Sublethal effects of botanicals on the growth and development of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

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The present study aimed to ascertain the sublethal effects of azadirachtin 1 EC, anosom 1 EC, derisom 2 EC and NSKE 5% on biological parameters and nutritional indices of *Spodoptera frugiperda*. The longest larval and pupal lengths were observed in all the treatments involving botanicals, which also significantly decreased adult longevity, fecundity and egg hatchability. Regarding nutritional indices, larvae treated with anosom recorded the least approximate digestibility index, efficiency of conversion of digested food and efficiency of conversion of ingested food of 64.7%, 13.2% and 8.4% respectively. Additionally, botanicals increased the percentage of defective and malformed adults. Thus, our findings suggest that these botanicals restrain the pests from causing damage and impede their further generations.

Keywords: Biological parameters, botanicals, nutritional indices, *Spodoptera frugiperda*, sublethal effects.

MAIZE is one of the most versatile emerging crops having wider adaptability. It is known as the 'queen of cereals' because of its highest genetic yield potential. Though several factors affect its higher yield, fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) dominates all others by threatening food security through its ability of destruction. This polyphagous devastating pest has been reported in over 353 plant host species causing 70% yield loss in the overall economy[1]. FAW is a ravenous lepidopteran pest native to tropical and subtropical regions of America[2,3]. Its invasion to the Indian subcontinent was first reported in Karnataka, feeding on maize leaf[4]. Since its invasion in India, farmers are majorly relying on chemical insecticides, and nearly 2.1 rounds of chemical insecticides are used for its management[5]. Increased pesticide applications could hasten the emergence of resistance, as has already occurred in many places[6]. Approximately 98% of pesticide compounds sprayed do not reach their respective target sites, contaminating the environment, leaving residues in food and posing health risks to the general public as well as harming natural adversaries and pollinators. Natural insecticides (botanicals) are suitable alternatives to plant protection chemicals with minimum negative risks[7,8]. The use of pesticides with a botanical origin goes back at least two millennia in China, Greece, Egypt and India[9]. Food consumption studies have a wide array of applications in areas such as growth, nutrition, behaviour, community structure, host–plant resistance and also to predict crop losses. Estimating nutritional indices using gravimetric methods developed by Waldbauer[10] helps elucidate quantitative changes in consumption and assimilation for several insects on a variety of diet regimes. Nutritional indices give a basis for community energy flow by using consumption and growth rate measures to develop simulation models for determining pest economic injury levels[11]. Botanicals affects larvae by causing growth inhibition and malformation, which can be quantified through indicators like digestibility utilization indices. In this study, we analyse the sublethal effects of botanicals on *S. frugiperda* in order to reveal their negative, non-lethal impact under laboratory conditions.

Materials and methods

An experimental set-up was laid out to study the effect of different botanicals on the growth and development of *S. frugiperda* using a completely randomized design (CRD) consisting of four treatments, each with 25 replications, in the laboratory at the Department of Entomology, College of Agriculture, Shivamogga, Karnataka, India. The experiments were conducted under laboratory conditions at 26° ± 2°C, 75–80% relative humidity (RH) and L16:D08 photoperiod.

FAW culture

Initially, different instars of FAW larvae were collected from the maize fields in and around the College of Agriculture,

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Shivamogga. The collected larvae were reared in a circular insect breeding dish (Himedia, Mumbai, India TCP030-90 × 40 mm diameter) providing maize leaves as food under laboratory conditions at 26° ± 2°C, 75–80% RH and L16:D08 photoperiod until pupation. The pupae were collected at regular intervals. After emergence, the moths were released in a cage (45 × 45 × 60 cm^3), and young 15-day-old maize plants were kept as an oviposition substrate. The males and females were fed on 10% (w/v) honey solution. To prevent desiccation, egg masses laid by females on maize leaves were removed and placed in an insect breeding dish layered with moistened filter paper. The eggs hatched after 2 or 3 days and the emerged larvae were fed with tender maize leaves until they reached the third instar.

Botanicals used for the study

The botanicals used in the study were neem seed kernel extract (NSKE) 5%, margosom 1 EC, anosom 1 EC and derisom 2 EC. Except for NSKE, all the botanicals were procured from Agri Life Pvt Ltd, Hyderabad, Telangana. NSKE was prepared manually in the laboratory by collecting seeds from 20-year-old neem trees.

Preparation of solutions

The preliminary dose-setting experiments were carried out to estimate the main concentration of the bioassay test and later assessed for LC_{30} values. Before selecting the testing concentration, initial screening toxicity was performed with several concentrations. For each bioassay, 5–7 testing concentrations which offered a mortality range of 10–90% were used to prepare the log concentration mortality regression lines. The sublethal doses were chosen based on the dose–mortality probit analysis.

The treatment suspensions were prepared with distilled water, and triton X-100 (0.5 ml/l) was added to facilitate solubilization. The required test compound was taken using a micropipette, and dispensed in 1 litre of water to get the required concentration. Maize leaf bits of approximately 5 × 4 cm were dipped in different treatments and were shade-dried for 10 min. Simultaneously, untreated control was maintained for comparison.

Determination of sub-lethal concentrations

The susceptibility of the third instar larvae to different botanicals analysed by leaf dip bioassay resulted in LC_{10}, LC_{30} and LC_{50} values. The larvae were placed individually in an insect breeding dish – circular (Himedia, TCP030-90 × 40 mm diameter) containing the treated leaf bits. Larvae were pre-starved for 4 h before releasing into the dish. Fresh treated leaves were given after 24 h. At 144 h after treatment, the mortality was assessed, and larvae that did not respond to brush strokes were considered dead. The mortality was corrected using Abbott's formula and the regression equation, sub-lethal concentration (LC_{30}), median lethal concentration (LC_{50}) and 95% confidence interval were calculated. NSKE 5%, margosom, anosom and derisom recorded the LC_{30} values of 4262.40, 2.48, 2.71, 38.34 mg/l respectively, and these values were taken for further studies.

Determination of biological parameters

A total of 25 larvae were used for each botanical, and the leaf discs were changed daily until pupation and observed till adult emergence. Biological parameters like larval period, pupal period, larval and pupal weight, larval survival (%), pupal survival (%), adult longevity (male and female), malformed adults (%), total life cycle, fecundity and egg hatch (%) were recorded. To assess fecundity, the egg mass was placed under a microscope. Scales and hairs from each egg mass were gently removed using a camel hairbrush, and the number of eggs laid per female was counted. The formulas given below were used to calculate several biological parameters.

$$\text{Larval survival (\%)} = \frac{\text{Number of larvae survived}}{\text{Total number of larvae}} \times 100$$

$$\text{Pupal survival (\%)} = \frac{\text{Number of pupae emerged into adults}}{\text{Total number of pupae}} \times 100$$

$$\text{Malformed adults (\%)} = \frac{\text{Number of malformed adults}}{\text{Total number of adults emerged}} \times 100$$

$$\text{Egg hatch (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs laid}} \times 100$$

The fresh weight of leaves was recorded before and after they were given to the larvae at 24 h intervals. Daily observations were recorded on the initial and final weight of the leaves, faecal matter weight, weight of the larvae (instar-wise) and duration of each instar, larval mortality and pupal weight.

Assessment of nutritional indices

Different growth and development indices such as consumption index (CI), relative growth rate (GR), efficiency of conversion of ingested food into body substance (ECI), efficiency of conversion of digested food into body substances (ECD) and approximate digestibility index (ADI) were estimated for larvae in different treatments by following

the method given by Waldbauer[10] using the following formulae:

$$\text{CI} = \frac{\text{Fresh weight of food eaten in grams } (F)}{\text{Duration of feeding in days } (T) \times \text{Mean fresh weight of larvae during feeding period in grams } (A)}$$

$$\text{GR} = \frac{\text{Fresh weight gain of larvae during the feeding period in grams } (G)}{\text{Duration of feeding in days } (T) \times \text{Mean fresh weight of larvae during feeding period in grams } (A)}$$

$$\text{ECI} = \frac{\text{Fresh weight gain of larvae during the feeding period in grams } (G)}{\text{Fresh weight of food eaten in grams } (F)} \times 100$$

$$\text{ECD} = \frac{\text{Fresh weight gain of larvae during the feeding period in grams } (G)}{\text{Fresh weight of food eaten in grams } (F) - \text{Weight of excreta in grams } (E)} \times 100$$

$$\text{ADI} = \frac{\text{Fresh weight of food eaten in grams } (F) - \text{Weight of excreta in grams } (E)}{\text{Fresh weight of food eaten in grams } (F)} \times 100$$

Statistical analysis

Data were statistically analysed using SPSS software v 16.0 (ref. 12). Probit analysis was performed using SPSS, from which slope and lethal concentration were calculated. The parametric data were analysed using one-way ANOVA, and mean separation was estimated by Tukey's test at $P > 0.05$.

Results and discussion

Biological attributes of fall armyworm on different botanicals

LC_{30} was chosen as a low lethal concentration to study sublethal effects because it is the mortality threshold (30%) recommended for the use of pesticides in integrated pest management[13].

Larval exposure to LC_{30} of different botanicals had a detrimental effect on the growth and development o*f S. frugiperda*, causing a significant reduction in larval weight, pupal weight, adult longevity, and prolonged larval and pupal period. The lowest larval (309.0 ± 7.5 mg) and pupal weights (133.2 ± 3.9 mg) were recorded in anosom, while the control larvae recorded 538.4 ± 6.4 and 342.5 ± 10.4 mg respectively (Table 1). NSKE 5%, anosom and derisom registered the lower larval survival of 72%, but azadirachtin recorded 76% (Table 2). Pupal survival was lower in anosom (66.6%), followed by derisom (72%), NSKE 5% (77.7%) and azadirachtin (78.9%) (Table 2). However, untreated larvae did not exhibit any mortality. Furthermore, anosom prolonged the larval and pupal periods up to 16.4 ± 0.1 and 9.8 ± 0.2 days respectively, while azadirachtin, NSKE and derisom were also on par with anosom (Table 1).

Botanicals also had a negative impact on adult longevity. The lowest female and male longevity periods of 6.0 ± 0.5 and 5.5 ± 0.2 days respectively, were recorded in larvae treated with anosom and were not significantly different from derisom, NSKE and azadirachtin. However, females and males in control had a longer longevity period of 10.4 ± 0.2 and 8.4 ± 0.1 days (Table 1). Larvae treated with anosom recorded the highest malformation in adults (75%), followed by NSKE 5% (53.3%), azadirachtin (57.1%) and derisom (61.5%), while the control larvae did not manifest any malformation (Table 2). The botanicals also had an extended effect on fecundity and egg hatchability percentage of 528.25 ± 14.37 eggs/female and 61.35 respectively, being lowest in the larvae treated with anosom, while other botanicals were on par with each other and control larvae recorded values of 990 ± 90.68 eggs/female and 93.40% respectively (Table 1).

Food consumption and utilization indices

The lowest CI was recorded in the NSKE 5% treatment (1.71 ± 0.06 g/g/day). However, the anosom-treated larvae registered a significantly higher index of (2.11 ± 0.09 g/g/day), which was on par with the control larvae (2.02 ± 0.05 g/g/day) (Table 3). GR was lowest in the NSKE 5% treatment (0.16 ± 0.00 g/g/day), which was similar to azadirachtin 1 EC (0.17 ± 0.01 g/g/day). Anosom and derisom treatments registered similar values (0.18 ± 0.01 g/g/day). ADI was high in all the treatments and was on par with the control. The ADI values recorded for anosom, NSKE, azadirachtin and derisom were 64.7 ± 1.5%, 67.7 ± 1.4%, 69.85 ± 0.96% and 71.2 ± 1.3% respectively. ECD was least in the larvae treated with anosom (13.2 ± 0.8%), which was followed by NSKE, azadirachtin and derisom with values of 14.11 ± 0.44%, 14.41 ± 0.69% and 14.5 ± 0.7% respectively. However, the control larvae recorded the highest ECD value of 16.9 ± 0.1% (Table 3). ECI was found to be minimum in larvae treated with anosom with the value of 8.4 ± 0.4%, which was on par with NSKE 5% (9.5 ± 0.2%), followed by azadirachtin and derisom (9.9 ± 0.4% and 10.2 ± 0.3% respectively). However, control larvae recorded higher values for all the nutritional indices.

Lower CI in the NSKE and azadirachtin treatments is due to azadirachtin. Azadirachtin is the main active ingredient, that acts on feeding and metabolism, resulting in reduced food consumption and growth, as it induces secondary

Table 1. Effect of different botanicals on the life cycle of *Spodoptera frugiperda*

Treatment	Dose (LC_{30}; mg/l)	Larval period (days)	Larval weight (mg)	Pupal period (days)	Pupal weight (mg)	Adult longevity (days)		Fecundity (number of eggs/female)	Egg hatchability (%)
						Female	Male		
		Mean ± SEm	Mean ± SEm	Mean ± SEm	Mean ± SEm	Mean ± SEm	Mean ± SEm	Mean ± SEm	
Azadirachtin	2.48	15.79 ± 0.16[b]	336.27 ± 9.05[a]	9.47 ± 0.22[a]	138.46 ± 4.18[a]	7.20 ± 0.20[a]	6.10 ± 0.27[a]	562.33 ± 11.55[a]	70.11
NSKE	4262.40	15.80 ± 0.16[b]	322.89 ± 9.17[a]	9.64 ± 0.23[a]	134.50 ± 2.57[a]	6.75 ± 0.48[a]	5.80 ± 0.25[a]	534.8 ± 15.58[a]	67.20
Anosom	2.71	16.44 ± 0.14[b]	309.05 ± 7.57[a]	9.83 ± 0.27[a]	133.25 ± 3.96[a]	6.00 ± 0.57[a]	5.56 ± 0.24[b]	528.25 ± 14.37[a]	61.35
Derisom	38.34	16.11 ± 0.11[b]	342.55 ± 10.49[a]	9.50 ± 0.23[a]	142.61 ± 3.84[a]	6.25 ± 0.25[a]	5.59 ± 0.20[ab]	622.75 ± 22.67[a]	74.66
Control	0	14.56 ± 0.18[a]	538.45 ± 10.46[b]	8.90 ± 0.12[b]	178.65 ± 3.28[b]	10.44 ± 0.21[b]	8.42 ± 0.19[c]	990 ± 90.68[b]	93.40

Values represent mean ± SEm of 25 replications.
Means (± SEm) with similar alphabets in the column are statistically not significant (Tukey's test: $P = 0.05$).

Table 2. Percentage of larval and pupal survivability, and malformed adults of *S. frugiperda* treated with botanicals

Treatment	Dose (LC_{30}; mg/l)	Larval survival (%)	Pupal survival (%)	Malformed adults (%)
Azadirachtin 1 EC	2.48	76.00	78.94	57.14
NSKE 5%	4262.40	72.00	77.77	53.33
Anosom 1 EC	2.71	72.00	66.66	75.00
Derisom 2 EC	38.34	72.00	72.00	61.53
Control	0.00	100.00	100.00	00.00

Table 3. Effect of different botanicals on consumption, digestion and growth of *S. frugiperda* larvae

Treatment	Dose (LC_{30}; mg/l)	CI (g/g/day)	GR (g/g/day)	ADI (%)	ECD (%)	ECI (%)
Azadirachtin	2.48	1.75 ± 0.08[a]	0.17 ± 0.01[a]	69.85 ± 0.96[ab]	14.41 ± 0.69[a]	9.97 ± 0.40[b]
NSKE	4262.40	1.71 ± 0.06[a]	0.16 ± 0.00[a]	67.76 ± 1.46[ab]	14.11 ± 0.44[a]	9.50 ± 0.23[ab]
Anosom	2.71	2.11 ± 0.09[b]	0.18 ± 0.01[a]	64.78 ± 1.52[ab]	13.28 ± 0.89[a]	8.43 ± 0.43[a]
Derisom	38.34	1.76 ± 0.09[a]	0.18 ± 0.01[a]	71.28 ± 1.39[b]	14.53 ± 0.74[a]	10.21 ± 0.37[b]
Control	0.00	2.02 ± 0.05[b]	0.24 ± 0.01[b]	71.06 ± 0.33[b]	16.96 ± 0.14[b]	12.05 ± 0.08[c]

CI, Consumption index (grams of food consumed per gram of body weight per day); GR, Growth rate (grams of biomass acquired per gram of body weight per day); ADI, Approximate digestibility index; ECD, Efficiency of conversion of digested food; ECI, Efficiency of conversion of ingested food. Values represent mean ± SEm of 25 replications.
Means (± SEm) with similar alphabets in the column are statistically not significant (Tukey's test: $P = 0.05$).

antifeedant effect[14]. In other lepidopteran species, the lowest CI of 0.43 was recorded in the third instar larvae of *Spodoptera litura* using NSKE 5% (ref. 15). In addition, reduced food intake and consumption rate were reported in *Spodoptera littoralis* when the larvae were treated at different concentrations of neemazal against second, third and fourth instars[16].

The larvae treated with anosom registered a significantly higher CI because acetogenin does not act as an antifeedant agent at sublethal concentrations[17]. Hence CI was higher, and there was no significant difference between control and anosom-treated larvae. A higher CI of 3.95 ± 2.23 g/g/day was reported in the second instar larvae of *S. frugiperda* when treated with squamocin, obtained from seeds extracts of *Annona cherimola*[18].

The lower growth rate is due to the principal compounds present in the botanicals, which interfere with the growth by disrupting the normal functions of the endocrine system and reducing the efficiency of converting food into biomass, in turn reducing the growth rate. NSKE 5% treatment recorded the least value of 0.16 ± 0.00 g/g/day. A similar study also recorded the lowest value of 0.13 in NSKE 5% than other neem products treated against third-instar larvae of *S. litura*[15]. Treatments anosom and derisom also recorded a closer value of 0.18 ± 0.01 g/g/day. A similar growth rate of 0.16 ± 0.05 g/g/day was recorded in second instar larvae of *S. frugiperda* treated against squamocin extracted from the seeds of *A. cherimola*[18].

ADI value increases as larvae experiences higher stress, stimulating them to eat relatively more and excrete more to compensate for reduced consumption and utilization of food to maintain growth rate[19]. Hence, larvae continued to feed on the corn leaves despite being treated with extracts, negatively affecting their development. The results were in accordance with a similar study which reported an ADI value of 68.94 when seed extracts of *Annona squamosa* (25%) were treated against third-instar larvae of *S. litura*[20].

ECD and ECI values did not differ significantly between treatments. ECD is the percentage of digested food that contributes to the weight gain of the insect. ECI is the

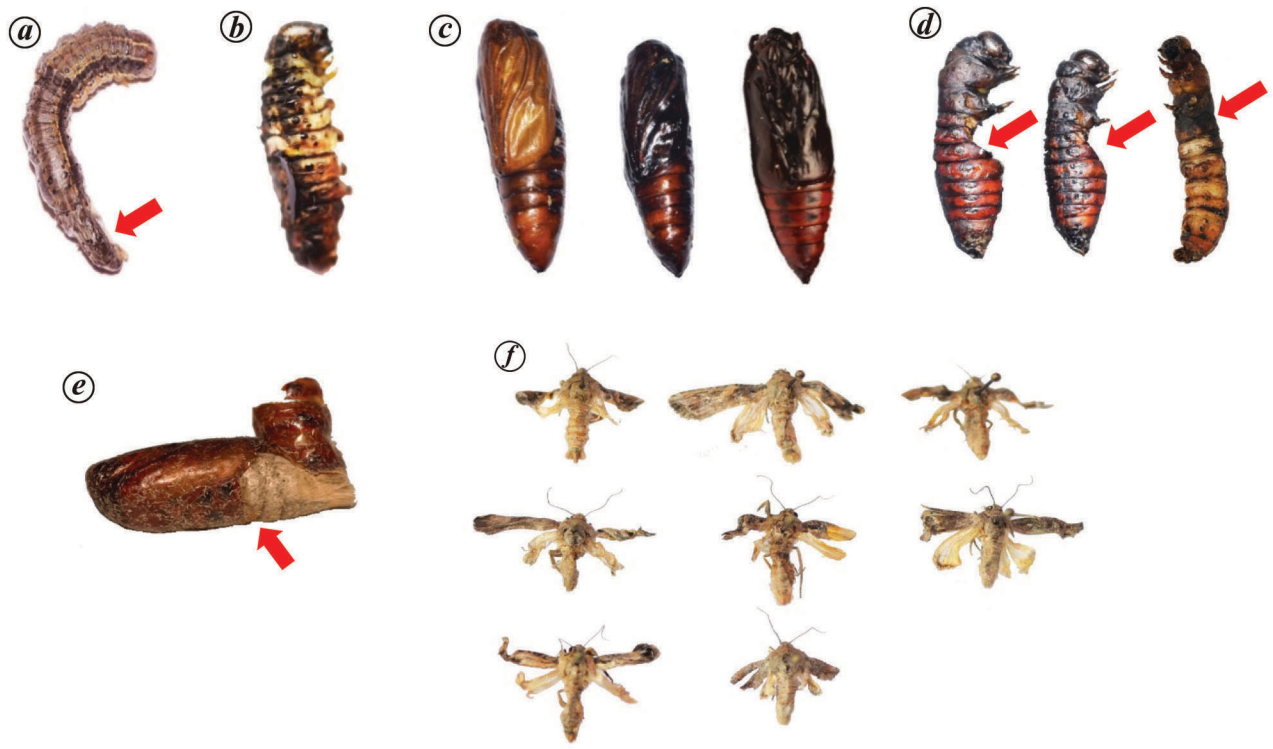

Figure 1. Sublethal effects of botanicals on fall armyworm, *Spodoptera frugiperda*: ***a***, Larvae unable to detach exuviae during the moulting process. ***b***, Death at pre-pupal stage. ***c***, Malformed and unemerged pupae. ***d***, Larval–pupal intermediates. ***e***, Pupal–adult intermediate. ***f***, Deformed wings in adults.

ability of the insect to utilize ingested food for its growth. When treated with plant extracts, larvae initially tend to ingest more food without showing any aversion to overcome the stress posed by active compounds from the plant extracts but failed to utilize the digested food for growth and development[20]. NSKE and azadirachtin treatments registered lower values because of the effect of azadirachtin on the normal gut function, which results in a flaccid semi-full gut, reducing in the efficiency of protein digestion.

Similar adverse effects were recorded on various biological parameters and consumption utilization indices treated with methanolic extract of mature and immature karanj seeds and its fractions on third instar larvae of *Plutella xylostella*[21]. These adverse effects are due to the principal compound karanjin, which acts as an antifeedant and interferes with the normal growth and development of the insects.

Sublethal effects

In all the treatments, larvae could not moult and remained in the same instars. They remained in the intermediate stage (larval–pupal) and failed to pupate or die during the pre-pupal stage (Figure 1 *b* and *d*). Some larvae which underwent moulting were incapable of detaching the exuviae and were observed as remnants attached to their abdomen (Figure 1 *a*).

Post-treatment, there were no significant differences in all botanical treatments, including the larval and pupal weight, survivability and deformity. The acetogenin annonin or squamocin (principal compound) acts on the physiology of *S. frugiperda* digestive system and related enzymes resulting in a substantial reduction in feeding conversion efficiency[22–24].

The most toxic effect of botanicals was exerted on the pupae, as many were malformed (Figure 1 *c–e*). Reduced larval and pupal viability was due to the action of the principal compound on insect energy production[25]. Acetogenins are considered potent complex I inhibitors (NADH: ubiquinone oxidoreductase) of the mitochondrial electron transport system and NADH oxidase of the plasma membrane, which induce cellular apoptosis (programmed cell death) as a result of ATP deprivation[26]. A previous study reported increased larval duration (22.84 ± 1.63 days) and decreased pupal weight (166.40 ± 4.14 mg) on treatment with anosom at its LC_{50} value (1352 mg/kg) against neonates of *S. frugiperda*[23]. Increased larval (26.47 ± 0.26 days) and pupal durations (12.83 ± 0.44 days) were recorded against third-instar larvae of *Helicoverpa armigera* treated with anosom[17]. In the case of azadirachtin, it affects the neurosecretory system and kills the insect by disturbing the ecdysteroid regulation than by being toxic to the insect. A lower amount of azadirachtin might remain in the body of the insect at the pre-pupal and pupal stages,

causing high mortality of pre-pupae and the formation of larva–pupa intermediates. Moulting disruption and deformities were also observed in NSKE and azadirachtin-treated larvae. In all the treatments, majority of the adults that emerged were malformed. The most common deformity was observed in the wings, as miniatured and abnormally curled wings (Figure 1 *f*). These findings are in agreement with those of another study, which reported prolonged larval and pupal durations in third-instar larvae of *S. littoralis* treated with different concentrations of azadirachtin[27]. The study also observed moulting disruption, anomalies, larval–pupal intermediates and wing deformities in adults caused by azadirachtin[27].

In all the treatments, male and female longevity, fecundity, and egg hatchability (%) were substantially lower and were on par with each other. Quality and quantity of nutrients obtained during larval feeding could influence the number of ovarioles per ovary and, by extension, reduce the potential for egg production[28]. A similar study reported reduced fecundity and egg hatchability in *S. frugiperda* treated with methanol extracts of medicinal plants of Annonaceae[29]. Azadirachtin interferes with the vitellogenin synthesis, and its intake by the developing oocytes disrupts ecdysteroid-regulated events in insects[30]. Similar results of reduced fecundity and fertility were also reported in *S. littoralis* treated with Aling (3.2% azadirachtin)[30]. Our results are also in accordance with another study, which reported lower fecundity of 66.4 eggs/moth, when treated with 0.1 µg of azadirachtin against *S. exempta* larvae and also decreased protein levels in ovaries, suggesting a reduced level of vitellogenesis[31]. Larvae treated with derisom also had similar effects because of the presence of the principal compound karanjin[21]. All the botanicals effectively induced sublethal effects and reduced larval and pupal viability in all the treatments.

Conclusion

All the botanicals tested in this study did not have any statistically significant difference between them and were effective in exerting their inhibitory action on the growth and development of FAW, besides inducing malformations. The multiple modes of action of these botanicals make it difficult for the pest to develop resistance, thus highlighting their importance in today's agriculture. However, there is a need for the development of efficient strategies for their slow active nature. More studies should focus on the sublethal effects of various botanicals for their effective use in Integrated Pest Management programmes.

Conflict of interest: The authors declare that they have no conflict of interest.

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