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Research Article

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Evaluation of *Bougainvillea glabra* (Nyctaginaceae) leaf extract and chitin synthesis inhibitor, flufenoxuron against *Spodoptera mauritia* BOISD. (Lepidoptera:Noctuidae): on larvicidal and pupicidal activity

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ABSTRACT

The present study was carried out to establish the properties of Bougainvillea glabra leaf extract and chitin synthesis inhibitor, flufenoxuron on larvicidal and pupicidal activity against the paddy army worm, Spodoptera mauritia. The methanol extract of B. glabra leaves showed larvicidal and pupicidal activity, after 24 h of exposure; against third- to sixth- instar larvae and pupae of S. mauritia, with obtained values of LD_{50}=5.340% in 3rd instar, 9.730 % in 4th instar,14.891% in 5th instar and 18.755 % in 6th and 21.468% in pupae respectively. The effect of chitin synthesis inhibitor, flufenoxuron againstthe third to sixth instar larvae and pupae with LD_{50} values in 3rd instar 2.088 %, 4th instar was 2.978 %, 5th instar was .4.37 %, and 6th instar was 5.946%, and pupae was .8.032 %, respectively. Moreover, combined treatment of the B. glabra and flufenoxuron LD_{50} values of 3rd instar was 0.638 %,4th instar was 1.571%, 5th instar was 2.475 %, and 6th instar was 4.768 %, and pupae was 8.266 %, respectively. The results showed the leaves extract of B. glabra and insect growth regulator, flufenoxuron are best choice for controlling Spodoptera mauritia. Hence, B. glabra and flufenoxuron can be considered for eco-friendly pest control programs.

Key words: *Bougainvillea glabra, Spodoptera mauritia, larvicidal, pupicidal.*

INTRODUCTION

Many noctuid moths are serious pests; among these species, the Rice swarming caterpillar or armyworm, *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae) is considered to be a sporadic pest which occasionally causes serious losses to rice crop. This insect is polyphagous and infests various graminaceous crops and weeds. Upland rice is its preferred host. For this reason, there is strong interest to find new methods for its control.

The recent control of intensive research is concerned mainly with avoiding the serious problems resulted from using harmful insecticides that cause harmful residues in the food chain and pollution of the surrounding natural enemies and pest resistance. Therefore, now it has become necessary to search for alternative means of pest control which can minimize the use of these synthetic chemicals[2]. The necessity to find environmentally safe insecticides as well as materials to combat species resistant to conventional pesticides has spurred increased interest in alternative insecticides such as use of plant extracts and insect

growth regulators (IGRs). IGRs are considered to have little human toxicity because humans do not make chitin and do not make or use the hormones insects use in moulting[17]. IGRs include JH mimics, Ecdysone mimics and Chitin Synthesis Inhibitors. The use of these compounds in insect control has considered as insect developmental inhibitor, which inhibits or prevents normal metamorphosis of immature stages to adult stages. Many of these compounds have been tested successfully against several insect species[4,14].

Botanical products are one of the most prominent alternatives for pest control strategy in current and future requirements. Recent study revealed some of the plant extracts shows anti-oxidant properties[15]. Survey on different plant families[7] since; the past two decades have promoted new sources for botanical insecticides that could possibly meet some of these demands. Many of these plant extracts have been shown to affect insect growth and behavior, acting asinsect growth regulators, antifeedants and toxicants[3]. Among the noted botanicals, *Bougainvillea glabra*are utilized as an effective natural dyeing agent and controlling sources to destroy the pests encountered in storage of rice. An added advantage, of these natural pest control agents are that they can be grown easily in the farmer's residential or cropping area. So, that the pesticides of natural origin that are economically viable and environmentally safe are easily available for the user[8,13]. Hence, in the present investigation an attempt has been made to evaluate the *B. Glabra* leaves extract and chitin synthesis inhibitor, flufenoxuron on the larvicidal, pupicidal activity of *Spodoptera mauritia.*

MATERIALS AND METHODS

Collection and maintenance of insect

The adult moths of *S. mauritia* were collected at night using fluorescent lights. They were kept in glass beakers covered with muslin cloth and were fed with a dilute solution of honey. They were allowed to lay eggs on the cloth. Larvae hatched out after 3-4 days. The larvae were reared in glass chimneys and were fed with fresh leaves of young paddy plants or leaves of the grass *Ischaemum aristatum.* When the larvae grew in size, they were kept in large plastic troughs with enough space for free movement. Care was taken to avoid extreme light and moisture as it may lead to mass death of the larvae. During dry season the cloth covering of troughs were wetted occasionally. The total larval period was found to range from17 to 19 days and consisted of 6 larval instars. The fully grown sixth instars larvae pupated. The pupae were kept in beakers for adult emergence. The female pupae took 7 days and the male pupae 8 days to moult into adults.

Collection and preparation of plant extract

B. glabra was collected from the calicut university campus, thenjippalam, malappuram, kerala, India. The plants were identified at Botany department (university of Calicut, then jippalam, malappuram, kerala, India. *B. Glabra* was washed with tap water and shade-dried at room temperature. The dried plant materials (leaves) were powdered (500 g) and were extracted with 1.5 litre of organic solvents of methanol using a soxhlet apparatus at 60–80°C for 8 hours (Vogel, 1978).The extract was concentrated under reduced pressure 22–26mm Hg at 45°C and the residue obtained was stored at 4°C.The extracts were filtered through a Buchner funnel with Whatman No. 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue were dissolved in 100 ml of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 5 to 25%, respectively.

Chemical compound: Flufenoxuron: (Pestanal) analytical grade

Chemicalname:N-[[[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2 fluorophenyl] amino]carbonyl]-2,6-difluorobenzamide.

Chemical (flufenoxuron) preparation

The chitin synthesis inhibitor, flufenoxuron used for the present study was purchased from Sigma Aldrich. The required quantity of flufenoxuron compound was diluted with acetone to prepare various concentrations and was adjusted to 0.1ppm, 2.5ppm, 5ppm, 10ppm respectively from working standard (10ppm).

Larval/pupal toxicity test

Laboratory colonies of *S. mauritia* larvae/pupae were used for the evaluation of larvicidal/pupicidal activity, after post treatment of the two tested compounds. Twenty-five individuals of 3rd to 6th instars larvae and pupae were topically treated with 5 µl of desired concentrations of plant extract, and flufenoxuron. Each tested concentration, was replicated thrice. The control was set up by administering 5µl of acetone. The larvae and pupae which were exposed without acetone served as vehicle. The control mortalities were corrected by using Abbott's formula[1]. The LD_{50} and LD_{90} were calculated from toxicity data by using probit analysis[6].

Statistical analysis

All data were subjected to analysis of variance; the means were separated using Duncan's Multiple Range Tests by (Alder and Rossler1977). SPSS (Statistical software package) 16.0 version was used. Results with $P<0.05$ were considered to be statistically significant.

RESULTS

Larval and pupal mortality of *S. mauritia,* was observed after the treatment of five concentrations (5%,10%,15%,20% &25%) of methanolic extract of *B. glabra* leaves. Forty percent mortality was noted in 3rd instar larvae by the treatment of *B. Glabra* at 5% concentration, whereas it has been noticed a gradual increase in the higher concentrations to *B. glabra* leaf extract treatment. Similar trend has been noted for all the instars of *S. mauritia* at different concentration of *B. Glabra* treatment (Table 1).In 4th instar larvae, after treatment to the given concentrations, it is observed 36 % mortality in the least concentration of 5% extract and following with a high rate of mortality in all respective concentrations of *B. glabra*. Similar observations are seen in 5th and 6th larval instars of *S. mauritia*. The treatment of 5%,10%,15%,20%,and 25% concentrations of *B. glabra* extract to pupae of *S. mauritia* exhibited 16,29,36,51 and 54 % mortality.

The present investigation also evaluated the effect of different concentrations (0.1,2.5,5.0,7.5 and 10ppm) of flufenoxuron on 3rd,4th,5th and 6th larval instars and pupae of *S. mauritia.* As depicted in (Table 2), fortyone percent mortality were observed in 3rd instar larvae after treatmentwith0.1ppm of flufenoxuron, whereas it has increased to 89% at 10ppm treatment. Similarly, 38% pupal mortality was evidenced in flufenoxuron treatment at 0.1ppm and it has been increased to 51% at10ppm. Same trend was also noted in all the instars of *S. mauritia* at different concentrations of flufenoxuron treatment (Table 2). The LD50and LD90 values were dose and time dependent.

The concentration at 5% *B. glabra* +0.1ppm flufenoxuron combination for 3rd instar larvae, mortality was recorded at 96% (Table 3). The LD50 value of 3rd instar was 3.73%, 4th instar was 4.72%, 5th instar was 5.55%, and 6th instar was7.66%. The LD90 values were also dose and time dependent.

Table:1 Effect of *Bougainvillea glabramethanol leaf extract against Spodoptera mauritia* on larval and pupal mortality rate

***S.mauritia* larval instars and pupae**	**% of larval and pupal mortality**					**LD50 (LD90)**	**95% confidence limit**		***x*2 (*df*= 3)**
	Concentration of *B. glabra* (%)						**LFL**	**UFL**	
	5	**10**	**15**	**20**	**25**		**LD50 (LD90)**	**LD50 (LD90)**	
3rdInstar	**40[a]**	**51[a]**	**60[a]**	**72[a]**	**89[a]**	5.340 (39.791)	8.041 (109.903)	10.074 (19.931)	1.04*
4thInstar	**36[ab]**	**44[ab]**	**53[b]**	**65[b]**	**76[b]**	9.730 (32.528)	6.668 (28.018)	11.900 (40.505)	1.33*
5thInstar	**32[bc]**	**39[b]**	**49[b]**	**62[b]**	**69[c]**	14.891 (40353)	12.523 (33.891)	17.238 (52.632)	1.49*
6th Instar	**27[cd]**	**34[c]**	**41[c]**	**53[c]**	**62[c]**	18.755 (46.249)	16.313 (38.011)	22.146 (62.938)	
Pupa	**16[d]**	**29[c]**	**36[d]**	**51[c]**	**54[d]**	21.468 (45.024)	19.109 (37.896)	25.023 (58.248)	1.947*

Control-Nil mortality, *LFL* = Lower Fiducidal Limit, *UFL* = Upper Fiducidal Limit, *x*2–*Chi*-square value, *df*- degrees of freedom, Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. *Significant at $P < 0.05$ level.

Table:2 Effect of flufenoxuron *against Spodoptera mauritia* on larval and pupal mortality rate

larval instars and pupae	**% of larval and pupal mortality**					**LD50(LD90)**	**95% confidence limit**		***x*2 (*df*= 3)**
	Concentration of flufenoxuron(ppm)						**LFL**	**UFL**	
	0.1	2.5	5	7.5	10		**LD50(LD90)**	**LD50(LD90)**	
3rd Instar	41[a]	54[a]	61[a]	74[a]	89[a]	2.088 (11.610)	0.784 (9.959)	3.040 (14.277)	2.337*
4th Instar	38[ab]	50[ab]	56[ab]	68[b]	78[b]	2.978 (32.528)	1.429 (28.018)	4.104 (40.505)	0.380*
5thInstar	34b[c]	46[b]	50[c]	61[c]	74[c]	4.379 (18.521)	2.948 (14.800)	5.637 (26.202)	0.562*
6th Instar	30[cd]	38[c]	49bc	56[c]	66[d]	5.946 (20.989)	4.622 (16.482)	7.557 (30.809)	0.365*
Pupa	25[d]	34[c]	42[d]	50[d]	56[e]	8.032 (24.354)	6.501 (18.625)	10.731 (37.958)	0.55*

Control-Nil mortality, *LFL* = Lower Fiducidal Limit, *UFL* = Upper Fiducidal Limit, *x*2–*Chi*-square value, *df*- degrees of freedom, Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. *Significant at $P < 0.05$ level.

Table:3 Effect of flufenoxuron & *Bougainvillea glabramethanol leaf extract against Spodoptera mauritia* on larval and pupal mortality

larval instars and pupae	% of larval and pupal mortality					LD50 (LD90)	95% confidence limit		*x*2 (*df*= 3)
							LFL	UFL	
	Concentration of flufenoxuron (ppm) + *B. glabra* (%)						LD50 (LD90)	LD50 (LD90)	
	0.1+5	2.5+10	5+15	7.5+20	10+25				
3rd Instar	49[a]	63[a]	73[a]	84[a]	98[a]	0.638 (8.259)	0.688 (7.188)	0.688 (9.836)	4.910*
4th Instar	44[b]	52[b]	68[b]	78[b]	86[b]	1.571 (11.54)	0.058 (9.841)	2.620 (14.352)	0.446*
5thInstar	41[b]	50[b]	58[c]	72[c]	81[c]	2.475 (13.918)	0.954 (11.621)	3.556 (18.013)	0.587*
6th Instar	30[c]	42[c]	50[d]	63[d]	70[d]	4.768 (16.853)	3.625 (13.908)	5.863 (22.287)	0.344*
Pupa	26[d]	32[d]	47[d]	56[e]	62[e]	8.266 (24.354)	14.085 (14.992)	5.054 (23.652)	01.280*

Control-Nil mortality, *LFL* = Lower Fiducidal Limit, *UFL* = Upper Fiducidal Limit, *x*2–*Chi*-square value, *df*- degrees of freedom, Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. *Significant at $P < 0.05$ level.

DISCUSSION

The available literature on the effects of different botanical extracts on different life stages of mosquitoes are plenty and many of these biopesticides exhibited larvicidal and pupicidal activity. But, studies on the botanicals against lepidopteran, are very much limited. Therefore, the present study focused the efficacy of the *Bougainvillea glabra* extract on the rice swarming caterpillar, *Spodoptera mauritia.* From the data obtained of this study, it is evidenced that the two compounds treated to different larval instars and pupae of *S. mauritia* exhibited larvicidal and pupicidal activity. Similar report is observed in ethanolic extracts from *M. citrifolia* plant and entomo pathogenic fungi *Metarhizium anisopliae,* exhibiting larvicidal and pupicidal activity against malaria vector, *Anopheles stephensi*[9]. The results of the present study are in agreement with the recent studies of botanical extract from eight plant species against *Hyblaea puera*[18]. In the present report we foundthe larval regulation in growth and development of major lepidopteran pest *S. mauritia* by methanolic extract of *B. glabra* leavesand chitin synthesis inhibitor flufenoxuron by topical application. The reduction in the life of larval and pupal weight in the present study might bedue to the treatment of compounds, affecting the physiological status. *Bougainvillea glabra* flower extract is known as an effective natural dyeing agent and act as a controlling agent to destroy the pests encountered in storage of rice[13]. Kalirajan *et al.,*[10] showed the flower extract of *Bougainvillea glabra* have potential to be a natural colouring agent and also considered as biopesticide. As it showed its potentiality, the flowers of this plant are an effective biopestides to destroy the insects often encountered from agricultural origin in the days to come. So that the pesticides of natural origin that are economically viable and environmentally safe are easily available for the user.

The toxicity of chitin synthesis inhibitor, flufenoxuron increased with concentration in the development stages of *S, mauritia,* showing larvicidal and pupicipal activity. On the basis of these data obtained, the highest concentration applied to the three development stages and pupae of *S. mauritia* were detrimental to the experimental specimen. The findings of our results are in accordance to the studies obtained by Reda, F.A. Bakr *et al*.,[5]. Recent observation of Khatter, N.A.,[11] provedlarvicidal,pupicidal and adulticidal

effect of two insect growth regulators flufenoxuron and juvenile hormone analogue methoprene against 3rd instar of *Agrotis ipsilon*.

The result of the present study revealed the larvicidal and pupicidal activity due to the insect growth regulator, flufenoxuron, in combination with the methanolic extract of *Bougainvillea glabra* showed its response with time and dose dependent manner. Further, it shows high mortality rate, in combined application of the two compounds, in treated larvae and pupae of *S. mauritia* rather in separate treatment of the compounds.

CONCLUSION

From the above study, it is been concluded, that the two compounds, *B. glabra*, flufenoxuron and their combinations have proved to be larvicidal and pupicidal activity in *S. mauritia*. The study also shows that the treatment of these compounds to *S. mauritia* exhibited dose dependent response in both larval instars and pupal stage.

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