Research Paper

Volume : 3 | Issue : 8 | Aug 2014 • ISSN No 2277 - 8179

Antifeedant and Larvicidal Activities Of Synthesized Silver Nanoparticles Using Aristolochia Indica Extract Against Helicoverpa Armigera Hübner (Lepidoptera: Noctuidae)

Zoology

KEYWORDS : Aristolochia indica Silver nanoparticles, Helicoverpa armigera, Antifeedant and Larvicidal activities

*** Dr. (Sr.) R. Regina Mary**	Assistant Professor, PG and Research Department of Zoology, Auxilium College (Autonomous), Vellore- 632 006. * Corresponding Author
C. Siva	Post Graduate and Research Department of Zoology, Auxilium College Autonomous, Gandhi Nagar, Vellore - 632 006, Tamil Nadu, India.
M. Santhosh Kumar	Post Graduate and Research Department of Zoology, Auxilium College Autonomous, Gandhi Nagar, Vellore - 632 006, Tamil Nadu, India.
K. Logeswaran	Post Graduate and Research Department of Zoology, Auxilium College Autonomous, Gandhi Nagar, Vellore - 632 006, Tamil Nadu, India.

ABSTRACT

In the present study, the antifeedant, larvicidal activities of synthesized silver nanoparticles (Ag NPs) using aqueous leaf extract of Aristolochia indica against third instar larvae of Helicoverpa armigera and were evaluated. Antifeedant, larvicidal activities of aqueous crude extracts and synthesized Ag NPs were studied using leaf disc no choice, leaf dipping methods respectively. The maximum antifeedant and larvicidal efficacy was observed in crude aqueous and synthesized Ag NPs against H. armigera larvae (LC50 = 127.49, 84.56 mg/L; 766.54, and 309.98 mg/mL), respectively. It is a novel, eco-friendly, simple, and cost-effective approach to synthesis of Ag NPs using A. indica to control the pest.

Introduction

The American bollworm, *Helicoverpa armigera* is one of the key pests having almost world-wide distribution. It is distributed in most of Asia, Australia, Africa and southern mediterranean region, including cotton producing countries such as India, China, Pakistan and Egypt (EPPO, 2005). Recently green Ag NPs have been synthesized using *Nelumbo nucifera* were tested against *Anopheles subpictus* and *Culex quinquefasciatus* (Santhoshkumar et al., 2011). In this study, the antifeedant and larvicidal effect of Ag NPs synthesized using the leaves of *A. indica* was assessed. Furthermore, these biologically synthesised NPs and crude aqueous extracts of *A. indica* were found to exhibit a significant pesticidal activity against *H. Armigera.*

Materials and methods

Plant material

A.indica L. (Aristolochiaceae) leaves were collected from Malaiyur Hills, Dharmapuri district. Tamil Nadu, India. The taxonomic identification was made by Dr. C. Hema, Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Vellore, India.

Preparation of *A. indica* leaf aqueous extract

Aqueous extract was prepared by mixing 100 g of dried leaf powder with 500 mL of water (boiled and cooled Milli Q water) under constant stirring using a magnetic stirrer (Minjas and Sarda, 1986). The suspension of dried leaf powder in water was left for 3 h, filtered through Whatman no. 1 filter paper, and the filtrate was stored in amber coloured air tight bottle at 10^0C and used within a week.

Synthesis of Ag NPs by *A. indica* leaf extract

A. indica plant leaf broth solution was prepared by taking 10 g of washed and finely cut leaves in a 250 mL Erlenmeyer flask along with 100 mL of deionized water and then boiling the mixture at 60^0C for 5min. After boiling, the solution was decanted, and 15mL of this broth was added to 85mL of 3mM aqueous $AgNO_3$ solution and the resulting solution became brown in colour. This extract was filtered through nylon mesh (spectrum), followed by Millipore hydrophilic filter (0.22µm) and used for further experiments.

Insect culture

Larvae of *H. armigera* were collected from the infested field of Nadazhagananthal, Tiruvannamalai district, Tamil Nadu, India, and cultured at room temperature (27 ± 2^0C) in the insectary and allowed to multiply as per the procedure of Kamaraj et al. (2008). The pest was identified by Dr. V. Rajagopal, Zonal Entomological Research Centre, Vellore, and Tamil Nadu.

Antifeedant activity of *H. armigera*

The fresh cotton leaf discs of 4 cm in diameter were punched using cork borer and dipped in 5, 10, 20, 30, 40 and 50 mg/mL concentrations of aqueous and synthesized Ag NPs extracts. The leaf discs treated with $AgNO_3$ (3 mM solution), Milli Q water was used as negative control and Azadirachtin at concentration of 5, 10, 20, 30, 40 and 50 mL/L was used as positive control. In each Petri dish, a wet filter paper was placed to avoid the early drying of the leaf discs and one third instars larva was introduced into each Petridish. Progressive consumption of leaf area by the treated and control larvae after 24 h was recorded using leaf area meter. Leaf area, eaten by larvae in treatment was corrected from the negative control. Five replicates were maintained for each treatment.

Larvicidal activity of *H. armigera*

The cotton leaf discs were dipped in different concentrations of aqueous extract, synthesized Ag NPs, AgNO3 solution and Azadirachtin. After 24 h of treatment, the larvae were continuously maintained on the non-treated fresh cotton leaves. Diet was changed every 24 h. Larval mortality was recorded after 96 h of treatment. Three replicates were maintained for each treatment with 10 larvae per replicate.

Statistical analysis

average Antifeedant and larval mortality data were subjected to probit analysis for calculating LC50, LC90 and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using the software developed by Reddy et al. (1992). Results with $p<0.05$ were considered to be statistically significant.

Results

In the present investigation, the leaf aqueous extract of *A. indica* and synthesized Ag NPs showed good Antifeedant ac-

tivity with 8.45, 13.16, 27.38, 41.33, 62.44, 72.22 % ; 22.20, 28.96, 33.87, 51.60, 78.61 and 92.40 % at 5, 10, 20, 30, 40 and 50 mg/mL, respectively. The aqueous extract of *A. indica* and synthesized Ag NPs exhibited the LC50 and LC90 values of 623.45, 1725.37 mg/mL and 365.72 and 968.33 mg/mL, respectively (Table 1). Synthesized Ag NPs showed strong antifeedant activity compared with aqueous extract against *H. armigera*. The positive control azadirachtin showed 97.28% potential antifeedant activity against *H. armigera* with LC50 and LC90 values of 348.98 and 955.39 mL/L, respectively (Table 2).

Larvicidal activity of aqueous extract of *A. indica* and synthesized Ag NPs showed 54.82, 68.16, and 87. 69 % and 64.04, 86.38 and 100% against *H. armigera* at 30, 40 and 50 mg/mL, respectively and the activity was statistically significant compared with control (Table 1). The LC50 and LC90 values were 766.54 and 1859.06 mg/mL for aqueous extract and 309.98 and 980.81 mg/mL for synthesized Ag NPs against *H. armigera*, respectively (Table 2). 100 % larval mortality was recorded with higher concentrations of synthesized Ag NPs and azadirachtin at 50 mL/L concentration with LC50 value of 297.03 and LC90 value of 855.98 mL/L.

Table 1
Antifeedant and larvicidal activity of *A. indica* aqueous extract, synthesized Ag NPs, Azadirachtin and Ag NO3 solutions against III instar larvae of *H. armigera*.

Concentrations (mg/mL)	% Mortality* (mg/mL) ± SD	
	Antifeedant	Larvicidal
Aqueous extract of A. indica 5	08.45 ± 3.19	16.60 ± 2.34
10	13.16 ± 1.36	27.07 ± 3.39
20	27.38 ± 4.48	36.53 ± 2.38
30	41.33 ± 2.85	54.82 ± 4.56
40	62.44 ± 1.94	68.16 ± 3.10
50	72.22 ± 4.61	87.69 ± 3.17
Synthesized Ag NPs 5	22.20 ± 2.97	27.19 ± 1.34
10	28.96 ± 3.50	31.33 ± 2.85
20	33.87 ± 4.63	36.53 ± 3.38
30	51.60 ± 5.95	64.04 ± 4.18
40	78.61 ± 5.73	86.38 ± 3.05
50	92.40 ± 3.17	100.0±0.00
Azadirachtin (mL/L)		
5	38.12 ± 1.88	45.87 ± 4.63
10	43.03 ± 2.85	53.24 ± 4.06
20	54.46 ± 2.51	66.45 ± 3.94
30	62.16± 3.10	74.81 ± 3.17
40	75.30 ± 2.10	84.07 ± 2.06
50	97.28 ± 4.91	100.0 ±0.00
Ag NO_3 (mg/mL) 50	04.28±0.83	02.63±0.42

Control (Distilled water); Nil mortality
*** Mean value of three replicates± SD**

Table 2 LC50 and LC90 of antifeedant and larvicidal activity of aqueous and synthesized Ag NPs using the extract of *A. indica* and Azadirachtin against III instar larvae of *H. armigera*.

Activities and Extracts	LC_{50}±SE (mg/mL)	95% Confidence limit		LC_{90}±SE (mg/mL)	95% Confidence limit		$\chi 2$ (df=4)
		Lower limit (LCL)	Upper limit (UCL)		Lower limit (LCL)	Upper limit (UCL)	
Antifeedant							
Aqueous	623.45±9.34	458.43	673.02	1725.37±24.33	1285.64	2248.14	15.36
Ag NPs	365.72±8.24	268.52	513.76	968.33±16.52	784.93	1090.24	6.82
Azadirachtin	348.98±9.15	321.84	668.20	955.39±15.34	778.31	1036.17	16.64
Larvicidal							
Aqueous	766.54±12.04	864.46	1296.92	1859.06±18.35	1377.30	2189.80	15.67
Ag NPs	309.98±8.98	282.77	547.20	980.81±28.33	788.08	1273.52	12.65
Azadirachtin	297.03±11.37	281.43	433.62	855.98±16.48	685.26	1136.17	13.58

LC50 Lethal concentration that kills 50% of the exposed larvae, LC90 lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit, LCL lower confidence limit; $\chi 2$ Chi-square, df degree of freedom, significant at P<0.05 level.

Discussion

Ag NPs nanoparticles produced using plants have been used in various applications for human benefit. The synthesized Ag NPs from *A. indica* showed more than 80% antifeedant and larvicidal activities against *H. armigera* at the concentration of 50 mg/mL. Baskar et al. (2011) reported that plant derivatives, leaf and root extracts of *Aristolochia tagala* showed higher antifeedant activity (56.06%), lethal concentration for feeding inhibition (3.69%), larvicidal (40.66%), pupicidal (28%), total mortality (68.66%) and prolonged larval–pupal duration (12.04-13.08 days) against *S. litura* were observed in ethyl acetate leaf extract at 5.0 % concentration. The aqueous

AgNO3 solution turned into yellowish brown colour within 1 hj with the addition of leaf extract of *A. indica*.

Conclusion

In conclusion, we propose an eco-friendly method for Ag NPs synthesized by the green chemistry approach using the aqueous leaf extract of *A. indica* within 30 minutes. The leaf extract of *A. indica* is environmentally benign and renewable and is capable of acting as both reducing and stabilizing agent. Synthesized Ag NPs showed potential antifeedant (92.40 %) and larvicidal (100 %) activities whereas aqueous extract showed good antifeedant (72.22 %) and larvicidal (87.69 %) activities against *H. armigera* with LC50 of 365.72 and 309.98 mg/mL; 623.45 and 766.54 mg/mL, respectively. In the present approach, we avoid the use of hazardous, toxic solvents and waste and this nanostructure showed excellent antifeedant and larvicidal activity against *H. armigera*. This synthesized nanoparticle could be used for the development of new botanical insecticidal formulation which can reduce crop damage as well as pest population.

REFERENCE

AL-Asady, A.A.B., Khalil, K. H., Barwari, S.S.M., 2012. Cytotoxic and Cytogenetics Effects of Aqueous, Methanolic and Secondary Metabolites Extracts of Capparis spinosa on Tumor Cell Lines in vitro. Jordan J. Biol. Sci.5, 15-30. | Baskar, K., Maheswaran, R., Kingsley, S., Ignacimuthu, S., 2010. Bioefficacy of Couroupita guianensis (Aubl) against Helicoverpa armigera (Hub) (Lepidoptera: Noctuidae) larvae. Span. J. Agric. Res. 8, 135–141. | Baskar, K., Sasikumar, S., Muthu, C., Kingsley, S., Ignacimuthu, S., 2011. Bioefficacy of Aristolochia tagala Cham. Against Spodoptera litura Fab. (Lepidoptera:Noctuidae). Saudi J. Biol. Sci. 18, 23–27. | EPPO., Distribution maps of Quarantine Pests, Helicoverpa armigera, 2005.Available from: <www.eppo.org/QUARANTINE/insects/Helicoverpa_armigera/ HELIAR_map>. | Ignacimuthu, S., Maria Packiam, S., Pavunraj, M., Selvarani, N., 2006. Antifeedant activity of Sphaeranthus indicus L. against Spodoptera litura Fab. Entomon. 31, 41–44. | Jbilou, R., Amri, H., Bouayad, N., Ghailani, N., Ennabili, A., Sayah, F., 2008. Insecticidal effects of extracts of seven plant species on larval development, alpha-amylase activity and offspring production of Tribolium castaneum (Herbst) (Insecta: Coleoptera: Tenebrionidae). Bioresour. Technol. 99(5), 959–964. | Kamaraj, C., Rahuman, A.A., Bagavan, A., 2008.Screening for antifeedant and larvicidal activity of plant extracts against Helicoverpa armigera (Hübner), Sylepta derogata (F.) and Anopheles stephensi (Liston). Parasitol Res 103:1361–1368. | Kranthi, K.R., Kranthi, S., Ali, S., Banerjee. S.K., 2000. Resistance to Cry1Ac δ-endotoxin of Bacillus thuringiensis in a laboratory selected strain of Helicoverpa armigera (Hubner). Curr. Sci. 78, 1001-1004. | Minjas, J.N., Sarda, R.K., 1986. Laboratory observations on the toxicity of Swartzia madagascariens (leguminaceae) extract to mosquito larvae. Trans. R. Soc. Trop. Med. Hyg. 80, 460–461. | Muthu, C., Baskar, K., Kingsley, S., Ignacimuthu, S., 2010. Bioefficacy of Atalantia monophylla (L.) Correa. against Earias vittella Fab. J. Cent. Eur. Agric. 11, 23–26. | Raja, N., Jeyasankar, A., Jeyakumar, S.V., Ignacimuthu, S., 2005. Efficacy of Hyptis suaveolens against lepidopteran pest. Curr. Sci. 88, 220–222. | Santhoshkumar, T., Rahuman, A.A., Rajakumar, G., Marimuthu, S., Bagavan, A., Jayaseelan, C., Zahir, A.A., Elango, G., Kamaraj, C., 2011. Synthesis of silver nanoparticles using Nelumbo nucifera leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol. Res. 108, 693-702.