Toxicity and Biological Effects of Jojoba oil, Lufenuron, and Protecto against *Spodoptera frugiperda* (Lepidoptera: Noctuidae), In Egypt.

Abo Elela, Mohamed R. G. and Ali R. El-Gabaly

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

ABSTRACT: In Egypt, nowadays maize crop faces a serious threat from the fall armyworm, *Spodoptera frugiperda* J. E. Smith. The current study set out to determine the toxicity of three insecticides against *S. frugiperda* larvae under laboratory conditions (27 C^0 and 55% RH.). The tested insectcidies included jojoba oil as a botanical extract, *Bacillus thuringiensis* (Protecto) as a bioinsecticide, and lufenuron as an insect growth regulator (IGR). The larvae were taken from the Qena Governorate's corn fields in Upper Egypt, and were reared under laboratory conditions (PPRI, Giza). All three insecticides showed concentration-dependent mortality rates through toxicity experiments, and jojobia oil recorded the maximum toxicity (LC_{50}: 2.521 ml/1000ml water), followed by lufenuron (LC_{50}: 4.842 ml/1000ml water) and protecto (LC_{50}: 6.9928 ml/1000ml water). In addition, the effect of these pesticides on the development of larval duration, pupal duration, % of emergence, male and female longevity, fecundity and % of hatchability was studied, and the obtained results recorded that the longest larval period and pupal period was observed with the treatment of jojoba oil, followed by lufenuron and protecto. This study makes sure that the pesticides under investigation are among those that are advised for use against armyworms.

Keywords: *Spodoptera frugiperda*, Protecto, Lufenuron , *Bacillus thuringiensis*, Toxicity, Biology, PPRI

1.INTRODUCTION:

One of the most important invasive polyphagous pests that inhabit over 353 plant species, including maize, sorghum, sugarcane, turfgrass, cotton and vegetable crops, is the autumn armyworm, *Spodoptera frugiperda* **(J. E. Smith) (Lepidoptera: Noctuidae) (Gamil, 2020)**. *S. frugiperda* has spread quickly over 44 West African nations since 2016 **(Kassie *et al.* 2020)**. The Food and Agriculture Organisation **(FAO) 2019** reported that *S. frugiperda* was initially recorded in Egypt in Komombo, Aswan Governorate, on maize fields in 2019. After that, in 2021, it spread in the governorate of Upper Egypt to north direction on maize and sorghum crops **(Mohamed *et al.*, 2022)**. It severely damaged maize and sorghum crops in Africa **(Hailu *et al.*, 2021)**, resulting in an annual production loss of up to 17.7 million tons **(Kassie *et al.*, 2020)**.The most common biological agent used to manage lepidopteran pests is the entomopathogenic bacterium, *Bacillus thuringiensis* **(Federici *et al.*, 2006)**. The primary mechanism of *Bacillus thuringiensis* insecticidal activity is the rupture of midgut epithelial cells by crystal protein**(s) (Honee and Visser, 1993)**. Chitinase may hydrolyze chitin in the peritrophic membrane and induce pore formation, increasing the quantity and rate of *B. thuringiensis* insecticidal chemicals that reach the insect midgut. Further research by **Granados *et al.* (2001)** revealed that the enhancin derived from the *Trichoplusia ni* granulovirus was also capable of breaking down the peritrophic membrane of a variety of lepidopteran larvae and boosting *B. thuringiensis* ability to combat lepidopteran larvae, including *Spodoptera exigua*. Therefore, one clear objective of increasing *B. thuringiensis* toxicity seems to be the breakdown of the peritrophic membrane. Insect growth regulators may prevent insects from developing physiologically by preventing feeding, moulting, and the production of new epidermis, all of which cause pests to die. Unlike traditional insecticides that work by acting on the nervous system, it works in a different way **(Zuowen, 2002)**. Reduced toxicity, reduced pollution, and minimal effects on beneficial organisms and natural enemies promote the development of sustainable agriculture, make it easier to produce food that is better for the environment, and enhance human health. Consequently, these are referred to be as "novel materials for insect control," "bio-regulators," "pesticides of the 21st century," and "third generation pesticides" **(Zuowen, 2002)**.

From the previous view the present work was conductef to evaluate bioinsectcide, botanical extract, and IGR on some biological aspects of the fall armyworm *S. frugiperda*,

2.MATERIALS AND METHODS:

2.1.Rearing insects and testing pesticides

The Department of Cotton, Plant Protection Research Institute, A.R.C. Giza, Egypt, collected fall armyworm larvae from maize fields in Qena Governorate, Upper Egypt. The method of growing insects adhered to the established guidelines that Dahi *et al.,* 2020 outlined. In this experiment, *S. frugiperda* larvae in their newly moulted fourth instars were used. To obtain the laboratory strain, fall armyworm larvae were raised in the lab for multiple generations. The effectiveness and potency of the three insecticides (protecto, lufenuron, and jojoba oil) against the fall armyworm were conducted. Common, trade names, chemical classes, and empirical formula of tested compounds were displayed, in Table (1).

Table (1) Common and trade names of tested compounds, their chemical classes and empirical formula

Common name	Trade name	Company	Chemical class	Empirical formula
Jojoba oil	Top healthy 60% EC	Top Chemical Factory for the Manufacture of Pesticides and Specialized Chemicals	Plant oils	C14H16Cl2N2O2
Lufenuron	Match®5% EC emulsifaible concentrates	Syngenta Crop Protection AG, Basel, Switzerland	Insect growth regulators	C17H8Cl2F8N2O3
Protecto®	Protecto® 9.4% WP	Wettable powder produced by the Plant Protection Research Institute, Biopesticide Production Unit, Dokki, Giza, Egypt	*Bacillus thuringiensis* var. kurstaki	Btk

2.2.Determining the half lethal concentration (LC_{50})

The larvicidal activity of the three tested insecticides was evaluated against the freshly forth instar larvae of *S. frugiperda* larvae by calculating their LC_{50} values. For every pesticide, the following concentrations were used: Protecto and jojoba oil: 1.25, 2.5,5,10, and 20 ml/1 ml water for lufenuron:0.1.0.2, 0.4,5,0.8,and 1.0 ml/ 1 ml water. The leaf dipping technique, as outlined by Aly *et al.,* 2023, was applied in this bioassay.

2.3.Biological Examinations

Several important biological aspects were examined in order to evaluate the biological impact of the calculated LC_{50} values of the three tested insecticides on *S. frugiperda*: larval duration, the length of the pupal stage, the percentage of larval mortality, the sex ratio (♂:♀), the percentage of adult emergence, the length of the adult life, the percentage of hatched eggs, the percentage of fertilized eggs, and fecundity (the total number of eggs deposited by a female).

2.4.Statistical analysis

Using the Statistical Package for Social Sciences (SPSS) programme, statistical analysis was carried out using the multiple range test of Duncan. A significant difference in the mean was observed when $P<0.05$. According to Finney, values of LC_{90} and LC_{50} acquired by probit analysis using LdP Line^{R} software **(Finney, 1971)**. Mortality percentage were computed according to **Abbott formula (1925)**.

3.RESULTS:

3.1.The toxicity values of *S. frugiperda* larvae in their fourth instars

The efficacy of the tested pesticides against the 4th instar larvae of *S. frugiperda* varied, according to the condensed results shown in Table "2". Jojoba oil showed the maximum toxicity (LC_{50} 2.521 ml/1000 ml water), followed by lufenuron (LC_{50} 4.842 ml/1000 ml water) and protecto (LC_{50} 6.9928 ml/1000 ml water) against 4th instar larvae.

Impact of the examined pesticides on some biological aspects of the larval, pupal, and adult stages of *S. frugiperda*

The acquired data in table (3) showed that jojoba oil, lufenuron, and protecto considerably delayed the larval development, as evidenced by the developmental course and reduction rates of the fourth instar larvae of *S. frugiperda*. The values of larval duration treated by jojoba oil, lufenuron, and protecto were, 12.8±0.62, 10.8±0.56 and 9.4±0.39 days, respectively, in comparison with the control values (8.1±0.56 days, Table 3). However, the highest pupation percentage was recorded in the treatment of lufenuron (54.0%), and the lowest percentage was induced by jojoba oil (47.3%), compared with the control (98.50%)(Table 3). The larval mortality % was the highest with jojoba oil followed by lufenuron and protecto as compared with the control value (100%)(Table 3). The current findings guaranteed that the normal larvae percentages (and the larvae malformation percentages) were 95.8, 100, and 100% following the treatment by jojoba oil, lufenuron, and protecto, respectively (Table 3).

Table 2: Toxicity values of jojoba oil, lufenuron, and protecto against 4th instar larvae of *S. frugiperda* under laboratory conditions, 27C^0 and 55% R.H.

Treatment	Concentrations ml/1000 ml water	Mortality (%)	LC_{50}
	1.25	33	
	2.5	47	
Jojoba oil	5	58	2.521
	10	66	
	20	75	
	0.1	22	
	0.2	25	
Lufenuron	0.4	32	4.842
	0.8	42	
	1.0	52	
	1.25	21	
	2.5	23	
Protecto	5	33	6.9928
	10	37	
	20	48	
Control	-	0	-

Table 3: Biological aspects of fourth-instar larvae of *S. frugiperda* larvae treated with LC_{50} of jojoba oil, lufenuron, and protecto pesticides under laboratory conditions, 27C^0 and 55% R.H.

Parameters	Control	Jojoba oil	Lufenuron	Protecto
Larval duration (days)	8.1±0.56b	12.9±0.62d	10.8±0.56c	9.4±0.39a
Pupation (%)	98.50	47.3	54.0	51.0
Larval mortality (%)	2.0	58.99	49.3	50.8
Normal larvae (%)	100.0	95.8	100.0	100.0
Malformed larvae (%)*	0	4.3	0	0

* There is a significant difference (P<0.05) between the means of the row after various letters.

*Malformed larvae displayed inadequate moulting of larvae into pupae, while other malformed larvae presented an intermediate shape between larvae and pupae

The LC_{50} doses of jojoba oil, lufenuron, and protecto considerably extended the pupal length in comparison to the control, as shown in Table 4. In contrast to the control group 9.62 days, the pupal duration for jojoba oil, lufenuron, and protecto was measured as 26.05, 18.72, and 13.05 days, respectively. LC_{50} of jojoba oil increased the pupal malformation percent (7.6%) followed by lufenuron (4.3%) compare to zero% in control. The results shown in Table "4" demonstrated that jojoba oil and lufenuron were the most effective insecticides against emergenc percentage of adult insects.

As shown in table (5) jojoba oil and lufenuron significantly increased the longevities of the male and female compared to the control group (9.75 and 10.00 days, respectively). Following the application of all pesticides, there was a considerable decrease in the average number of eggs (fecundity) and hatched eggs (Table 5).

Table 4: Biological characteristics of *S. frugiperda* pupae developed from treated larvae with LC_{50} of jojoba oil, lufenuron, and protecto pesticides under laboratory conditions, 27C^0 and 55% R.H.

Parameters	Control	Jojoba oil	Lufenuron	Protecto
Pupal duration (days)	9.62±0.06d	26.05±0.16a	18.72±0.20b	13.05±0.25c
Normal pupae (%)	100.0	94.8	97.5	100.0
Malformed pupae (%)*	0.0	7.6	4.3	0.0
Emergence (%)	100.0	86.0	95.0	100.0

There is a substantial difference (P<0.05) in the means of the row when various letters are followed.
*While some malformed pupae showed distinct constrictions at the head and thorax, malformed pupae were seen to have humpbacks.

Table 5: Biological aspects of *S. frugiperda* adult stages developed from treated as fourth instar larvae with LC_{50} of jojoba oil, lufenuron and protecto pestcidies under laboratory conditions, 27C^0 and 55% R.H.

Biological aspects	Control	Jojoba oil	Lufenuron	Protecto
Sex ratio (%) (♂: ♀)	1:1	1:1	0.7:1	0.8:1
Adult longevity (days)	9.88±0.13c	14.53±0.31a	12.0±0.54ab	10.88±0.38b
Female longevity (days)	9.75±0.25c	13.75±0.25a	12.63±0.25a	11.00±0.58b
Male longevity (days)	10.00±0.00c	14.63±0.71a	11.35±1.03ab	11.55±0.25bc
Fecundity	1305.8±63.7a	1099.8±21.6b	1031.3±65.2b	1028±23.0b
Hatchability %	93.0	77.0	84.0	82.0

There are significant differences (P<0.05) betweenthe means of each row after different letters.

4.Discussion:

The current study determined the toxicological effects and ensuing physiological effects of using bioinsecticides: jojoba oil, and protecto, along with the insect growth regulator lufenuron, against forth instar larvae of *S. frugiperda*. This investigation determined the potential toxicity of tested pesticides as well as the effects that might follow, taking into consideration the different variables including concentration, chemical makeup, and the stage at which the treated larvae were developing. The results of the study highlight on the different effects of various tested pesticides. It was clear that the toxicity of these insecticides is concentration-dependent, with high concentrations resulting in the highest rates of larval mortality. In the treatment of jojoba oil alone, the LC_{50} concentration resulted in an increase in larvae mortality, a decrease in pupation percentage, and larval deformity. These insecticides have physiological effects that go beyond just killing larvae. Lufenuron and protecto treatments resulted in increase of pupal deformity, prolonged pupal stage duration, and prolonged larval development. Furthermore, an increase in pupae mortality was seen as a result of the tested pesticides (jojoba oil and lufenuron only). Also, longevity was increased by the studied insecticides, while, hatchability rates and egg deposition were decreased, suggesting possible consequences for population expansion and pest management tactics. Mechanisms of action and target areas of the tested insecticides can be used to explain this variation in toxicity. As an inhibitor of chitin synthesis, lufenuron prevents the development of the exoskeleton of insects **(Lv *et al.*, 2022)**, which ultimately results in growth suppression and death. **(Moawad and Sadek, 2018)** found that the treated larvae had longer larval and pupal durations due to the effect of jojoba oil. These results corroborate and validate our earlier study by Aly *et al.,* 2023, which noted changes in larval growth, developmental schedules, and survival rates of *S. frugiperda* larvae in their second instar after treatment with lufenuron, emamectin benzoate, and Bt k. In conclusion, these laboratory experiments confirm how crucial it is to choose the tested pesticides carefully in order to manage fall armyworms effectively. The application of laboratory results to real field circumstances, however, needs to be carefully considered, even though they offer vital insights into the toxicity and impacts of pesticides. Therefore, in order to confirm and improve upon laboratory findings, more research trails in real-world settings are essential.

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السمية والتأثيرات البيولوجية لزيت الجوجوبا واللوفينورون والبروتكتو ضد دودة الحشد \ فى مصر

محمد أبو العلا – على ربيع الجبلى

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقى – الجيزة - مصر

الملخص العربى:

يواجه محصول الذرة في مصر تهديدًا خطيرًا من دودة الحشد الخريفية. تهدف الدراسة الحالية إلى تحديد مدى نجاح ثلاثة مبيدات حشرية ضد يرقات دودة الحشد الخريفية في طورها الرابع في تحت الظروف المعمليه (٢٧م ٥٥٪ رطوبه نسبيه). وشملت كل من: زيت الجوجوبا (زيت نباتى)، و مبيد حشري حيوي يحتوى على البكتيريا الممرضة للحشرات (*Bacillus thuringiensis*) باسم (بروتكتو ٩,٤٪ تركيز قابل للاستحلاب), و مبيد حشري حيوي باسم (لوفينورون ٥٪ تركيز قابل للاستحلاب) كمنظم لنمو الحشرات(IGR) . و تم أخذ اليرقات من حقول الذرة بمحافظة قنا في صعيد مصر، ثم تم تربيتها في المعمل (معمل قسم بحوث دوده ورق القطن- معهد بحوث الوقايه). أظهرت جميع المبيدات الحشرية الثلاثة معدلات موت تعتمد على التركيز من خلال تجارب السمية، حيث أظهر زيت الجوجوبيا أقصى سمية (LC_{50}: 2.521ml/L) ، يليه اللوفينورون(LC_{50}: 4.842 ml/L)) ومركب بروتكتو: (LC_{50}: 6.9928 ml/L) . بالإضافة إلى ذلك، تمت دراسة تأثير هذه المبيدات على تطور اليرقات و فتره العذراء و نسبه الخروج و عمر الذكر و الانثى و الحصوبه و نسبه الفقس. ولوحظ ان أطول فترة للعمر اليرقى وفتره العذراء مع زيت الجوجوبا، يليه اللوفينورون والبروتكتو، بهذا الترتيب. تأكد هذه الدراسة أن المبيدات قيد الدراسه هي من التي الموصى باستخدامها ضد دودة الحشد.