Research Article

Bioassay comparative dose efficacy of Lufenuron on the biology of *Spodoptera litura* (F.) on cauliflower

Hakim Ali Sahito^{1*}, Mumtaz Hussain Mahar¹, Bhugro Mal², Tasneem Kousar¹, Abdul Qadir Malik³ and Wali Muhammad Mangrio¹

1. Shah Abdul Latif University, Khairpur Mir's, (66111) Sindh-Pakistan

2. Arid Zone Agriculture Research Institute PARC, (75270) Karachi-Sindh-Pakistan

3. Entomologist, District Health Office, ICT, (45500) Islamabad-Pakistan

*Corresponding author's email: hakim.sahito@salu.edu.pk

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Abstract

The common cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), was first described by Johan Christian Fabricius in 1775, is a nocturnal moth, polyphagous in nature, destructive insect pest, damaging economically important crops, and decreasing the yield completely. The research was conducted to control through sub-lethal doses of Lufenuron under laboratory conditions where mortality was determined. The results indicated that the Lufenuron treatment caused mortality, reduction in larval and pupal weight. The copulation time, fecundity, and fertility decreased as compared to control. The maximum mortality was found on dose one after one week and minimum on dose five after 24 hours. The maximum larval weight reductions were recorded in dose one after one week, and dose three after 24 hours with the longest larval and pupal period in dose one and the shortest in dose five as compared to control. The maximum pupal weight reduction was recorded in dose one and minimum in dose five as the shortest adult longevity of *S. litura* in dose one and the longest in dose five thus; the longest duration of life-cycle in dose one and the shortest in dose five. The shortest copulation time in dose one and the highest in dose five with the minimum fecundity found in dose one and maximum in dose five whereas; minimum fertility of eggs in dose one and maximum in dose five as compared to control. It is concluded that the Lufenuron (IGR) is effective by their sub-lethal concentrations on *S. litura* however, the most preference can be given to which caused highly sub-lethal effects on its biology.

Keywords: Cauliflower; Different doses; Lufenuron; Laboratory conditions; *S. litura*

Introduction

Cauliflower, a member of the family Brassicaceae and about 2,000 years ago originated in the Mediterranean and the oldest as 6th BC. Most of the European

scholars documented that during the 16th century in Egypt and Turkey cauliflower contribute positively to human health because of its high glucosinolate content [1]. Cauliflower is the most important vegetable,

and Pakistan ranked the top ten cauliflower producing country with 13.6 thousand harvested areas, 234.4 thousand tons of production [2]. As far as the nutritional values are concerned the 100gm piece of cauliflower contains calories 25 to 30 gm, moisture 90.8gm, carbohydrate 5gm, protein, and dietary fiber 2gm, calcium 33mg, magnesium 20mg, sodium 53mg, potassium 113mg, oxalic acid 19mg, vitamin A, vitamin C, and other nutrient essentials [3]. This vegetable contains a high content of folate water, dietary fiber, and vitamin C, and fewer amount carbohydrates but enriches with nutritional value and helpful for weight loss. The cauliflower production is produced about 212.12 thousand tons in Pakistan and ranks 8th position but in the province of Sindh contributes 13.314 thousand tons [4].

The cauliflower is attacked by as many insect pests, many diseases, and disorders [5]. But *S. litura* (F) is a destructive pest and this pest insect widely found in sub-tropical and tropical parts of the world but frequently in China, Pakistan, India harming about one hundred and fifty varieties of host plants of which forty varieties are reported from India [6]. The tobacco caterpillar is considered the most harmful pest in the rainy season and caused yield reduction [5]. Most of the larvae of the pest insects are destructive to their host plants [7] and frequently lay the eggs on the surface of the leaves [8]. Same way, larvae of this pest insect feed plant leaves later on almost parts of the host plants [5]. Thus; [9] used Lufenuron insecticide on the larval instars of *S. litura* in cauliflower. They used the dip bioassay method under laboratory conditions in which the results showed that high LC₅₀ values for limited time exposure might be due to its slow-acting as a chitin synthesis inhibitor.

The tobacco caterpillar is a pest insect of several valuable crops including, groundnuts, maize, cucurbits, cruciferous

vegetables, potato, tobacco, and soybean. At least, 128 countries reported with the most universally dispersed of all Lepidoptera [5]. The insect growth regulators were first used in 1956 to control the insect population through interference in their reproduction or disturbance in metamorphosis [10]. The insect growth regulators are chemical insecticides which are chitin synthesis inhibitors by which the analogs of juvenile hormones interfere with the molting process through which insects can't grow [11]. The population of pests' can be controlled by the use of insect growth regulators through them cause genitalia abnormality, then mating process inhibited and fertile offspring do not produce [12].

The pest population encounters sub-lethal concentrations of pesticides and many biological and behavioral abnormalities found in insect populations occur due to sub-lethal doses of insecticides as a reduction in feeding and growth rate, adult fecundity, fertility, longevity, abnormal behavior and sometimes increased pupal weight [13]. By the application of different insecticides controlled two major pests [9]. The toxicity and field resistance effects of Lufenuron under laboratory conditions on the second and fourth larvae of the Tobacco caterpillar and documented that this insecticide is meaningful on the second and fourth larval [14]. In cutworm [15] reported the impacts of sub-lethal concentrations at 0.1, 0.5, and 1.2 µg mL⁻¹ of chitin synthesis inhibitors on larval development but dose-dependent effects of sub-lethal concentration found on third-stage larvae which subjected in length and weight reduction in instars, pupae, adult and a maximum number of the larvae died when they reach at molting stage [15]. The present scientific work was performed to find out the proper bioassay competence of Lufenuron against Tobacco caterpillar on cauliflower vegetable.

Materials and Methods

Collection of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) from field conditions

The larvae of tobacco cutworm, *S. litura* were collected from the cauliflower farmer field condition which was cultivated under date palm tree orchards located at district Khairpur and brought under Entomology laboratory, DPRI, SALU-Khairpur in the year, 2019 and kept at a constant room temperature of $25\pm 2^{\circ}\text{C}$ and relative humidity 50-60%. The 4th stage larvae of *S. litura* were collected from culture to observe the sub-lethal effect of Lufenuron. The experiment was initiated with 600 number of 4th instar larvae. These larvae were treated with Lufenuron at five variable doses. The experiment was comprised of four treatments with five concentrations.

Experimental design under laboratory conditions

There were five concentrations (sub-lethal doses) of insect growth regulators Lufenuron Dose: (100 ppm) (75 ppm) (50 ppm) (40ppm) (25 ppm) dissolve in one liter water. Lufenuron 5% EC Dose: 1680, 1440, 1200, 960, and 720 ppm dissolved in one-liter water. Each treatment concentration of dissolved insecticide was replicated three times for further confirmation. Lufenuron solution was prepared in the bucket and fresh cauliflower leaves of standard size were dipped into each concentration for up to 15 seconds, later on, those were kept to be smoothly dried in laboratory corridor shade. Treated food was given to ten larvae of each concentration of 4th instar to feed. After 48 hours of post-treatment, normal untreated food was provided to the surviving larvae. Treated surviving larvae were kept in plastic bottles with moist soil for pupation when the adults emerged out, they were transferred into emerging cages for egg-laying, during that period 40% honey solution immersed cotton role was provided for moths feed and

replaced daily until insects were alive. The freshly laid eggs were counted and transferred into Petri dishes having blotting papers. Fresh food was provided to the newly hatched larvae daily. The observation was recorded on surviving insects after the treatment with sub-lethal effects of neem oil and recorded the mortality, duration of the life cycle, larval growth, pupal weight, pupal period, sex ratio, copulation time, fecundity, and fertility.

Data analysis

The percentage formulation was calculated using numbers of reduced insect stages (larval mortality and larval weight reduction) divided by total numbers of insects *S. litura* that emerged successfully. The larval populations were compared by analysis of variance and means were separated by LSD test ($P=0.01$). Finally, the mean numbers of tobacco cutworms were calculated and statistically analyzed through the statistics software, 8.1 USA versions.

Results

This work was performed to estimate the bioassay dose efficacy of Lufenuron on the biology of *S. litura* i.e., larval mortality, larval weight, larval period, pupal period, pupal weight, adult longevity, total life period, sex ratio, copulation time, fecundity, and fertility under laboratory conditions. The mortality percentage after 24 hours varied significantly ($F= 8.30$; $DF=4, 24$; $P=0.002$) after the application of Lufenuron at different doses. At 1st dose, the highest mortality (30.00) percent was recorded at 75 ppm followed by (26.67 at 100 ppm), (10.00 at 50 ppm), (10.00 at 40 ppm), and (3.33 at 25 ppm), respectively. At dose two, the highest percent mortality after 48 hours was recorded at (40.00 at 100 ppm) followed by (36.67 at 75 ppm), (30.00 at 50 ppm), (16.67 at 40 ppm), and (13.33 at 25 ppm) in which all treatments found with a significant difference. The highest mortality percentage after 72 hours was recorded after application

at dose three (53.33 at 100 ppm) followed by (40.00 at 75 ppm), (36.67 at 50 ppm), (26.67 at 40 ppm) and (13.33 at 25 ppm), subsequently; all treatments with a significant difference. At dose four, the maximum mortality percentage after 96 hours was recorded after application (73.33 at 100 ppm) followed by (46.67 at 50 ppm), (43.33 at 75 ppm), (43.33 at 40 ppm), and (26.67 at 25 ppm) found all treatments with a significant difference. The highest mortality percentage after one week was recorded after application at dose five (86.67 at 100 ppm) followed by (73.33 at 75 ppm), (46.67 at 50 ppm), (46.67 at 40 ppm), and (33.33 at 25 ppm). All treatments were observed with a significant difference.

The larval weight reduction percentage after 24 hours of *S. litura* varied non-significantly ($F= 0.56$; $DF=4, 24$; $P=0.6477$) after the application of insecticide. At dose one, the highest larval weight reduction percentage was recorded (27.62 at 100 ppm) followed by (30.96 at 75 ppm), (13.76 at 40 ppm), (12.64 at 50 ppm), and (6.45 at 25 ppm) where all treatments with significantly dissimilar to each other. At dose two, the highest larval weight reduction percentage after 48 hours was recorded (41.00 at 100 ppm) followed by (38.68 at 75 ppm), (32.93 at 50 ppm), (19.59 at 40 ppm), and (18.73 at 25 ppm) found significantly different. The highest larval weight reduction percentage after 72 hours was recorded after application of dose three (50.42 at 100 ppm) followed by (46.62 at 75 ppm), (40.32 at 50 ppm), (27.88 at 40 ppm), and (22.87 at 25 ppm) with significantly different. At dose four, the mortality percentage, all treatments were significantly different from each other. The maximum larval weight reduction percentage after 96 hours was recorded after application of (76.11 at 100 ppm) followed by (71.33 at 75 ppm), (53.28 at 50 ppm), (46.92 at 40 ppm), and (37.86 at 25 ppm). The highest larval weight reduction

percentage after one week was recorded after application at dose five (77.85 at 100 ppm) followed by (77.61 at 75 ppm), (57.66 at 50 ppm), (51.54 at 40 ppm) and (45.01 at 25 ppm) as described in under given (Table 1).

The larval period of *S. litura* varied significantly ($F= 264.71$; $DF=5, 35$; $P=0.002$) after the application of insecticides (Table 2). The maximum larval period was recorded in (28.83 at 100 ppm) followed by (27.67 at 75 ppm), (26.23 at 50 ppm), (24.90 at 40 ppm), (23.77 at 25 ppm), and control (14.23 at 00 ppm). The pupal period varied significantly ($F= 78.73$; $DF=5, 17$; $P=0.002$) after application of insecticide. The highest pupal period was recorded in (14.66 at 100 ppm) followed by (12.66 at 75 ppm), (11.800 at 50 ppm), (10.36 at 40 ppm), (8.76 at 25 ppm), and control (6.80 at 00 ppm). The pupal weight was also significantly different ($F= 209.96$; $DF=5, 17$; $P=0.002$) after the application of insecticide. The pupal weight was highly reduced (46.83 at 100 ppm) followed by (43.33 at 75 ppm), (41.03 at 50 ppm), (38.27 at 40 ppm), (35.53 at 25 ppm), and control (24.03 at 00 ppm). The developmental time was also found significantly different ($F= 210.86$; $DF=5, 17$, $P=0.002$) after the application of insecticide. The maximum developmental time was recorded in (46.83 at 100 ppm) followed by (43.33 at 75 ppm), (41.03 at 50 ppm), (38.27 at 40 ppm), (35.53 at 25 ppm), and control (24.03 at 00 ppm). The adult longevity was also found significantly different ($F= 41.27$; $DF=5, 17$; $P=0.002$) after the application of insecticide. The minimum longevity of *S. litura* was recorded in (6.63 at 75 ppm) followed by (6.93 at 100 ppm), (8.13 at 50 ppm), (9.533 at 40 ppm), (9.53 at 25 ppm), and control (11.83 at 00 ppm). The total life period was also found significantly different ($F= 223.73$; $DF=5, 17$; $P=0.002$) after the application of insecticide. The highest total

life period was recorded in (53.73 at 100 ppm) followed by (49.967 at 75 ppm), (49.16 at 50 ppm), (47.46 at 40 ppm), (45.03 at 25 ppm), and control (35.86 at 00 ppm), respectively.

Through the different treatment dose of Lufenuron male with an average found more susceptible at 0, 3, 4, 5, 8 and when

compared with control at 10 while as; female at 4, 5, 10, 11, 11, 20 and average with sex ratio, 0:4, 3:5, 1:2.5, 5:11, 8:11 and when compared with control as described at an average 1:2 of different doses, respectively, further the descriptions are given in (Table 3).

Table 1. Percentage larval mortality and weight reduction of *S. litura* (F.) after 24, 48, 72, 96 hours, and one week treated with Lufenuron

Treatment	Dose	Concentration in ppm	(%) Larval mortality	(%) Larval weight reduction
24 hours	D1	100	26.67	27.62
	D2	75	30.00	30.96
	D3	50	10.00	12.64
	D4	40	10.00	13.76
	D5	25	3.33	6.45
48 hours	D1	100	40.00	41.00
	D2	75	36.67	38.68
	D3	50	30.00	32.93
	D4	40	16.67	19.59
	D5	25	13.33	18.73
72 hours	D1	100	53.33	50.42
	D2	75	40.00	46.62
	D3	50	36.67	40.32
	D4	40	26.67	27.88
	D5	25	13.33	22.87
96 hours	D1	100	73.33	76.11
	D2	75	43.33	71.33
	D3	50	46.67	53.28
	D4	40	43.33	46.92
	D5	25	26.67	37.86
One week	D1	100	86.67	77.85
	D2	75	73.33	77.61
	D3	50	46.67	57.66
	D4	40	46.67	51.54
	D5	25	33.33	45.01
Control	Distal water	00	0.00	0.00

Table 2. Larval period, pupal period, pupal weight, development time, adult longevity, and total life period of *S. litura* (F.) after 24, 48, 72, 96 hours, and one week treated with Lufenuron

Treatment	Dose	Concentration in ppm	(%) Larval weight reduction
Larval period	D1	100	28.83
	D2	75	27.67
	D3	50	26.23
	D4	40	24.90
	D5	25	23.77
Control	Distal water	00	14.23
Pupal period	D1	100	14.66
	D2	75	12.66
	D3	50	11.80
	D4	40	10.36
	D5	25	8.76
Control	Distal water	00	6.80
Pupal weight	D1	100	46.83
	D2	75	43.33
	D3	50	41.03
	D4	40	38.27
	D5	25	35.53
Control	Distal water	00	24.03
Development time	D1	100	46.83
	D2	75	43.33
	D3	50	41.03
	D4	40	38.27
	D5	25	35.53
Control	Distal water	00	24.03
Adult longevity	D1	100	6.93
	D2	75	6.63
	D3	50	8.13
	D4	40	9.53
	D5	25	9.53
Control	Distal water	00	11.83
Total life period	D1	100	53.73
	D2	75	49.96
	D3	50	49.16
	D4	40	47.46
	D5	25	45.03
Control	Distal water	00	35.86

Table 3. The sex ratio shows that the males of *S. litura* are more susceptible to Lufenuron treated insecticide as compared to females

Treatment	Dose (ppm)	Total pupae	Emerged	Not emerged	Male (average)	Female (average)	Sex ratio
Lufenuron	100	4	4	0	0	4	0:4
	75	8	8	0	3	5	3:5
	50	15	14	1	4	10	1:2.5
	40	16	16	0	5	11	5:11
	25	20	19	1	8	11	8:11
Control	00	30	30	0	10	20	1:2

The copulation time of *S. litura* was observed with a significant difference ($F=0.03$; $DF=4, 29$; $P=0.998$) after the application of insecticide (Table 4). The minimum copulation time was recorded in (16.83 at 25 ppm) followed by (19.66 at 40 ppm), (21.33 at 50 ppm), (23.33 at 75 ppm), (23.83 at 100 ppm), and control (57.00 at 00 ppm). The fecundity of females also varied significantly ($F=0.02$; $DF=4, 29$; $P=0.999$) after the application of insecticide. The lowest fecundity of females was recorded in

(320.67 at 100 ppm) followed by (334.00 at 75 ppm), (362.00 at 50 ppm), (397.67 at 40 ppm), (433.67 at 25 ppm), and control (1178.00 at 00 ppm). The fertility percentage of females of *S. litura* varied significantly ($F=0.02$; $DF=4, 29$; $P=0.999$) after the application of insecticide. The lowest fertility percentage of females was recorded (44.423 at 100 ppm) followed by (50.42 at 75 ppm), (56.76 at 50 ppm), 59.50 at 40 ppm), (65.144 at 25 ppm), and control (93.07 at 00 ppm), respectively.

Table 4. Copulation time, Fecundity, Fertility of *S. litura* (F.) after 24, 48, 72, 96 hours, and one week treated with Lufenuron

Treatment	Dose	Concentration in ppm	(%) Larval weight reduction
Copulation time	D1	100	23.83
	D2	1680	23.33
	D3	250	21.33
	D4	2500	19.66
	D5	75	16.83
Control	Distal water	00	57.00
(F= 0.03; DF= 4, 29; P= 0.998)			
Fecundity	D1	100	320.67
	D2	1680	334.00
	D3	250	362.00
	D4	2500	397.67
	D5	75	433.67
Control	Distal water	00	1178.00
(F= 0.02; DF= 4, 29; P= 0.999)			
Fertility	D1	100	44.423
	D2	1680	50.420
	D3	250	56.761
	D4	2500	59.508
	D5	75	65.144
Control	Distal water	00	93.073
(F= 0.02; DF= 4, 29; P= 0.999)			

Discussion

Keeping in mind the above detailed described facts, research was conducted to assess the efficacy of Lufenuron against tobacco cutworm *S. litura*. As we know, the use of cauliflower is beneficial and substantial protection against cancer, diabetes, and cardiovascular disease. Phytochemicals of plants contain anti-oxidant, anti-inflammatory, and anti-proliferative contents and these properties are essential against cancer. Due to these potential properties, a research study was carried out to protect the crop through different techniques by the insecticides from the vigorous pest. These results are agreed with the [16] they described the importance of cauliflower vegetable, although the nutritional profile of cauliflower. India ranks second largest vegetable producer in the world but about 0.44% of vegetable production contributes by Pakistan and in the response of cauliflower Pakistan ranks 19th position in the world [17]. This kind of vegetable contains a high concentration of glucosinolates, which are metabolized into isothiocyanates. A research report was published in Medicinal chemistry sheds light that how isothiocyanates of cauliflower put forth their activities of anti-cancer. Cauliflower is better at returning our investment for farmers with plenty of production [18].

The biology of *S. litura* (F.) affected by the lufenuron, larval mortality, and weight reduction percentage recorded after 24, 48, 72, 96 hours, and one week. All results were dose-dependent, the maximum mortality and larval weight reduction percentage recorded in higher doses as compared to lower and after 24 and 48 hours. The mortality, as well as larval weight reduction, did not record in control but their larval weight increased. The pupal weight of treated larvae also decreased as compared to control. The developmental time, larval, pupal, and all

life cycle stages by the use of insecticides increases compared to control, the males are more susceptible to insecticide as compared to females. The copulation time, fertility, and fecundity decreased as compared to control. Zhu *et al.* [15] reported documented that impacts of sub-lethal concentrations 0.1, 0.5, and 1.2 $\mu\text{g mL}^{-1}$ of inhibitor hexaflumuron on the development and growth of larvae and kept attention to estimate the proportion of carbohydrate glyceride and trehalose, hemocytes inside the hemolymph in the cutworm and third-stage larvae were subjected to the sub-lethal concentrations. For parasitic wasps, earwigs, honeybees, predaceous mites, and ants this type of IGR is safer and beneficial [19].

In our study, the biology of *S. litura* (F.) positively affected by lufenuron, caused mortality, larval and pupal weight reduction, incassation in the duration of the life cycle as larval and pupal periods, developmental time and total life period, decreased copulation time, fecundity and fertility. According to [20], due to the sub-lethal effects of insecticides on *S. littoralis*, the duration of the life cycle increased, pupal weight, copulation time, adult longevity weight gain (food consumption) decreased as compared to control. According to the experiment on sub-lethal effects of insecticides by [21] both duration and development of larval and pupal stages of *S. exigua* were prolonged and larvae and pupae weight reduced and fecundity, pupation rate, and fertility were decreased, as compared to control. The insects are affected both negatively and positively by the use of insecticides as according to [22] studied the sub-lethal effects of carbaryl, monocrotophos, and endosulfan on *S. litura* (F). With sub-lethal concentrations of these insecticides, leaves were treated [23] reported the most advantage of insect growth regulators is that it is non-toxic or less toxic to the environment, invertebrates,

vertebrates including fish, birds, humans, wildlife, etc. Development of resistance and cross-resistance, lethal or sub-lethal effects on non-targeted species are some disadvantages of these types of chemicals. Persons are also confused by their slow effects on insect pests because they do not an immediate knockdown, comparatively conventional insecticides are safe to the environment.

Conclusion and Recommendations

Since lufenuron has better results against *S. litura* (F.) by their sub-lethal effects, therefore that should be applied for control. Farmers are advised to wait a few days for results until the Lufenuron has delayed the molting process, which later causes mortality at molting time. This should be used in alteration with other compounds against *S. litura* (F.) such as polo/Pegasus or Agree / Truex (*Bacillus thuringiensis*) to avoid resistance mechanism. Due to environmental safety, they can be regarded as suitable for the International pest management program. It is recommended to investigate the sub-lethal effects of this insecticide generation after generation of insect pests. The same experiment should be repeated in field conditions and more insect growth regulators (insecticides) should be tested for their sub-lethal effects on the biology of *S. litura* (F.).

Authors' contributions

Experiments designed and conceived by: HA Sahito & MH Mahar, Experiments performed by: AQ Malik & T Kousar, Data analyzed by: WM Mangrio, Contributed tools/ analysis/ materials by: Bhugro Mal, Research article written by: HA Sahito & WM Mangrio.

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