



Efficacy of Solvent Extracts of *Nelumbo nucifera* Gaertn (Nelumbonaceae) and *Melia dubia* Cav (Meliaceae) against Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Spodoptera frugiperda is a significant pest of economic importance due to its high rate of reproduction, potential for damage and capacity to consume several types of plants. It has become resistant to numerous chemical pesticides. It is challenging to control this pest in field due to the

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lack of host plant resistance and inadequate management techniques. Bioactive molecules of plant origin hold potential alternative for the chemical pesticides. In the present study, leaves of *Nelumbo nucifera* and *Melia dubia* were extracted with acetone, ethyl acetate and benzene. All the solvent extracts of *N. nucifera* and *M. dubia* exhibited larval, pupal and adult malformation of *S. frugiperda*. At higher doses, these plant extract exerted medium antifeedancy. In regarding, insect growth regulatory (IGR) activity, maximum of 33.33% larval malformation at 7% benzene extract of *M. dubia*, 53.33% pupal malformation at 7% benzene extract of *N. nucifera* and 33.33% adult malformation at 5% ethyl acetate extract of *N. nucifera* was recorded. In comparing all the extracts, the benzene extract of *N. nucifera* showed maximum IGR activity against *S. frugiperda* at 5%.

Keywords: Anti- insecticidal; juvabione; montmoribant condition.

1. INTRODUCTION

One of the primary issues in the agricultural sector is insect pest, which results in 20-40% losses in global agricultural output [1,2]. *Spodoptera frugiperda* 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 [3]. 353 plant species have been reported as hosts by Montezano et al. [4]. It is a significant pest of economic importance due to its high reproductive rate and their nature of damage [5,6,7].

For their management and constant agricultural output, farmers prefer to use synthetic pesticides as quick-fix pest control options [8]. *S. frugiperda* is highly adaptable and well known to evolve resistance against synthetic pesticides [9]. It is challenging to control this pest in the fields due to the lack of *S. frugiperda* resistance in host plants and inadequate management 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 [10,11-14]. 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.

For this purpose, in this study, two plants namely, Lotus, *Nelumbo nucifera* Gaertn. (Family: Nelumbonaceae) and Maha Neem, *Melia dubia* C. (Family: Meliaceae) have been selected to test their anti- insect properties (antifeedancy, insecticidal and insect growth regulatory activity) against *S. frugiperda*.

2. MATERIALS AND METHODS

2.1 Mass Culturing of Test Insect for the Bioassay

The egg masses collected from the infested field were placed in plastic cups (200ml capacity) along with pieces of fresh maize leaf and covered by mesh. Parasitized egg masses were discarded totally. Upon hatching, the larvae were transferred to plastic buckets (7L capacity) @ 25 nos of first instar larvae of *S. frugiperda* 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 the larvae were fed with fresh maize shoots. 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. The larvae which were about to pupae were collected from the multi-cavity trays and placed in the plastic cups (200ml capacity) containing sand. After emergence, the adults were sexed and released in the oviposition cages @ 1:1 (male: female) ratio. Five per cent honey solution in a cotton wicked vial was kept as food. After 24h, maize shoots kept in a conical flask containing water was placed in the cage. The egg masses collected from the oviposition cages were incubated for 24h and surface sterilized with sodium hypochlorite (0.05%). Either maize or castor leaves were given as feed [15].

2.2 Preparation of Plant Extract

Leaves of *Nelumbo nucifera* and *Melia dubia* were collected and placed in paper bags of A3 size affixed with the common/vernacular name of the plant on the cover. The plants brought to the laboratory were rinsed with water; wiped off and shade dried for 15 to 20 days. The dried leaves were powdered using Wiley-Mill (Pearl Lab

Instruments Co.) individually and stored at -20 °C in a deep freezer.

2.3 Preparation of Solvent Extracts

Solvent extracts of *M. dubia* and *N. nucifera* were obtained by following cold solvent extraction technique [16]. The solvents used for extraction were acetone (polarity index-5.1, boiling point- 56°C), ethyl acetate (semi-polarity index-4.4, boiling point-77.1°C) and benzene (non- polarity index-2.7, boiling point-80.09°C).

50 g of *M. dubia* and *N. nucifera* leaf powder were formed into thimbles and placed inside 1 L stoppered round-bottom flasks and filled with 500 mL of the appropriate solvent. The flasks were then left for 72 hours at room temperature. Next, the thimbles were carefully taken out and the extracts were concentrated under reduced pressure in a rotary vacuum pump (Rotoevaporater, India). The resulting mesilla were placed in a tiny glass vials covered with foil to keep light out and preserved in a deep freezer at -20 °C.

2.4 No-Choice-Poison Food Bio Assay

The bioassays were conducted in the Phyto-insecticides laboratory of our department during 2021 to assess the antifeedant, insecticidal and growth regulatory properties of above said plants. The screening was done with solvent extracts of the above said plants at a range of concentration such as 1, 3, 5, 7 and 9%.

A total of seventeen treatments including absolute control and positive control (treated with 0.15% azadirachtin) 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.

2.5 Antifeedant Assay

Leaf discs were treated with 200 µL of solvent extract at different concentrations (1, 3, 5, 7, and 9%) using a blunt glass rod on both the adaxial and abaxial sides and air dried. The antifeedant experiment was ended when the leaves were completely fed in control. Then, the leaf area left out in the treatments 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 [17].

Percent leaf area protection over control = % leaf area protection in treatment-% leaf area protection in control / 100-% leaf area protection in control x 100

2.6 Insecticidal Assay

Leaf discs (14.5 cm²) treated with 200 µL of solvent extract at respective concentration (1, 3, 5,7 and 9%) and air dried were used to feed the larvae. 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.

2.7 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.

2.8 Statistical Analysis

Data from the studies were analysed using analysis of variance (ANOVA) under CRD using the Gomez and Gomez's specified techniques [18]. Necessary data transformation made before analysis and the computer-based WASP Agristat package used for the calculation.

List 1. Leaf area protection

Antifeedancy rating scale		
Per cent leaf area protection	Antifeedancy	Rating
> 80	Strong Inhibition	++++
50-79	Medium Inhibition	+++
20-49	Weak Inhibition	++
< 19	Insignificant inhibition	+

3. RESULTS AND DISCUSSION

3.1 Efficacy of *N. nucifera* against *S. frugiperda*

From the data represented in Table 1, a maximum of 73.65% leaf area protection over an absolute control was observed at 9% benzene extract, indicating medium antifeedancy. Additionally, medium inhibition against *S. frugiperda* larvae was observed at 5, 7, and 9% in benzene and acetone extracts, as well as at 7 and 9% in ethyl acetate extracts and in the positive control. 100% of the leaf area was fed in an absolute control. Weak inhibition of less than 50% leaf area protection over an absolute control was noted at 1 and 3%.

In terms of IGR activity, abnormalities were recorded in all concentrations of solvent extract of *N. nucifera* (Table 2). At 3, 5 and 9% of acetone extract and at 7 and 9% of benzene extract, the highest larval deformity of 26.67% was recorded. It was followed by acetone extract at 7%, ethyl acetate extract at 9% and benzene extract at 3, 5, 7 and 9%, in which, each of them have recorded 20.00% larval malformation. In *N. nucifera* treatment, there was a size reduction of the larval segments which became shrunken and leaving the head portion. After four days, they didn't feed the normal leaves provided and resulted in montmoribant condition. Our findings were in line with Indumathi and Arivudainambi [19] reported that *N. nucifera* showed 100 per cent insect growth regulatory activity against larval stages of *Spodoptera litura* (Fab.) and they have found that, the treated third instars were continuously engaged in moulting without melanization. The larvae were lived for about nine days and then died. Larval mortality of 26.67% was observed at 9% acetone and 7 and 9% of benzene extract. The highest pupal malformation of 53.33%, was observed at 9% benzene extract. The malformed pupae were intermediate between pupae and adult. Maximum of 26.67% adult malformation was noted at 1 and 7% in acetone extract and at 5% in benzene extract. In the adult, wings were folded and look like a roof. According to Sridhar and Rajeev [20] and Imana pal and Purnima Ray [21], alkaloids including diuricine, lotusine, nuciferine, pronuciferine, linensinine, isolinensinine, roemerine, nelumbine, neferine, gluteolin, hyperfine, and rutin were found in *N. nucifera* and it may be the cause of the malformation. Per cent normal adult emergence in acetone and ethyl acetate extract at 9% and in

benzene extract at 5, 7 and 9% was found to be zero. This juvobione activity became greater with increasing in the concentrations. Santhoshkumar et al. [22], conducted a bioassay with solvents like acetone, chloroform, ethyl acetate, acetone, hexane, methanol and water extracts and synthesized silver nanoparticles of *N. nucifera* against 4th instar larvae of *A. subpictus* and *C. quiquefasciatus* mosquitoes at 50µg/l. Among them, methanol extract and silver nanoparticles of *N. nucifera* gave cent per cent larval mortality in both mosquito species at 24 and 48 hrs of exposure. Their studies were in line with our results of larval malformation in various solvent extract of *N. nucifera*.

3.2 Efficacy of *M. dubia* against *S. frugiperda*

In Table 3, the larvae fed with 9% acetone extract exerted 74.65% leaf area protection over an absolute control and recorded as maximum among all the treatment. Per cent leaf area protection over an absolute control was in the range between 53.71% and 74.65% was recorded at 5, 7 and 9% in acetone extract and 7 and 9% in ethyl acetate and benzene extract and in positive control. They exhibited medium antifeedancy against *S. frugiperda* larvae. Weak inhibition was observed in the remaining solvent extract of *M. dubia*. The Meliaceae family is renowned for being a reliable source of secondary metabolites. Inconfirmity with Carpinella et al. [23], in their study, they have reported that limonoid, active compound from *M. dubia* showed an antifeedant activity and growth regulating activity against *Spodoptera* species.

From the data presented in Table 4, acetone extract at 9% exerted maximum of 40.00% larval malformation and 33.33% larval mortality. In total, larval malformation of 26.67% was noted in three treatments, 20.00% in another three treatments, 13.33% in five treatments, 6.67% in the remaining three treatments and zero per cent in an absolute control. The larvae intended to be pupae become black coloured and half moulted pupal skin was found on the dorsal side of the larvae. In accordance with Gopal et al. [24], reported that *M. dubia* leaf extracts possess larvicidal activity and recorded the growth inhibitory activity and deterrence activity as it inhibited the growth in a dose dependent manner. Pupal malformation of 46.67% was found highest at 9% benzene extract. Next to that, 40.00% was observed at 7% acetone and 9% ethyl acetate extract. Less than 26.67% of

pupal malformation was recorded in the remaining treatments. The maximum of 20.00% adult malformation was observed at 3% acetone and 1% benzene extract. Adult malformation was found zero at 9% benzene extract and in an absolute control. Normal adult emergence of more than 50% was observed in six treatments and less than 50% in the remaining eleven treatments. Malformed wings and intermediate between pupae and adult were observed. In line with Bhuiyan et al. [25] reported that, extracts of

M. dubia have growth inhibitors, antifeedants, stomach poisons and make moulting disorders and morphological defects in a number of pests. Triterpenoids, which have a plethora of bioactivities, including insecticidal action, are the primary bioactive chemical compounds in meliaceae plants might be the reason for the above anti- insect properties [26]. Similar results were observed in the aqueous extract of *N. nucifera* and *M. dubia* against *S. frugiperda* [27].

Table 1. Antifeedant effects of solvent extract of *N. nucifera* against *S. frugiperda*

S. No	Solvent Extract	Percent leaf area fed	Percent leaf area protection over control	Antifeedant rating
1	Acetone 1%	74.38 (59.603) ^c	25.62	++
2	Acetone 3%	63.47 (52.819) ^d	36.53	++
3	Acetone 5%	43.93 (41.512) ^f	56.07	+++
4	Acetone 7%	30.58 (33.565) ^{ij}	69.42	+++
5	Acetone 9%	27.46 (31.593) ^{jk}	72.54	+++
6	Ethyl acetate 1%	76.08 (60.733) ^{bc}	23.92	++
7	Ethyl acetate 3%	78.26 (62.224) ^b	31.07	++
8	Ethyl acetate 5%	60.86 (51.276) ^d	39.14	++
9	Ethyl acetate 7%	46.38 (42.923) ^{ef}	53.62	+++
10	Ethyl acetate 9%	31.89 (34.375) ^{hi}	68.11	+++
11	Benzene 1%	73.79 (59.216) ^c	26.21	++
12	Benzene 3%	61.35 (51.564) ^d	38.65	++
13	Benzene 5%	48.64 (44.220) ^e	51.36	+++
14	Benzene 7%	35.42 (36.518) ^{gh}	64.58	+++
15	Benzene 9%	26.35 (30.875) ^k	73.65	+++
16	Positive control (0.15% azadirachtin)	38.62 (38.418) ^g	61.38	+++
17	Absolute control	100.00 (84.705) ^a		-
CD (0.05%)		2.346		

Values are mean of three replications
Values in parentheses are arc sine transformed
Values with various alphabets differ significantly

Table 2. IGR effects of solvent extract of *N. nucifera* against *S. frugiperda*

	Solvent Extract	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	Acetone 1%	0.00 (2.306) ^d	6.67 (14.965) ^d	20.00 (26.565) ^e	26.67 (31.093) ^b	46.67 (43.091) ^c
2	Acetone 3%	6.67 (14.965) ^c	26.67 (31.093) ^a	20.00 (26.565) ^e	20.00 (26.565) ^c	26.67 (31.093) ^e
3	Acetone 5%	13.33 (21.413) ^b	26.67 (31.093) ^a	26.67 (31.093) ^d	13.33 (21.413) ^d	20.00 (26.565) ^f
4	Acetone 7%	13.33 (21.413) ^b	20.00 (26.565) ^b	33.33 (35.262) ^c	26.67 (31.093) ^b	6.67 (14.965) ^h
5	Acetone 9%	20.00 (26.565) ^a	26.67 (31.093) ^a	33.33 (35.262) ^c	20.00 (26.565) ^c	0.00 (2.306) ⁱ
6	Ethyl acetate 1%	0.00 (2.306) ^d	6.67 (14.965) ^d	20.00 (26.565) ^e	13.33 (21.413) ^d	53.33 (46.909) ^b
7	Ethyl acetate 3%	6.67 (14.965) ^c	13.33 (21.413) ^c	20.00 (26.565) ^e	13.33 (21.413) ^d	46.67 (43.091) ^c
8	Ethyl acetate 5%	13.33 (21.413) ^b	20.00 (26.565) ^b	20.00 (26.565) ^e	33.33 (35.262) ^a	13.33 (21.413) ^g
9	Ethyl acetate 7%	20.00 (26.565) ^a	26.67 (31.093) ^a	33.33 (35.262) ^c	13.33 (21.413) ^d	6.67 (14.965) ^h
10	Ethyl acetate 9%	20.00 (26.565) ^a	26.67 (31.093) ^a	46.67 (43.091) ^b	6.67 (14.965) ^e	0.00 (2.306) ⁱ
11	Benzene 1%	13.33 (21.413) ^b	6.67 (14.965) ^d	20.00 (26.565) ^e	20.00 (26.565) ^c	40.00 (39.231) ^d
12	Benzene 3%	13.33 (21.413) ^b	20.00 (26.565) ^b	26.67 (31.093) ^d	13.33 (21.413) ^d	26.67 (31.093) ^e
13	Benzene 5%	20.00 (26.565) ^a	20.00 (26.565) ^b	33.33 (35.262) ^c	26.67 (31.093) ^b	0.00 (2.306) ⁱ
14	Benzene 7%	20.00 (26.565) ^a	26.67 (31.093) ^a	46.67 (43.091) ^b	6.67 (14.965) ^e	0.00 (2.306) ⁱ
15	Benzene 9%	20.00 (26.565) ^a	26.67 (31.093) ^a	53.33 (46.909) ^a	0.00 (2.306) ^f	0.00 (2.306) ⁱ

	Solvent Extract	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
16	Positive control (0.15% azadirachtin)	13.33 (21.413) ^b	13.33 (21.413) ^c	26.67 (31.093) ^d	6.67 (14.965) ^e	46.67 (43.091) ^c
17	Absolute control	0.00 (2.306) ^d	0.00 (2.306) ^e	0.00 (2.306) ^f	0.00 (2.306) ^f	100.00 (86.456) ^a
	CD (0.05%)	0.464	0.378	0.327	0.429	0.932

Values are mean of three replications
 Values in parentheses are arc sine transformed
 Values with various alphabets differ significantly

Table 3. Antifeedant effects of solvent extract of *M. dubia* against *S. frugiperda*

S. No	Solvent Extract	Percent leaf area fed	Percent leaf area protection over control	Antifeedant rating
1	Acetone 1%	68.35 (55.772) ^c	31.65	++
2	Acetone 3%	60.15 (50.860) ^e	39.85	++
3	Acetone 5%	46.29 (42.871) ^f	53.71	+++
4	Acetone 7%	31.86 (34.357) ^{ij}	68.14	+++
5	Acetone 9%	25.35 (30.220) ^l	74.65	+++
6	Ethyl acetate 1%	74.87 (59.926) ^b	25.13	++
7	Ethyl acetate 3%	68.39 (55.797) ^c	31.61	++
8	Ethyl acetate 5%	62.32 (52.136) ^{de}	37.68	++
9	Ethyl acetate 7%	40.05 (39.258) ^g	59.95	+++
10	Ethyl acetate 9%	29.77 (33.059) ^{jk}	70.23	+++

S. No	Solvent Extract	Percent leaf area fed	Percent leaf area protection over control	Antifeedant rating
11	Benzene 1%	77.33 (61.581) ^b	22.63	++
12	Benzene 3%	69.88 (56.722) ^c	30.12	++
13	Benzene 5%	64.35 (53.344) ^d	35.65	+++
14	Benzene 7%	35.55 (36.596) ^{hi}	64.45	+++
15	Benzene 9%	26.54 (30.998) ^{kl}	73.46	+++
16	Positive control (0.15% azadirachtin)	38.62 (38.418) ^{gh}	61.38	+++
17	Absolute control	100.00 (84.702) ^a		-
CD (0.05%)		2.343		

*Values are mean of three replications
 Values in parentheses are arc sine transformed
 Values with various alphabets differ significantly*

Table 4. IGR effects of solvent extract of *M. dubia* against *S. frugiperda*

S. No	Solvent Extract	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	Acetone 1%	6.67 (14.965) ^c	13.33 (21.413) ^e	13.33 (21.413) ^f	13.33 (21.413) ^b	53.33 (46.909) ^d
2	Acetone 3%	6.67 (14.965) ^c	26.67 (31.093) ^c	13.33 (21.413) ^f	20.00 (26.565) ^a	33.33 (35.262) ^f
3	Acetone 5%	13.33 (21.413) ^b	20.00 (26.565) ^d	26.67 (31.093) ^d	13.33 (21.413) ^b	26.67 (31.093) ^g
4	Acetone 7%	13.33 (21.413) ^b	20.00 (26.565) ^d	40.00 (39.231) ^b	20.00 (26.565) ^a	6.67 (14.965) ⁱ
5	Acetone 9%	20.00 (26.565) ^a	40.00 (39.231) ^a	33.33 (35.262) ^c	6.67 (14.965) ^c	0.00 (2.306) ^j
6	Ethyl acetate 1%	0.00 (2.306) ^d	6.67 (14.965) ^f	6.67 (14.965) ^g	13.33 (21.413) ^b	73.33 (58.907) ^b
7	Ethyl acetate 3%	6.67 (14.965) ^c	6.67 (14.965) ^f	13.33 (21.413) ^f	6.67 (14.965) ^c	66.67 (54.738) ^c
8	Ethyl acetate 5%	6.67 (14.965) ^c	13.33 (21.413) ^e	20.00 (26.565) ^e	13.33 (21.413) ^b	46.67 (43.091) ^e
9	Ethyl acetate 7%	20.00 (26.565) ^a	26.67 (31.093) ^c	13.33 (21.413) ^f	13.33 (21.413) ^b	26.67 (31.093) ^g
10	Ethyl acetate 9%	20.00 (26.565) ^a	20.00 (26.565) ^d	40.00 (39.231) ^b	13.33 (21.413) ^b	6.67 (14.965) ⁱ
11	Benzene 1%	0.00 (2.306) ^d	6.67 (14.965) ^f	13.33 (21.413) ^f	20.00 (26.565) ^a	66.67 (54.738) ^c
12	Benzene 3%	13.33 (21.413) ^b	13.33 (21.413) ^e	20.00 (26.565) ^e	6.67 (14.965) ^c	53.33 (46.909) ^b
13	Benzene 5%	13.33 (21.413) ^b	13.33 (21.413) ^e	26.67 (31.093) ^d	13.33 (21.413) ^b	33.33 (35.262) ^f
14	Benzene 7%	20.00 (26.565) ^a	33.33 (35.262) ^b	20.00 (26.565) ^e	13.33 (21.413) ^b	13.33 (21.413) ^h
15	Benzene 9%	20.00 (26.565) ^a	26.67 (31.093) ^c	46.67 (43.091) ^a	0.00 (2.306) ^d	6.67 (14.965) ⁱ

S. No	Solvent Extract	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
16	Positive control (0.15% azadirachtin)	13.33 (21.413) ^b	13.33 (21.413) ^e	26.67 (31.093) ^d	6.67 (14.965) ^c	46.67 (43.091) ^e
17	Absolute control	0.00 (2.306) ^d	0.00 (2.306) ^g	0.00 (2.306) ^h	0.00 (2.306) ^d	100.00 (87.694) ^a
	CD (0.05%)	0.479	0.387	0.360	0.450	0.405

*Values are mean of three replications
 Values in parentheses are arc sine transformed
 Values with various alphabets differ significantly*

4. CONCLUSION

From the results obtained, the solvent extracts of *N. nucifera* was found more effective than *M. dubia*. In *N. nucifera*, the benzene extract recorded the maximum anti-insect activities against *S. frugiperda*. Further research has to be undertaken to know their mode of action and the newer, more effectual and eco-friendlier compound has to be identified through characterization. Formulation and distribution among the farmers have to be done. These bioinsecticides ought to be quite powerful, and they could be crucial in IPM campaigns against the fall armyworm. A benefit of bioinsecticides is that they are less hazardous to creatures other than the target pests, in addition to having better insecticidal activity than some chemical insecticides.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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