhibition of growth with increasing doses of azadirachtin in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (54). Disruptions in the metamorphosis of *S. frugiperda* larvae treated with three neem-based formulations ranging from 0.34–1.10 % were recorded (55). The larvae failed to pupate and died due to incomplete moulting.

Conclusion

Neem-based formulations showed pronounced sublethal effects on *S. frugiperda*, significantly prolonging larval and pupal durations while reducing pupal weight, pupal size, adult emergence, adult size and adult longevity. These formulations also disrupted normal morphogenesis in larvae, pupae and adults, leading to impaired metamorphosis. Affected pupae exhibited darkening, reduced size and mortality before emergence, whereas adults displayed smaller body size and deformities such as twisted antennae and wings, hindering flight and mating. Among the tested formulations, the commercial neem preparation containing 0.15 % active ingredient demonstrated the highest efficacy in inducing these sublethal and morphological abnormalities. The combined safety of neem-based formulations for nontarget organisms and mammals, along with their physiological effects on *S. frugiperda*, underscores their potential as promising eco-friendly tools for inclusion in integrated pest management programs and for conducting field-scale evaluations aimed at sustainable control of this invasive pest.

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Authors' contributions

GS carried out all the experiments in the study and drafted the original manuscript. AKC conceptualised the research area. AK performed the analysis. JJ formally analysed and reviewed the manuscript. All authors read and approved the final manuscript for publication.

Compliance with ethical standards

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