Entomology and Applied Science Letters
Volume 5, Issue 4, Page No: 112-118

Available Online at: www.easletters.com

ISSN No: 2349-2864

Possible Chitin Inhibitor Effects of *Melilotus Indicus* (L.) Extraction on *Spodoptera Littoralis* (Boisduval) Under Laboratory Conditions

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ABSTRACT

Yellow sweet-clover, Melilotus indicus (L.), is known for its medicinal and ethnomedicinal uses. A very few studies have focused on the efficacy of it on pest control. Herein, the biological effects of sublethal concentrations of M. indicus extract in comparison with three insect growth regulators (IGRs), novaluron, lufenuron, and diflubenzuron against the life cycle of Spodoptera littoralis (Boisd.) were evaluated under laboratory conditions. All tested compounds, especially M. indicus, prolonged the duration of the larval and pupal stages comparing to that of the untreated control. The emerged adult percentage was highly reduced by 91.35%, 78.96%, 77.33%, and 62.46% after larval feeding with the higher concentration of M. indicus extract, diflubenzuron, novaluron, and lufenuron; respectively. M. indicus extract and diflubenzuron caused high increment in malformation in larvae, pupa, and adults. The efforts should be implemented to get better understanding about the molecular basis of its possible unknown mode of action on growth hormones to be continued in the future.

Keywords: *Melilotus indicus,* insect growth regulators (IGRs), *Spodoptera littoralis*, botanical pesticides, integrated pest management (IPM).

HOW TO CITE THIS ARTICLE: Mohamed Ahmed Ibrahim Ahmed, Ahmed Mahmoud Mohamed Ahmed, Ah-med Mohamed Abdel-Rahman Mohamed Amro; Possible Chitin Inhibitor Effects of *Melilotus Indicus* (L.) Extraction on *Spodoptera Littoralis* (Boisduval) Under Laboratory Conditions, Entomol Appl Sci Lett, 2018, 5 (4):112-118.

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Received: 14/08/2018
Accepted: 07/12/2018

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisduval), is considered a devastating insect pest in Egypt which diminishes the quality and quantity of the cotton production and the yield as well [1-5]. However, the frequent use of conventional insecticides results in the appearance of insecticide resistance issues and environmental complications. Therefore, inspecting a contemporary method for controlling *S. littoralis* can be considered substantial [6, 7].

Interestingly, the application of botanical pesticides for crop protection and insect pests control has been paramount. However, numerous researchers have experimented and developed plant extracts as pesticide alternatives to be used against insects [8-10].

Generally, the plant kingdom includes about half-million plant species, which can produce about 30,000 secondary metabolites [11]. The production of these secondary natural chemicals is mainly related to the presence of molecules and activation of special genes [12].

Chemically, these compounds have been classified into five main categories, nitrogen compounds, terpenoids, phenolics, proteinase inhibitors, and regulators of the growth [13]. Plants play pivotal roles in ecological systems [14]. Plants' active byproducts fall into several categories, including feeding deterrents, growth regulators, toxins, and repellents.

Some of the mentioned compounds have been produced for the purpose of preventing attack from phytophagous (plant eating) insects. Thus, they provide alternatives to act as insecticides

[15]. Also, many of them are efficient to control mosquitoes and biting insects related to Diptera, besides the volatile components which release due to an action of consequence herbivory [16]. Essential oils are among the best-known substances tested against insects [17].

These compounds play the role of fumigants [18], insecticides by contact [19], showing repellent effects [20] and act as anti-feedants [21], and might affect the parameters of biological control like the rates of growth [22], life span and reproduction [23]. *M. indicus* is a naturally-grown annual herb as a weed of cultivations in urban gardens of Egypt.

M. indicus is also found in Asia, Europe and throughout Arabia, and has been introduced to many countries around the world [24]. Actually, Coumarin, herniarin, umbelliferene, and scopoletin have been identified in *M. indicus* plant. Additionally, the presence of â-sitosterol, a sterol or triterpene alcohol, choline and an aromatic compound have also been reported [25].

In this study, the biological effects of sublethal concentrations of *M. indicus* extract were assessed in comparison with three insect growth regulators (IGRs), novaluron, lufenuron, and diflubenzuron against the life cycle of *S. littoralis* under laboratory conditions.

MATERIALS AND METHODS

Spodoptera littoralis (Boisd.)

A laboratory susceptible strain of *S. littoralis* was reared in the laboratory of Plant Protection Department Research building, Faculty of Agriculture, Assiut University. The rearing regime was described before [1, 26].

Compounds

Novaluron (99.6%), lufenuron (99.7%), and diflubenzuron (98.1%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Preparation of M. indicus extracts

M. indicus leaves, stems and fruits were washed thoroughly with distilled water, after that dried in the shade for 3 days at room temperature. The dried tissues were uniformly ground. Three hundred grams of the dry powder were extracted for 4 days in 1 Liter of absolute methanol [27].

The Whatman No. 1 filter paper was used to filter the separated extract, and evaporated till dryness by using the rotary evaporator at 40 °C. The thick extracted mass was then dried at room temperature, and dissolved in 200 ml of distilled water, then stored in a refrigerator at 4 °C to be ready for the experimental use.

Biological bioassay

The effects of tested compounds on certain biological parameters of *S. littoralis* were determined using three sub-lethal concentrations for each compound. The treated instar and its subsequent developmental stages were determined as follows: twenty newly molted 4th instar larvae of *S. littoralis* were used for each compound. Three replicates were tested for each compound; twenty larvae in each replicate were fed on castor bean, *Ricinus communis'* leaves were treated with different concentrations. In control test, the leaves were treated with distilled water only. All larvae were allowed to feed for 24-h on the treated leaves, then endorsed to complete their life-cycle on fresh leaves. Some biological aspects such as: larval mortality percentage, larval duration, pupation percentage, pupal mortality, pupal duration, emerged moth percentage, and total inhibition of adult emergence were determined.

Statistically analysis

All the obtained experimental data were statistically analyzed using *t*-test ($P<0.05$) by using IBM SPSS statistics 24 program (SPSS Inc., Chicago, IL, USA).

RESULTS

The results in (Table 1) showed the sublethal effects of the tested compounds on certain biological parameters on 4th larval instar of *S. littoralis*.

Larval stage

Significantly, all compounds, protracted the duration of the larval stage than that of the untreated control (Table 1). The data implied that the larval duration was 10.92, 11.82, 12.12, and 10.13 days at the lowest tested concentrations, respectively. However, the larval duration was 15.61, 14.92, 14.23, and 13.40 days for *M. indicus* extract, novaluron, lufenuron, and diflubenzuron with the higher concentrations; respectively. It appeared that larval span was correlated with the compound concentration.

Further, the percentage of larval mortality had raised by increasing the concentration. Data in (Tables 1) revealed that the *M. indicus* extract

was proved to be the most effective compound at the highest tested concentrations (22.22%) followed by novaluron (17.68%), diflubenzuron (12.75%), and lufenuron (7.75%). However, little changes were shown at the lowest tested concentrations of *M. indicus* extract (3.43%), followed by lufenuron (2.91%), diflubenzuron (2.07%), and novaluron (1.97%); respectively.

Pupal stage

The pupal duration was impressed in all treatments compared with the untreated control (Table 1). However, all tested compounds protracted the pupal durations which were 16.93, 16.82, 14.58, and 11.82 days at the highest concentrations compared to the untreated control for *M. indicus* extract, novaluron, diflubenzuron, and lufenuron; respectively. These results may be due to the delaying of molting process.

Table 1. Effect of selected compounds on certain biological parameters on 4th instar larvae of *Spodoptera littoralis.*

Compounds	Conc. (µg/ml)	Larval stage		Pupal stage			Adult stage
		Mortality %	Mean duration (days) ± S.E.	Pupation %	Mortality %	Mean duration (days) ± S.E.	Emerged adult%
Control	-	0.0	9.07 ± 0.41^{a*}	100	0.0	6.14 ± 0.65^{a}	100
M. indicus extract	10	3.43	10.92 ± 0.31^{a}	96.57	7.87	11.67 ± 0.62^{b}	30.73
	50	19.71	11.06 ± 0.52^{b}	80.29	12.16	12.58 ± 0.50^{b}	15.42
	100	22.22	15.61 ± 0.43^{c}	77.78	17.89	16.93 ± 0.71^{c}	8.65
Novaluron	10	1.97	11.82 ± 0.21^{b}	98.03	9.22	12.99 ± 0.34^{b}	29.76
	50	14.08	12.23 ± 0.73^{b}	85.92	20.93	14.02 ± 0.41^{c}	23.62
	100	17.68	14.92 ± 0.84^{c}	82.32	27.11	16.82 ± 0.58^{c}	22.67
Lufenuron	10	2.91	12.12 ± 0.65^{a}	97.09	3.58	8.04 ± 0.62^{a}	42.79
	50	5.52	13.80 ± 0.72^{b}	94.48	5.72	9.64 ± 0.67^{b}	39.07
	100	7.75	14.23 ± 0.68^{b}	92.25	7.59	11.82 ± 0.96^{b}	37.54
Diflubenzuron	10	2.07	10.13 ± 0.54^{b}	97.93	9.98	11.85 ± 0.39^{b}	30.09
	50	10.88	11.47 ± 0.61^{b}	89.12	18.22	13.96 ± 0.53^{c}	25.78
	100	12.75	13.40 ± 0.69^{c}	87.25	24.17	14.58 ± 0.71^{c}	21.04

* The same letter within the same column for each compounds is not significantly different with $P \leq 0.05$ at each stage

Additionally, in case of pupae resulted from 4th instar larvae treated at lower concentrations, there was an increase in pupation percentage (98.03%, 97.93%, 97.09%, and 96.57% for novaluron, diflubenzuron, lufenuron, and *M. indicus* extract, respectively). However, at higher concentrations, there was a decrease in pupation percentage (92.25%, 87.25%, 82.32%, and 77.78% for lufenuron, diflubenzuron, novaluron, and *M. indicus* extract; respectively).

The pupal mortality had intensified by increasing the concentration for tested compound. The novaluron proved to be the most potent compound at the highest tested concentrations (27.11%) followed by diflubenzuron (24.17%), *M. indicus* extract (17.89%), and lufenuron (7.59%);

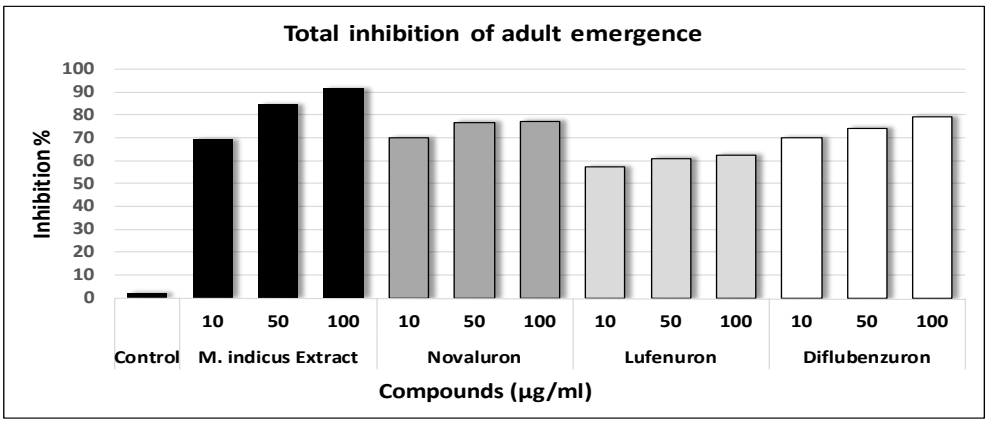

Figure 1. Total inhibition (expressed in percentage) of the adult emergence after the treatments with different concentrations of selected compounds.

Table 2. Types of different malformations as effected by tested chitin synthesis inhibitors on 4th instar larvae of *Spodoptera littoralis.*

Types of malformations		Compounds			
		M. indicus extract	Novaluron	Lufenuron	Diflubenzuron
Larvae	Larvae could not able to discard the old cuticle	+	+	+	+
	The production of abnormal chitin deposition	+	+	+	+
	Swollen of the posterior part of larvae abdomen	-	-	-	+
	Extrusion of molting fluid from treated larvae	+	+	+	+
Pupa	Pupal-larval intermediates	+	+	+	+
Adult	Adults adhering to pupal exuvium	+	-	-	-
	Adults with malformed wings	+	-	-	+

respectively. In contrast, this trend was slightly changed at the lowest tested concentrations to be diflubenzuron (9.98%), followed by novaluron (9.22%), *M. indicus* extract (7.87%), and lufenuron (3.58%).

Adult stage

(Tables 1 and figure 1) display the percentages of adult emergence and the total inhibition of adult emergence. However, the emerged adult percentage was highly reduced by 91.35%, 78.96%, 77.33%, and 62.46% after larval feeding with the higher concentration of *M. indicus* extract, diflubenzuron, novaluron, and lufenuron; respectively.

The data in (Table 2 and figure 2) exhibited that the use of *M. indicus* extract and diflubenzuron highly increased malformation especially in larvae, pupa, and adults. Interestingly, the types of different malformations as affected by tested compounds on 4th larval instar of *S. littoralis* were increased by increasing the concentrations.

Figure 2. Types of malformations (A) larvae; (B) pupae, and (C) adult, resulted from the application of *M. inidcus* on *Spodoptera littoralis.*

DISCUSSION

In general, plant extracts have long been attempted to be attractive alternatives for manufacturing conventional pesticides for pest management. However, the plentiful of plant extracts have been considered to be used in pest control such as antifeedants, repellents, and toxicants, but little commercial success has been ensued for plant extracts that interrupt insect physiology. In this study, the light was shed on the possible chitin inhibitor effects of *M. indicus* extract on *S. littoralis.* The studies that focused on the effects of *M. indicus* extract in pest control are lacking.

In agreement with the findings of the present study, Hatem et al., (2011) [28] revealed that larval duration was significantly prolonged compared to the control (16.05 days) when they continuously fed the 4th larval instar of *S. littoralis* on the untreated fresh leaves of the tested weeds using *M. indicus* as a host plant. In the same trend, El-zoghby et al., (2011) [29] found that there was a potent effect of *M. indica* petroleum ether extract on sperms of the 6th instar larvae of *S. littoralis* as an evident reduction in the numbers of the eupyrene sperm bundles, the absence of axoneme, and the presence of more than reticular appendages and non-differentiated sperms which affected the life cycle of *S. littoralis*. Pavela & Chermenskaya, (2004) [30] demonstrated that *M. indicus* indicated an anti-feedant property when tested on 3rd instar larvae of *S. littoralis.*

Since *M. indicus* represented the possible chitin inhibitor effects on 4th instar larvae of *S. littoralis* based on the present study, the reasons could be due to the elevation of the levels of carbohydrate hydrolyzing enzymes, for instance, chitinase, lactic dehydrogenase (LDH), acid & alkaline phosphatases, and/or trehalase enzyme. Furthermore, trehalase enzyme plays a critical role in the creation of the glucose for chitin construction. This process has been considered as a necessary physiological process so that the inhibition of carbohydrate hydrolyzing and trehalase enzyme may cause the malformations according to this study [31, 32]. Moreover, protease and chitinase enzymes that assimilated the essential constituents of the old endocuticle have been responsible for the processing of separate epidermal cells from the old cuticle by molting fluid secretion and ecdysal membrane formation which are mainly affected. Thus, the disturbance of the chitinase and protease enzymes that caused by *M. indicus* extract was supposedly the reason of the malformations [33, 34].

CONCLUSION

To sum up, *M. indicus* extract represented prospective chitin inhibitor effects on the specific biological parameters on 4th larval instar of *S. littoralis*. Certain assessment should be carried out to focus on the possible effects of *M. indicus* extract on major hormones that are involved in molting processing such as ecdysone, 20-hydroxy-ecdysone (molting hormones), and juvenile hormone. However, it is very important to associate *M. indicus* extract in the integrated pest management (IPM) and integrated resistance management (IRM) programs because it is less harmful to the environmental aspects, and it has a more compatible pest control system.

Compliance with ethical standards

Conflict of interest: The authors declared that they had no conflict of interest.

Human and animal rights

This article does not contain any studies with human participants or animals performed by any of the authors.

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