

Anti- insect properties of Certain Plant extracts against fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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Abstract

Ten plants of pesticidal value, suggested by a group of organic farmers in Perambalur district of Tamil Nadu, were subjected to laboratory bioassay to validate their anti- insect potency against *Spodoptera frugiperda*. Out of those ten plants, at 5% aqueous extract, *Cleistanthus collinus* showed the maximum antifeedancy. The highest larval malformation of 33.33% was observed at 5% aqueous extract of *N. nucifera*. The maximum of 26.67% pupal malformation was recorded in *W. tinctoria* and *M. dubia*. The highest adult deformity was noted in *N. nucifera*, *E. agallocha* and *M. dubia* (33.33% each) extracts. After observation taken on malformation, about 86.67% deformity were recorded at various stages in *N. nucifera* and *M. dubia*, and exhibited the lowest normal adult emergence of 13.33% was observed,

Keywords: *Spodoptera frugiperda*, plant extracts, antifeedant, repellent, insect growth regulatory activity

Introduction

Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) indigenous to the tropical regions of the western hemisphere from Argentina to the United States of America has been reported for the first time in India during July 2018, from the maize fields of Karnataka and severe damage was noticed in Chikkaballapur, Hassan, Shivamogga, Davanagere and Chitradurga districts due to the variety of host plants and the favorable environment and climate [1,2,3,4]. In the year of establishment, maize production fell by 3.2% (27.8 million tonnes) in India and there is a further decline in production in the subsequent years due to the attack of FAW [5,6].

It is a highly polyphagous pest, as it feeds on a wide range of economically important crops including cotton (*Gossypium hirsutum* L.) (Malvales: Malvaceae), corn (*Zea mays* L.) (Cyperales: Poaceae) and many other grass crops [7]. About 186 host plants were recorded from 42 different families for *S. frugiperda*. However; recently 353 plant species have been reported as hosts by Montezano and their team [8]. It is a significant economic pest due to its high rate of reproduction, potential for damage, and capacity to consume several types of plants [9,10,11]. FAW larvae, unlike other army worms, have specialized mouthparts with notched cutting edges that allow them to feed on silica-rich vegetation. Furthermore, because of the cannibalistic behavior, older larvae feed on younger larvae and can outcompete competitors of the same species as well as competitors of other species within the same genus, ensuring their survival [12].

For their management and constant agricultural output, farmers prefer to use synthetic pesticides as quick-fix pest control options [13]. *S. frugiperda* has become resistant to numerous artificial pesticides. It is challenging to control this pest in the fields due to the lack of *S. frugiperda* resistance in host plants and inadequate control techniques. Many researchers are exploring insecticidal plants for the management of FAW, with some promising findings, but could not identify the chemical basis of action [14]. However, testing of plant extracts against this insect is still ongoing globally to determine the various effects of botanicals on

this pest and to develop a cost-effective and environmentally friendly biopesticide. In this present study, we concentrate on botanicals suggested by organic farmers in the Perambalur district during survey and already proven botanical against *Spodoptera litura* for the management of *S. frugiperda*.

Materials and methods

Collection of plant materials

Ten plants were chosen and collected from the Kolli hills and other regions in and around Chidambaram. The plant pieces that will be harvested were collected and packed in A3 paper bags. On the cover of the bag, a paper sticker with the plant's common/vernacular name was inscribed on it. They were cleaned with water, wiped down and then shade dried for 15 to 20 days. Using an electric blender, the dried plant materials were powdered, and then the bags were sealed. They were preserved in a deep freezer at - 20 °C at Phyto-insecticide laboratory, Department of Entomology for further analysis.

Mass culturing of test insect for the bioassay

The egg masses collected from the infested field were placed in plastic cups (200ml capacity) and covered by mesh. Parasitized egg masses were discarded totally. Upon hatching, the larvae of *S. frugiperda* were transferred to plastic buckets (4 cm height x 21 cm length) @ 25 larvae per bucket, and covered by using gada cloth and elastic band. The culture was maintained at 25± 2 °C, 65 ± 5% RH and a photoperiod of 12:12h L:D. Every day fresh maize shoots were given as feed to the larvae. From third instars, due to cannibalistic behaviour larvae were reared individually in multi-cavity trays of 24 cells. Maize shoots were supplied twice a day until pupation. From the multicavity trays, the larvae that were about to pupate were removed and put in the plastic cups with sand. After emergence, the adults were sexed and released in the oviposition cages @ 1:1 ratio. Five per cent honey solution in a cotton wicked vial was kept as food. After 24h, conical flask containing maize shoots along with water for

oviposition was placed in the cage. The egg masses that were removed from the oviposition cages were surface sterilised with sodium hypochlorite (0.05%) after a 24-hour incubation period. Either maize or castor leaves were given as feed.

Aqueous Extraction of selected plants

Powders of the selected plants were individually formed into 50g thimbles using Whatman No. 40 filter paper and extracted with distilled water at room temperature for 72 hours, after which the thimbles were carefully removed and the extracts were collected. 100mL of distilled water was used to make a 50% stock solution for a 50g thimble. Various concentrations were prepared from the stock and utilised in preliminary bioassays.

No-choice-poison food bio assay to assess the anti-insect properties of aqueous extracts of selected plants

The bioassays were conducted in the Phyto-insecticides laboratory of our department during 2021 to assess the antifeedant, insecticidal and growth regulatory properties of these plant extracts. The screening was done with aqueous extracts at 5%.

A total of twelve treatments including absolute control and positive control (treated with 0.15% azadirachtin) with three replications were followed in each bio-assay. Uniform sized (14.5 cm²) leaf discs prepared from the castor leaves collected from the pesticide-free pot culture yard were taken. Five newly shed, 3 h pre-starved, third instar were used per replication. Three replications were maintained per treatment.

Antifeedant assay

A 200 µL of aqueous extract of respective plant at 5% were smeared on both adaxial and abaxial surfaces of leaf discs using a blunt glass rod each separately and air dried. The antifeedant experiment was terminated when the leaves in control were completely fed. The leaf area left out in the treatments for the antifeedant assay were measured using Leaf area meter (Systronicis- Leaf Area Meter Z11) and the average per cent leaf area protection over control was calculated and rated as per the scale given below.

$$\text{Percent leaf area protection over control} = \frac{\% \text{ leaf area protection in treatment} - \% \text{ leaf area protection in control}}{100 - \% \text{ leaf area protection in control}} \times 100$$

Calculated per cent leaf area protection	Rating
> 80	Strong Inhibition (++++)
50-79	Medium Inhibition (++++)
20-49	Weak Inhibition (++)
< 19	Insignificant inhibition (+)

Insecticidal assay

Leaf discs (14.5 cm²) treated with 200 µL of aqueous extract of respective plant at 5% and air dried were used to feed the larvae. Five newly shed, 3 h pre-starved, third instar were used per replication. Three replications were maintained per treatment. The mortality of the larvae in treatments and control were recorded once in 12 h and fresh treated leaf discs were supplied. The study was continued upto pupation.

Insect growth regulatory assay

The methodology described in antifeedant assay, was followed in this assay and after 24h of exposure the larvae were fed with fresh leaves and reared until they emerged as adults. Every 24 hours, observations were made on the mortality and malformations of various stages, and cumulative percent mortality and malformations was calculated.

Statistical Analysis Data from the studies were analysed using analysis of variance (ANOVA) under CRD using the Gomez and Gomez's specified techniques [15]. Necessary data transformation made before analysis and the computer-based WASP Agristat package used for the calculation.

Result and Discussion

At 5% concentration, highest feeding inhibition was recorded in *C. collinus* leaf extract which showed 86.30% leaf area protection over absolute control. The antifeedancy rated as strong inhibition against *S. frugiperda* larvae and ranked first among the ten plants taken. This might be as a result of the poisonous substance found in the leaf, such as the aryl naphthalene lignin lactones Diphyllin and its derivative Cleistathin A and B [16,17]. Medium antifeedancy was observed in positive control (0.15% azadirachtin), *N. nucifera* and *M. dubia* with 33.34%, 42.64% and 46.32% leaf area fed and 66.66%, 57.36% and 53.68% leaf area protection over the absolute control respectively. Both the positive control and *M. dubia* belongs to Meliaceae family and they were known to deter the feeding in insects [18]. *W. tinctoria*, *E. agallocha* and *B. longiflora* exerted weak inhibition. These plants recorded 74.32%, 78.85% and 76.64% leaf area fed and 25.68%, 21.15% and 23.36% leaf area protection over control respectively. The lowest leaf area protection over absolute control was noted in *C. colocynthis*, *C. lancifolius*, *X. strumarium* and *C. phomidis* recorded 6.73%, 5.01%, 4.76% and 4.12% respectively and were considered to possess insignificant inhibition. In absolute control, per cent leaf area fed was 100% (Table 1). Among the ten plants taken, except *C. collinus*, in remaining all the plant extract treated leaves, the percent leaf area fed was maximum and they were further accessed for their juvabione activity.

At 5%, per cent larval mortality observed in these twelve treatments was in the range between 0% in absolute control and maximum of 33.33% in *C. collinus*. The highest larval malformation of 33.33% was observed at 5% aqueous extract of *N. nucifera*. The reason behind the malformation may be the alkaloids such as diuricine, lotusine, nuciferine, pronuciferine, linensinine, isolinensinine, roemerine, nelumbine, neferine, gluteolin, hyperfine and rutin were found in *N. nucifera* [19,20]. It was followed by *M. dubia* recorded 26.67% larval malformation. 20.00% in *E. agallocha* and *B. longiflora*, 13.33% in *W. tinctoria*, *C. lancifolius* and *C. colocynthis* and 6.67% in *X. strumarium* and positive control was recorded. There was no larval malformation in the remaining treatments. The maximum of 26.67% pupal malformation was recorded in *M. dubia*. These findings were in line with Carpinella and his team [21] reported that limonoid from *M. dubia* shows an antifeedant activity and growth regulating activity against *Spodoptera* species. In next to this, *N. nucifera* exerted 20.00% pupal malformation. Adult malformation was found highest of

20.00%, when the larvae were fed with the leaves treated with *N. nucifera* and *M. dubia*. Remaining treatments exhibited less than 15% adult malformation. Cent per cent adult survival was found in absolute control. After

observation taken on malformation, about 86.67% deformity were recorded at various stages, when treated with aqueous extract of *N. nucifera* and *M. dubia* and they exhibited the lowest normal adult emergence of 13.33% (Table 2).

Table 1: Screening for antifeedancy of selected plants against *S. frugiperda*

S.No	Aqueous extract (5% concentration)	Per cent Leaf area fed	Per cent Leaf area protection over control	Antifeedant rating
1	<i>Nelumbo nucifera</i>	42.64 (40.766) ^f	57.36	+++
2	<i>Cleistanthus collinus</i>	13.70 (21.705) ^h	86.30	++++
3	<i>Wrightia tinctoria</i>	74.32 (59.558) ^e	25.68	++
4	<i>Conocarpus lancifolius</i>	94.99 (77.162) ^{bc}	5.01	+
5	<i>Xanthium strumarium</i>	95.24 (77.502) ^{bc}	4.76	+
6	<i>Excoecaria agallocha</i>	78.85 (62.629) ^d	21.15	++
7	<i>Citrullus colocynthis</i>	93.27 (75.024) ^c	6.73	+
8	<i>Melia dubia</i>	46.32 (42.889) ^f	53.68	+++
9	<i>Clerodendrum phomidis</i>	95.88 (78.420) ^b	4.12	+
10	<i>Barleria longiflora</i>	76.64 (61.105) ^{de}	23.36	++
11	Positive control (0.15% azadiractin)	33.34 (35.265) ^g	66.66	+++
12	Absolute Control	100.00 (85.067) ^a	-	-
CD (P=0.05)		2.635		

Values are mean of three replications

Values in parentheses are arc sine transformed

Values with various alphabets differ significantly

Table 2: Insecticidal and IGR effects of botanicals against *S. frugiperda*

S. No	Aqueous extract (5% concentration)	Cumulative Per cent Larval mortality	Cumulative Per cent Larval malformation	Cumulative Per cent Pupal malformation	Cumulative Per cent adult malformation	Cumulative Per cent Normal adult emergence
1	<i>Nelumbo nucifera</i>	13.33 (21.413) ^c	33.33 (35.262) ^a	20.00 (26.565) ^b	20.00 (26.565) ^a	13.33 (21.413) ^h
2	<i>Cleistanthus collinus</i>	33.33 (35.262) ^a	6.67 (14.965) ^e	6.67 (14.965) ^d	0.00 (2.306) ^d	53.33 (46.909) ^f
3	<i>Wrightia tinctoria</i>	13.33 (21.413) ^c	13.33 (21.413) ^d	20.00 (26.565) ^b	13.33 (21.413) ^b	40.00 (39.231) ^g
4	<i>Conocarpus lancifolius</i>	6.67 (14.965) ^d	13.33 (21.413) ^d	6.67 (14.965) ^d	6.67 (14.965) ^c	66.67 (54.738) ^d
5	<i>Xanthium strumarium</i>	6.67 (14.965) ^d	6.67 (14.965) ^e	6.67 (14.965) ^d	6.67 (14.965) ^c	73.33 (58.907) ^c
6	<i>Excoecaria agallocha</i>	13.3 (21.413) ^c	20.00 (26.565) ^c	13.33 (21.413) ^c	13.33 (21.413) ^b	40.00 (39.231) ^g
7	<i>Citrullus colocynthis</i>	6.67 (14.965) ^d	13.33 (21.413) ^d	13.33 (21.413) ^c	6.67 (14.965) ^c	60.00 (50.769) ^e
8	<i>Melia dubia</i>	13.33 (21.413) ^c	26.6 (31.093) ^b	26.67 (31.093) ^b	20.00 (26.565) ^a	13.33 (21.413) ^h
9	<i>Clerodendrum phomidis</i>	6.67 (14.965) ^d	0.00 (2.306) ^f	6.67 (14.965) ^d	6.67 (14.965) ^c	80.00 (63.435) ^b
10	<i>Barleria longiflora</i>	13.33 (21.413) ^c	20.00 (26.565) ^c	13.33 (21.413) ^c	13.33 (21.413) ^b	40.00 (39.231) ^g
11	Positive control (0.15% azadiractin)	26.67 (31.093) ^b	6.67 (14.965) ^e	13.33 (21.413) ^c	13.33 (21.413) ^b	40.00 (39.231) ^g
12	Control	0.00 (2.306) ^e	0.00 (2.306) ^f	0.00 (2.306) ^e	0.00 (2.306) ^d	100.00 (87.694) ^a
CD (P=0.05)		0.445	0.473	0.442	0.494	0.355

Values are mean of three replications

Values in parentheses are arc sine transformed

Values with various alphabets differ significantly

Conclusion

Based on the result obtained, *C. collinus* showed the highest antifeedancy and *M. dubia* and *N. nucifera* exhibited more prominent juvabione activities. Further research has to be undertaken and the newer, more effectual and eco-friendlier compound has to characterize from these plants and formulated to distribute among the farmers. These bioinsecticides ought to be quite powerful, and they could be crucial in IPM campaigns against the fall armyworm

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Conflicts of interest

The authors declare no conflict of interest.

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