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Plant Extract Induce Change in Biological and Biochemical Parameters of *Schistocerca* Gregaria (Forsk)

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Abstract

The desert locust, *Schistocerca gregaria*, is the most dangerous herbivore species of locust. The present investigation aimed to throw light the effect of methylene chloride extracts of *Azadirachta indica*, and *Euphorbia granulate* on the 2nd instar nymph of *Schistocerca gregaria* under laboratory condition. The results showed that the methylene chloride extract of *A. indica*, are the most potent extracts against *Schistocerca gregaria*. The present study was also extended to conduct the insecticidal effect of the most potent extracts *Azadirachta indica*, and *Euphorbia granulate* methylene chloride extract on *Schistocerca gregaria*. The present result showed that *Azadirachta indica*, and *Euphorbia granulate* extract caused not only mortality in larval stages, but also caused effect on pupal and adult duration, adult fecundity and egg fertility. Also extracts of *Azadirachta indica*, and *Euphorbia granulate* extract treatments have resulted in emergence of deformed pupae and adults.Also, the data showed that Marked biochemical changes however, being recognized in pest as marked decrease in total lipids, total protein and glucose contents. The activity of both ALAT and ASAT are also being highly affected.

Keywords: *Schistocerca gregaria*, *Azadirachta indica*, *Euphorbia granulate* plant, Biochemical studies.

Contents

1. Introduction

The desert locust, *S. gregaria* (Forsk) is the most dangerous herbivore species of locust. Specialist and generalist insect herbivore species often differ in how they respond to host plant traits and these responses can include weakened or strengthened immune responses to pathogens [1]. It is one of the serious pests attacking vegetables and wide varieties of other field crops in Egypt and other different countries. Several reports have emphasized the control of each pest using organic and inorganic compounds. Organophosphorous, organochlorine, carbamate and pyretheriod compounds are the most compounds used [2, 3]. Also, the inorganic chemicals have been widely used. Many different countries search for less dangerous pesticides by using the naturally occurring herbs that can be applied effectively in habitats [4, 5]. Many different countries search for less dangerous pesticides by using the naturally occurring herbs that can be applied effectively in habitates [4-6]. Many workers reported that plants are considered one of the richest sources which can be used as pest control agents [7, 8]. In Egypt, attempts have been done to monitor insecticidal activity of different plants extracts against many insects [6, 9].

Many studies have reported that plants are one of the richest sources which can be used to pest control agents. They attended to use extract as toxicants, repellents synergists, growth regulators and antifeedants for many insect pests [6, 7]. In Egypt, attempts have been done to monitor insecticidal activity of different plants extracts against many insects [1, 5, 8-10]. The aim of the present investigation is to determine insecticidal effects of two different organic solvents of *Azadirachta indica* and *Euphorbia granulate* against the 2nd instar nymph of *Schistocerca gregaria.* The present study was also extended to evaluate the most potent promising plant extracts on the biological and biochemical parameters of the studied pest.

2. Materials and Methods

2.1. Experimental Insects

Desert Locust (*Schistocerca gregaria*, Family: Orthoptera) The susceptible strain of locust. *Schistocerca gregaria* was obtained from Desert Locust Research Department. Ministry of Agriculture. Dokki. Giza being used throughout the present investigation. It was reared on clover leaves. The tests were carried out on the 2th instars nymph.

2.1.1. Tested Plants

The plant used in this study *Azadirachta indica* (Meliaceae) and *Euphorbia granulate* (Euphorbiaceous) were collected from fields of Jeddah , Saudi Arabia during flowering stages and identified in Botany department. Faculty of Science, Cairo University. The collected plant leaves were left to dry in air and then in an oven at 50°C [11]. Plants were left to dry in air and then in an oven at and powdered by a mixer.

2.1.2. Extraction

Solvent (methylene chloride) was used for extraction. A stock extract was prepared soaking 200 g of the plant powder in 800 ml of methylene chloride for 4 days at room temperature (25±1±C),. The combined extract was filtered over anhydrous sodium sulphate. The solvent was then evaporated under reduced pressure using a rotator evaporator at 30^{O}. Afterwards, the remaining powder was soaked again in 800 ml of methylene chloride by the same procedure. The extracted solutions were left away for complete dryness to obtain the crude extracts. The extracts of *A.indica, E. granulate* were carried out by Freedman, et al. [12] method with some modification the crude extract was transferred quantitively to a clean and weighted flask and kept in the refrigerator until used for Biological and toxicological investigation.

2.2. Preparation of the Tested Concentrations

Consider the crude extract as 100% a known weight of the crude was added to a similar volume of the solvent (acetone) to obtain stock solution. Stock solution of each extract was made prior to use. Successive dilutions were carried out to obtain the tested solutions.

2.3. Bioassay

The different concentrations of each plant extract were tested on the 2^{nd} instar nymph of *S. gregaria*. Leaf dipping technique was used. The same sizes of clover leaves for *S. gregaria* were dipped in each tested concentration of plant extracts and in the control for 20 seconds and left to dry. The dried leaves were put singly in plastic cups. Tens insects were transferred to each cup and allowed to feed on the treated leaves for one day. Three replicates for each concentration were done. After 24h surviving insects were transferred to clean cups and supplied daily with untreated leaves until the end of experiment. Group of insects was left unexposed under the same laboratory conditions as control. Mortality was recorded daily until the end of experiment and corrected according to Abbott [13]. Mortality values 24 days after exposure were analyses by profit analysis (LDP line) to obtain LC_{25}, LC_{50} LC_{90} and slope for each extract according to method adopted by Finney [14]. The most effective plant extracts were selected for father experiments.

2.4. Biological Studies

A leaf dipping bioassay method was adapted to evaluate insecticidal activity of plant extracts against the 2^{nd} instar larvae of *S. littoralis.* LC_{25} of *A. indica* and *E. granulate* extracts were used. Eighty of starved larvae, distributed in four replicates (20 larvae / replicate) were used and treated according to method described by Baker, et al. [15]. 24 hrs inspections were carried out for all treatments and control to record mortality percentages. Then daily inspections were carried out until adult emergence occurred. Larval mortality %, larval duration, pupation %, pupal duration and pupal malformation were recorded. Adult emergence %, total inhibition of adult emergence %,

fertility %, fecundity, sterility % and in addition malformations was recorded. Adult fecundity was determined by placing one female and one male together in a glass jar of 75 c.c capacity provided with a piece of cotton soaked in 10% sugar solution and was internally covered with soft sheet of paper for ovipositor. The jars were inspected daily for counting the number of laid eggs. To determine the fertility, two or three patches having not less than 100 eggs were collected during the first 3 days of ovipositor and incubated under the laboratory conditions until hatching and the percentage of hatchability was recorded.

2.5. Biochemical Studies and Sampling

After feeding on treated leaves with sublethal concentration (LC_{25}) of the extracts for 24 hours, alive insects were collected and allowed to feed on normal leaves after 7, 14, 21 and 28 days for *S. gregaria*. A specific number of pests was taken and subjected directly for biochemical assays.The collected pests after each time intervals. The homogenates were centrifuged at 3500 r p m. for 10 minutes and the supernatants were filtered through glass wool to remove fatty materials and kept in deep freezer at-20^{O}C till use for determination of glucose, total protein and lipid concentration and aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) activites. Aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) activites were determined according to the method of Reitman, et al. [16]. Total protein was determined according to the method of Weichselbaum [17]. Glucose concentration was determined according to the method of Trinder [11] and Total lipids were determined according to the method of Zollner, et al. [18].

2.6. Statistical Analysis

The data were expressed as means ± standard errors. The statistical significance of differences between individuals means were determined by using one way ANOVA test. Levels of significance of each experiment was stated to be significant at ($P \leq 0.05$), high significant at ($P \leq 0.01$) and very high significant at ($P \leq 0.001$). The oviposition deterrent index (O.D.I) was calculated according to Lundergren [19].

3. Results

3.1. Toxicity Testing

The result recorded in table (1) showed that, the A. indica plant was the only one affected on 2 instar nymph of *S. gregaria*. The recorded LC_{50} and LC_{90} values of A. indica plant extract were 6 and 11ppm (mg/ml), respectively. On the other hand, the recorded LC_{50} and LC_{90} values of *E. granulate were* 7.4 and 13ppm, respectively.

3.1. Biological Studies

Results recorded in Table (2) showed that plant extract had a significant effect on the larval and pupal duration as the mortality increase. The larval mortality increased to 30 % at LC_{25} of *A. indica* while pupal mortality was 20 % at LC_{25} of *E. granulate*. Pupal durations showed significant increase. Especially for pupae which showed very high increase elongation at LC_{25} of *E. granulate* compared with control. On the other hand, the pupation percentages were greatly reduced to 50 at LC_{25} of *A. indica* and 70 % at LC_{25} of *E. granulate*. The percentage of the adult emergence was decreased to 40 at LC_{25} of *A. indica.* The present results indicated that there was a significant inhibition of adult emergence percentages 60%,30% at *A. indica* and *E.granulate*,respectively compared with control 0.0 %.

The results tabulated in Table (3) showed that plant extract induced a reduction in both fecundity and fertility. The mean number of eggs deposited by females resulted from treated larval with plant extract were lower significantly than the control. Average numbers of laid eggs were 966 and 1188 eggs/females at *A. indica* and *E.granulate*, respectively. While the mean number of eggs deposited by untreated female were 1385eggs/females. The percent of egg hatching was high significant affected by treatments. The hatchability percentages of eggs were 74.2 and 91 %, respectively for females emerged from treated by $LC_{25}$of *A.indica* and *E.granulate*, respectively, compared with 100% eggs/ females of control.

3.2. Biochemical Effects

The activity of aspartate amion transferase in the whole body homogenate of *S. gregaria nymph* was determined after treatment with LC_{25} of *A. indica* and *E. granulate* extracts are given in Table (4). From the recorded data it was noticed that, the LC_{25} of *A. indica* showed significant elevation of the enzyme activity throughout all tested periods.

Post 1, 2 and 3 weeks and changed by 11.2, 17.42% and 21.4, respectively in comparison to the control value. The data recorded in Tables (5) showed, the effect of the LC_{25} of *A. indica* and *E. granulate* extracts on the activity of ALAT of S. gregaria nymph. The recorded revealed a fluctuated significant change in enzyme activity during the different time intervals. It was noticed that, the LC_{25} of *A. indica* showed significant elevation of the enzyme activity throughout all tested periods. Post 1, 2 and 3 weeks and changed by 17.7, 22.12% and 23., respectively in comparison to the control value.

The data recorded in table (6) showed the effect of LC_{25} extracts of *A. indica* and *E. granulate* on the total protein content of *S. gregaria* nymph. It was clear from the recorded data that was a continuous decrease in the level of total protein of treated nymph post exposure to LC_{10} of A. indica and *E. granulate* extracts. The maximal decrease however, was recorded after 3 weeks post exposure, and the calculated percentage differences were -36.88% and -24.6522% for *A. indica* and *E. granulate*,respectively as compared to the control level.

The data given in tables (7) showed the effect of LC_{25} of *A. indica* and *E. granulate* extracts on glucose concentration of *S. gregaria* nymph. The data recorded showed that was a highly significant decrease in glucose concentration after treatment with LC_{25} of the two tested extracts during most time intervals. At 1,2 and 3 weeks, the percentage of reduction were -17.1%, -26.14and -39.18%,respectively, for A. indica extract and -8.55%, -17.6%, and

-32% respectively, for *E. granulate* extract .the maximal reduction however was achieved after 3 weeks for both tested extracts.

The total lipid concentration in *S. gregaria* nymph homogenate after treatment with LC_{25} of the extracts of *A. in*dica and *E. granulate* were given in table (8). From The recorded data, there was a significant decreased effect on the total lipid after treatment with LC_{25} of the two extracts during all the tested time intervals. .At the 1, 2 and 3 weeks post exposure and as compared to the control level the percentage changes were -22.88%, -35.11%and -44.94%for A. indice and -12.14%, -23.4%and -37.1% for *E. granulate* exposure, respectively . The maximal highly significant decrease, however, was attained after 3 weeks.

4. Discussion

Schistocerca gregaria is one of the serious pests attacking varieties of field crops and vegetables in different countries including Egypt [20]. Since the mortality potency of the investigated plant extracts is not the same as that caused by conventional insecticides and because no initial change was observed, On the basis of the LC_{50} values of the tested plant extracts post 24 days on 2nd nymphal stage of *S. gregaria*. It was found that, the maximum effect was given with *A.indica* followed by *E. granulate extracts*. This is in accordance with the findings of other investigators using different plants insecticides including *A.indica* [20, 21]. According to Stark and Rangus [22], neem act slowly and one week may be a short interval for its evaluation against aphids. Also, as reported by Schmutterer [23] and Lowery, et al. [24], neem extracts had no direct contact toxicity.

The high insecticidal potency of *A. indica and E. granulate* can be attributed to several factors including plant specific differences of the extracted active ingredients, types of the extracted products, differences in their mode of action, Method of penetration and the behavioral characteristics of the studied pests [23, 25].

It is now well established that in many plants including the tested plants especially *A. indica* and *E. granulate* the activity is due to the presence of saponin components [26] triterpenoid components [27], Alkaloidscomponents [28]. Tannins compounds [29] although the effect seems to be very specific dependent. Further work is necessary to understand better the mechanisms of increasing toxicity by extract formulation, since various authors have postulated the effect of different plant extract on the feeling behavioral pattern of different insects [4].

In the present study indicated that there were positive correlations with the tested concentrations of *A. indica* and *E. granulate extracts*. The mortality in the larval stage was clearly due to the mounting disrupting effect of the plant extract. This effect is mainly induced by inhibiting chitin formation [30], thereby causing abnormal endocuticular deposition and abortive mounting [31]. Furthermore higher concentrations have ant feeding effect. Therefore, feeding impairment of treated larvae could lead to prolongation of the larval instars and subsequently leading to a reduction in the percentage of pupation and adult emergence. Gelbic, et al. [32] showed that larval instars of *S. littoralis* treated with lufenuron unable to complete the molting process and died in the old larval cuticle, likewise, flufenoxuron caused similar effect on *S. littoralis* treated larvae [15]. Pupal mortalities in this study were obvious; there were dose-dependent effect on pupation and pupal mortalities. The same results were found by [33-35].

It was obvious that the percent of inhibition of adult emergence were in positive relation with the tested concentrations of *A. indica* and *E. granulate extracts*. These results were also found by Osman and Mahmoud [36] who demonstrated that the emergence of Egyptian cotton leafworm moth affected by treatments larvae with lufenuron compared to the control. The reduction in fecundity and egg hatchability of *S. gregaria* nymph following treated with A. indica and *E. granulate extracts*. These results was also found by Abdel Rahman, et al. [30].

Reduction in fecundity may be due to the reduction in longevity and the number of oocytes per ovary and the reduction in oviposition period Soltani and Mazouni [37]. In addition to the above factors the maturation of an insect egg depends on the materials that are taken up from the surrounding haemolemph and materials synthesized by the ovary. These materials include protein, lipids and carbohydrates all of which required for embryonic structure [38]. Subsequently the reduction in total number of eggs per female could be due to interference of the IGR with oogenesis. They induce decrease in the concentration of yolk proteins, carbohydrates, lipids and inhibition in both DNA and RNA synthesis in the ovaries of females treated as larval instars, moreover they caused vacuolation of nurse cells and oocytes of the ovaries [39].

Reduction in the percentage of egg- hatch obtained in the present study may be due to defects in the differentiation of oocytes and sperms Meola and Mayer [40] and Date obtained from the biochemical effect of the tested extracts of *A. indica and E. granulate* at sublethal dosis also confirms different degrees of action on total protein content, total lipid content, glucose content and transaminases activity of *S. gregaria*.

In the present study, the activity of ALAT of insect decreased and the activity of ASAT increased throughout all the tested periods. Elevation of ASAT after exposure to different toxic agents in invertebrate and vertebrate animals has been investigated. the present study, the greater and continuous release of ASAT might be due to the necessity of enhanced deamination of aspartic acid for the process of gluconeogensis especially under conditions of impaired carbohydrate metabolism and/or a potential induced damage to parenchymal cells as reported by Rawi et al.,1996. On the other hand, the higher decrease in ALAT activity compared to that of ASAT suggest that with the use of extract of both plants, the reaction involving oxaloacetate seems to gain more importance than other pyruvate [25]. Data obtained also showed different pattern effect on total protein contents of the studied pest. At the tested dose level of *A. indica and E. granulate* the recorded values in pests showed marked decreases of the total protein content. Extensive work has been carried out in order to determine how various toxic agents affect protein synthesis. A diminution in the rate of ATP synthesis and inhibition of RNA synthesis are also the main causes of decreased total protein content [41]. Also, Rawi, et al. [42] have reported that protein leakage during intoxication may arise from reduced body weight. Conversion of protein to amino acids, degradation of protein to release energy or to the direct effect of the tested extracts on the amino acids transport of the cell. It seems from the present study that in 2nd nymphul stage of S. gregaria the tested plant extracts may caused proteolysis which yielded high level of amimo acids especially aspartate through greater release of ASAT which might be related to the higher

content of aspartic acid.The behavioral pattern changes of glucose level post treatment showed significant decreases in pest. The recorded effect was more pronounced with the effect of *A. indica* extracts than *E. granulate*. These findings coincide with those of Abo El-Ela, et al. [43]. Chitra and Reddy [44] showed reduction in carbohydrate content of different instar larvae when treated with *Ammi majus*, *Apium graveolens*, *Melia azedarach* and *Vince rosea* extracts.Also, as reported by another investigator Hashem, et al. [45], amylase is the most sensitive enzyme to the action of several molluscicdes. Otherwise the inhibition of the enzyme activity will in turn reduce glucose level in both pests through decreasing the hydrolytic rate of glycogen.Regarding the total lipid content, a number of toxic agents have been found to cause disturbances of fats in different body organs of both vertebrate and invertebrate animals Rawi, et al. [42]. Data obtained in the present work disclosed a significant reduction in the lipid contents of the pest through out all the tested periods. These findings are in agreement with those obtained by Mostafa [46],

Table-1. Toxicological evaluation of plant extract against the 2nd instar nymph of *S. gregaria.s*

	Toxicity of match Ppm			Slop function
	LC_{25}	LC_{50}	LC_{90}	
Azadirachta indica	3	6	11	2.1
Euphorbia granulate	4.2	7.4	13	2.5

Table-2. Effect of plant extract on some biological aspects of the 2nd instar nymph of *S. gregaria.s*

Conc. (ppm)	Larval mortality % ±S.E	Larval duration (days) ±S.E	Pupation % ±S.E	Pupal mortality % ±S.E	Pupal duration (days) ±S.E.	Emerged moths % ±S.E	Total inhibition of adult emergence %
Control	0	10±0.41	100±0.0	0	7±0.41	100±0.0	0
LC_{25} of *Azadirachta indica*	30±0.3***	13±0.11*	50±0.2***	10±0.3***	11 ±0.51**	40±0.8***	60±1.4***
LC_{25} of *Euphorbia granulate*	20±0.2**	11±0.31	70±0.23*	**5.5±0.16	8 ±0.11*	70±0.52**	30±1.2**

Table-3. Effect of plant extract on fecundity, fertility and sterility against adults of the 2nd instar nymph of *S. gregaria.s*

Conc. (ppm)	No. of eggs/female (fecundity) ±S.E	Egg hatching (fertility) % ±S.E	Sterility % ±S.E
0.0	1385±30.48	100±0.0	0.0
LC_{25} of *Azadirachta indica*	966±21.12***	74.2±1.5**	42.1±1.51***
LC_{25} of *Euphorbia granulate*	1188±25.22**	91 ±0.43*	23.6±1.4**

Table-4. The activity of ASAT in *Schistocerca gregaia* nymph treated with the extract of *Azadirachta indica* and *Euphorbia granulate*.

Formulated extract of	Dose mg/ml	Exposure period (weeks)					
	LC_{25}	week	Change %	2weeks	Change %	3 weeks	Change %
	Control	20.23±1.12		17.22±1.3		18.22±0.2	
Azadirachta indica	6	22.5±0.62	11.2	20.22**±1.6	17.42	22.12*±0.6	21.4
Euphorbia granulate	7.4	20.82*±1.3	2.19	19.11*±1.2	10.9	21.2**±0.5	16.4

Values are presented as Mean±SE.
-Nonsignificant P> o.o5
*Significant P<0.05
** high significant P<0.01

Table-5. The activity of ALAT in *Schistocerca gregaia* nymph treated with the formulated extract of *Azadirachta indica* and *Euphorbia granulate*

Formulated extract of	Dose mg/ml	Exposure period (weeks)					
	LC_{25}	1	Change %	2	Change %	3	Change %
	Control	22.32±0.14		22.6±0.0.12		22.8±0.11	
Azadirachta indica	6	26.28±0.12	17.7	27.6**±0.4	22.12	28.2*±0.41	23.7
Euphorbia granulate	7.4	24.2*±0.2	8.4	24.8*±0.3	9.7	25.1**±0.32	10.1

Values are presented as Mean±SE.
-Nonsignificant P> o.o5
*Significant P<0.05
** high significant P<0.01

Table-6. Effect of extract of *Azadirachta indica* and *Euphorbia granulate* on total protein concentration of *Spodoptera littoralis* larvae

Formulated extract of	Dose mg/ml	Exposure period (day)					
	LC_{25}	1	Change %	5	Change %	10	Change %
	Control	50.5±0.33		50.4±0.22		50.7±0.33	
Azadirachta indica	6	41.22**±0.45	-18.38	36.22**±1.33	-28.13	32±0.3	-36.88
Euphorbia granulate	7.4	44.11**±0.7	-12.65	40.2**±0.4	-20.24	38.2**±1.1	-24.65

Values are presented as Mean±SE., -Nonsignificant P> o.o5, *Significant P<0.05, ** high significant P<0.01

Table-7. Effect of extract of *Azadirachta indica* and *Euphorbia granulate* on glucose concentration of *Spodoptera littoralis* larvae

Formulated extract of	Dose mg/ml	Exposure period (weeks)					
	LC_{25}	1	Change %	2	Change %	3	Change %
	Control	70.2±0.67		70.4±0.44		70.7±0.34	
Azadirachta P<0.01*indica*	6	58.22*±1.1	-17.1	52**±1.3	-26.14	43**±0.44	-39.18
Euphorbia granulate	7.4	64.2**±0.65	-8.55	58**±0.6	-17.6	48**±0.82	-32

Values are presented as Mean±SE.. -Nonsignificant P> o.o5, *Significant P<0.05, ** high significant

Table-8. Effect of formulated extract of *Azadirachta indica* and *Euphorbia granulate* on total lipid concentration of *Spodoptera littoralis* larvae

Formulated extract of	Dose mg/ml	Exposure period (day)					
	LC_{25}	1	Change %	5	Change %	10	Change %
	Control	9.22±0.45		9.4±0.11		8.9±0.22	
Azadirachta indica	6	7.11**±0.18	-22.88	6.1**±0.09	-35.11	4.9**±0.14	-44.94
Euphorbia granulate	7.4	8.1**±0.14	-12.14	7.2**±0.14	-23.4	5.6**±0.11	-37.1

Values are presented as Mean±SE., -Nonsignificant P> o.o5, *Significant P<0.05, ** high significant P<0.01

References

[1] E. Lampert, "Influences of plant traits on immune responses of specialist and generalist herbivores," *Insects*, vol. 3, pp. 573-592. Dio: 10.3390/insects3020573, 2012.

[2] M. A. Fouda, K. S. Ghoneim, and A. S. Bream, "Biological activity of fenoxycarb (Ro13-5223) against house fly, musca domestica," *J. Egypt. Ger. Soc. Zool.*, vol. 2, pp. 277-288, 1991.

[3] J. T. Steveans and D. D. Summer, *Herbicides. Papes 13171408 in W.J.Hayes. Jr., and E.R. Laws, Jr., (Eds). Handbook of pesticide toxicology*. San Deign, Calif: Academic Press, 1991.

[4] M. Brumo, A. M. Piozzi, F. Puech, S. Rosselli, and M. S. J. Simmonds, "Neoclerodane diterpenoids from teucrium poloum sub sp. Poloum and their antifeedant activity," *Biochemical Systematics and Ecology*, vol. 2, pp. 1051-1056, 2003.

[5] M. M. Sadek, "Antifeedant and toxic activity of adhatoda vesica leaf extract against spodoptera littoralis (Lep. Noctuidae)," *J. Applied Entomology*, vol. 127, pp. 396-404, 2003.

[6] M. Nakatani, S. A. M. Abd El-Galeil, J. Kurawaki, H. Okamura, T. Iwagawa, and M. Doe, "Antifeedant ring B and D opened limonoids from khaya senegalensis," *J. of Natural Products*, vol. 64, pp. 1263-1265, 2001.

[7] G. H. Schmidt and S. Assembe-Tsoungui, "Effect of oral application of melia fruit extract on growth, development and gregarization of the desert locust schistocerca gregaria (Forsk) (Caelifera, Acridiae)," *Zeitschrift fur Pflanzenkrankheiten und Pflanzenachutz*, vol. 109, pp. 38-56, 2002.

[8] N. A. Farag, "Effect of haphyllum tuberculatum extracts on an insect on an insect pest spodoptera littoralis and the predator, coccinella und ecimpunctata," *Ann. of Agric. Sci. Cairo.*, vol. 47, pp. 1097-1105, 2002.

[9] M. R. Strand, "The insect cellular immune response," *Insect Science*, vol. 15, pp. 1-14, 2008.

[10] F. Vogelweith, D. Thiery, B. Quagliett, Y. Moret, and J. Moreau, "Host plant variation plastically impacts different traits of the immune system of a phytophagous insect," *Functional Ecology.*, vol. 25, pp. 1241–1247, 2011.

[11] P. Trinder, "Determination of glucose in blood using glucose oxidase with an alternative oxygen accepter," *Ann. Clin. Biochem.*, vol. 6, p. 24-27, 1969.

[12] B. Freedman, L. J. Nowak, W. F. Ewalek, E. C. Berry, and W. D. Guthrie, "A bioassay for plant derived pest control agents using European corn borer," *J. Econ. Ent.*, vol. 72, pp. 541-545, 1979.

[13] W. S. Abbott, "A method of computing the effectiveness of an insecticide," *J. Econ. Ent.*, vol. 18, pp. 265-267, 1925.

[14] D. J. Finney, *Propit analysis*, 3rd ed. London: Cambridge Univ. Press, 1971.

[15] R. F. A. Baker, N. M. El-Barky, M. F. AbdElaziz, M. H. Awad, and M. E. Abd El-Halim, "Effect of chitin synthesis inhibitors (Flufenoxuron) on some biological and biochemical aspects of the cotton leafworm S. littoralis bosid (Lepidoptera: Noctuidae)," *Egyptian Academic Journal of Biological Sciences*, vol. 2, pp. 43-56, 2012.

[16] S. Reitman, S. Frankel, and J. Amer, "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases," *Clin. Path.*, vol, 28, pp. 56, 1957.

[17] T. E. Weichselbaum, *Amer. J. Clin. Path., Tech. Sect.*, vol. 10, p. 40, 1946.

[18] N. Zollner, and K. Kirsch, "Colorimetric method for determination of total lipids with sulfophosho-vanillic mixture" *Z. Ges. Exp. Med.*, vol. 135, p. 545, 1962.

[19] L. Lundergren, "Natural plant chemicals acting as oviposition deterent on cabbage butter flies, pieris rapae and P. napi," *Zool. Seri.*, vol. 4, pp. 250-258, 1975.

[20] M. A. M. Khawas and H. A. S. Abd El-Gawad, "The efficiency of two plant extracts (Fenugreek and Lupine) and commercial biofungicide (Biofly) on the cotton leafworm, spodoptera littoralis (Boisd) (Lepidoptera:Noctuidae) larvae as a new approach of control," *J. Egypt. Ger. Soc.Zool.*, vol. 37, pp. 39-57, 2002.

[21] N. Badr, A. M. Sondos, and S. Abd El-Haleem, "Effect of seed oil extracts on the different developmental stages of the Egyptian cotton leafworm, spodoptera littoralis (Boisd)," vol. 10, pp. 39-50, 2000.

[22] J. D. Stark and T. M. Rangus, "Lethal and sublethal effect of the neem insecticide formulation, margosan-o on the pea aphid," *Pestic. Sci.*, vol. 41, pp. 45-47, 1994.

[23] H. Schmutterer, "Properties and potential natural pesticides from the neem tree, azadirachta indica," *Ann. Rev. Ent.*, vol. 35, pp. 271-297, 1990.

[24] D. T. Lowery, M. B. Isman, and N. L. Brard, "Laboratory and field evaluation of neem for the control of aphids (Homoptera: Aphididae)," *J. Econ. Ent.*, vol. 86, pp. 23-28, 1993.

[25] C. Roger, C. Vincent, and D. Coderra, "Mortality and predation efficiency of coleomegilla maculate lengi timb. (Col.,Coccinellidge) following application of neem extracts (Azadirachta Indica A. Juss.,Meliaceae)," *J. App. Ent.*, vol. 119, pp. 439-443, 1995.

[26] S. M. Rawi, H. El-Gindy, and A. Abd El-Kader, "New possible molluscicides from calendula micrantha officinalis and ammi majus. II: Molluscicidal, physiological and egg laying capacity against B. alexandrina and B. truncates," *J. Ecotoxicology and Environment Safety*, vol. 35, pp. 261-267, 1996.

[27] H. Schmutterer, "Potential of azadirachtin containing pesticides for integrated pest control in developing and industrialized countries," *J. Ins. Phys.*, vol. 34, pp. 713-719, 1988.

[28] A. Kogam, "Natural chemicals in plant resistance to insect," *Towa State J. of Research*, vol. 60, pp. 501-527, 1986.

[29] J. A. Klock and B. Chan, *Chemistry and technology of agrochemical formulations*. Boston/London: Kluwer Academic Published Dordrecht, 1986.

[30] S. M. Abdel Rahman, E. M. Hegazy, and A. E. Elwey, "Direct and latent effects of two chitin synthesis inhibitors to S. littoralis larvae (Boisd)," *Journal of Agricultural & Environmental Sciences*, vol. 2, pp. 457–464, 2007.

[31] R. Mulder and M. J. Gijswigt, "The laboratory evaluation of twopromising new insecticides which interfere with cuticle deposition," *Pesticide Science*, vol. 4, pp. 737 –745, 1973.
[32] I. Gelbic, M. M. Adel, and H. M. Hussein, "Effects of nonsteroidal ecdysone agonist RH-5992 and chitin biosynthesis inhibitor lufenuron on S. alittoralis (Boisduval, 1833)," *Central European Journal of Biology*, vol. 6, pp. 861- 869, 2011.
[33] N. S. Butter, G. Singh, and A. K. Dhawan, "Laboratory evaluation of the insect growth regulator lufenuron against H. paarmigera on cotton," *Phytoparasitica*, vol. 31, pp. 56-60, 2003.
[34] D. Biddinger, L. Hull, H. Huang, B. Mcpheron, and M. Loyer, "Sublethal effects of chronic exposure to tebufenozide on the development, survival and reproduction of the tufted apple bud moth," *Journal of Economic Entomolology*, vol. 99, pp. 834-842, 2006.
[35] S. G. Salokhe, J. K. Pal, and S. N. Mukherjee, "Effect of sublethal concentrations of flufenoxuron on development, growth and reproductive performance of tribolium castaneum," *Journal of Invertebrate Reproduction & Development*, vol. 43, p. 141, 2008.
[36] M. A. M. Osman and M. F. Mahmoud, "Effects of bio-rational insecticideson selected biological aspects of the Egyptian cotton leafworm S. littoralis (Boisd) (Lepidoptera: Noctuidae)," *Journal of Plant Protection Research*, vol. 49, pp. 147-154, 2009.
[37] N. Soltani and N. S. Mazouni, "Diflubenzuron and oogenesis in the codling moth cydia pomonella," *Pesticide Science*, vol. 34, pp. 257-261, 1992.
[38] M. R. Kanost, J. K. Kawooya, J. H. Lan, R. O. Ryan, M. C. Van Heusden, and R. Ziegler, *In advanced insect physiology. (Eds). PD. Evans and V.B. Wigglesworth* vol. 22. London: Academic Press, 1990.
[39] E. H. Shaurub, Ahmed, Z. A. Samira, and E. M. El-Naggar, "Impacts of pyriproxyfen and extracts of schinus terebinthifolius, on development, reproduction and reproductive organs in S. littoralis," *Journal of the Egyptian German Society of Zoology*, vol. 27, pp. 57-82, 1998.
[40] S. M. Meola and R. T. Mayer, "Inhibition of cellular proliferation of imaginal epidermal cells by diflubenzuron in pupae of the stable fly," *Science*, vol. 207, pp. 985-987, 1980.
[41] L. Nabih, Z. El-Dardiri, A. El-Ansary, and S. A. Ahmed, "Kinetic properties of two transaminases and lactate dehydrogenase in fresh water snails specific intermediate host for human schistosomiasis," *Cell. Mol. Biol.*, vol. 36, p. 375, 1990.
[42] S. M. Rawi, H. El-Gindy, A. M. Haggag, A. Abou El Hassan, and A. Abdel Kader, "Few possible molluscicides from calendula micrantha officinalis and ammi majus plants. I. Physiological effect on B. alexandrina and B. truncatus," *J. Egypt. Ger. Soc. Zool.*, vol. 16, pp. 69-75, 1995.
[43] R. Abo El-Ela, N. Helmy, Q. Monairy, and H. Salah, "Effect of certain plant extracts on some biochemical aspects of the housy fly larvae musca domestica (Dipetra Muscidae)," *Bull. Ent. Soc. Egypt, Econ. Ser.*, vol. 22, pp. 17-25, 1997.
[44] K. C. Chitra and T. S. V. Reddy, "Effects of annona squamosa L. seed extract on protein metabolism of spodoptera liture," *Fab. Insect Environmental*, vol. 6, pp. 39-40, 2000.
[45] A. M. Hashem, A. El- Ansary, and L. M. Faddah, "Effect of some organophorus insecticides on amylases and cellulases in biophalaria alexandrina snails," *Egypt. J. Bilh.*, vol. 2, pp. 19-35, 1993.
[46] S. A. Mostafa, "Biochemical effect of some chemical compound on spodoptera littoralis(Boisd)," P.H.D. Thesis, Fac.Agric. Al-Azhar Univ. Egypt., 1993.