Insecticidal activity of *Calotropis procera* extracted groups on some biochemical aspects of the house fly, *Musca domestica* vicina (Diptera: Muscadae)

Najat A. Khatter and Faten F. Abuldahab

King Abdulaziz University, Faculty of Science for Girls- Biology Department Jeddah, Saudi Arabia
najat.khatter4@gmail.com

Abstract: *Calotropis procera* (Family: Asclepidaceae) is known to contain alkaloids, steroids and resinous substance. Fresh leaf extract of milkweed juice showed larvicidal properties against *Musca domestica* larvae. The methanolic extracted groups (clalactin, calotoxin and calotropin) were tested at the dose of 80 µg / larvae, topically to 3rd larval instar. The effect of latex active groups is most probably enzymatic in nature. A significant increase in the mean total carbohydrate content was noticed as a result of calotoxin treatment, the increase was 89.6% and 29.75% after 24 and 48 hrs, respectively. The tested groups were arranged according to their efficiency in increasing of total lipid contents as follow Calactin, Calotropin and calotoxin. An increase in lipid contents was observed and explained as, the toxic groups increase the conversion rate of carbohydrates to lipids and stored in fat tissues. It is evident that all tested groups cause reduction in total protein content the groups were arranged ascendingly according to their efficiency in decreasing the mean total protein as follows : Calotoxin, Calotropin and Calactin. The results of this study suggest the utility of *C. procera* extracted groups as potential technology for control of *M. domestica* larvae in breeding places.
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Key words: Toxicity – metabolism – larvicide – medicinal plants – house fly – pests – proteolytic enzymes – alkaloids.

1. Introduction

The use of chemical pesticides is a risk factor. Besides many insect-vectors and reservoirs of diseases have developed resistance to a wide range of insecticides (WHO, 1986). So, many authors, (Morsy *et al.,* 1998) worldwide screened the effect of the different indigenous medicinal plants and herbs in pest control. The target fly species and that have minimal detrimental effects on the environment have become a must. Thus, fly control through the use of effective, biodegradable, economically feasible botanical extracts is an important alternative control strategy. The potential use of phytochemicals from weeds and wild-growing plants offers an additional economic incentive for their production and development. Some plant species accumulate bioactive chemicals differentially in various parts of the plant, such as leaves, flowers, seeds, roots and bark. Such phytochemicals may produce larvicidal, pupicidal and adulicidal effects, most behaving like general toxicants (Quadri and Rao, 1977; 1980; Ahmed *et al*., 1981; Chavan and Nikam, 1982a; b; Kubo *et al.,* 1984; Jacobson, 1985; El-Nagger and Mosallam, 1987; Naqvi, 1987; Miller and Chamberlain, 1989; Green *et al*., 1991; Gbewonyo and Candy, 1992; Abdl-Hamid, 1993; Al-Moajel, 1993; Perich *et al*., 1994; Nassar, 1995 ; El-Shazly *et al*., 1996 and Khatter, 2010).

Calotropis procera (Family: Asclepidaceae) is an evergreen plant inhabiting Gassim farms and probably other regions in Saudia Arabia. It has been included among toxic plants in Gassim region (Omar, 1987). It is slow growing weed commonly found in dry, arid, uncultivated soils. *C. procera* leaves latex and flowers have been used in Unani and Ayurvedic medicines. Latex of *C. procera* has also been shown to to have ovicidal property against mosquitoes (Mittal and Subbarao, 2003). The latex contents have been analyzed by various researchers and a list of components, (Chopra, 1933 and Bruce *et al*., 1985), include gutta-percha rubber, proteolytic enzymes, alkaloids, carbohydrates, amino acids, hydrocarbons resembling petroleum etc. The latex contains proteolytic enzymes.

The aim of the present study is to estimate the effect of extracted toxic groups of *Calotropis procera* latex on the physiological aberration induced as a result of the treatment with these groups, total carbohydrate content, total protein content and total lipid content in the homogenated 3rd larval instar of *Musca domestica* vicina.

2. Material and Methods:

The tested plant (*Calotropis procera*)

This plant was reported to be insecticidal (Farnsworth *et al.,* 1975). Its latex is very poison and yielded five crystalline bodies ; calactin C29 H40 O9, calotoxin C29 H40 O10, calotropin C29 H20 O11, uscharidin C29 H38 O9 and uscharin C31 H41 O8.

Extraction and separation of pure groups by soaking method:

The extraction of pure crystals from latex of *C. procera* was carried out according to Court *et al.*(1964) as follows: Samples (300 ml) were immediately collected from the plant and soaked in ethanol 95%

(300 ml) for 48 hours at 40-60°C on a thermostatic water bath, then it was filtered off (A). The latex coagulate was resoaked in ethanol 95% (400 ml) in the same manner and filtered off (B) . The two filtrates (A+B) were mixed together and kept in a freezer at 0-4°C for 48 hrs as crude latex and filtered off (C), the precipitate (In) was found to be white crystals (5.98 gm, m.p. 84-88°C range). The filtrate (C) was kept under cooling for further 48 hrs and filtered off (D), giving further portion of white crystals (If1) (1.8 gm, m.p. 82-90°C range). It was found that some crystals did not melt up to 290°C The filtrate (D) was also kept under cooling (0-4°C) for three weeks and filtrated off (E) to give pale yellow flakes precipitate II (1.12 gm, m.p. 84-90°C). However, If1, If2 and II portions are considered as solid mixture of crude latex cardenolides.

The filtrate (E) was concentrated to about one third of its volume using rotary evaporator, the concentrate (F) was kept under deep cooling for 48 hrs, giving a brownish yellow precipitate (3.77 gm) which was dissolved in acetone and the insoluble part was separated and dried to give pale yellow crystals, Calactin, (0.8 gm, m.p. 248-249°C). Another portion of concentrate (F) was further concentrated using rotary evaporator, producing an oily brown layer which was filtered off and clear filtrate was kept on water bath at 40-60°C for one hour and then cooled (0-4°C) to produce calotoxin . Silvery crystals, calotropin, (0.65 gm, m.p. 221.5°C) were produced.

Test insects

a- Sources of colony

Adult susceptible strain of house fly *M. domestica vicina* used in the present study were obtained from well established colony originated from King Abdulaziz University, Faculty of Science for Girls-Biology Department.

b- Rearing technique

Egg masses were used to maintain a colony in the laboratory under constant conditions of temperature and humidity (27 ± 2°C and 60 ± 10% R.H.). Each egg mass was placed in a clean petri dish (10 cm diameter), previously constant technique described by (Lelwallen, 1954). Full grown larvae were allowed to pupate in clean glass petri-dishes. Following emergence, the adults were provided with a piece of cotton soaked in 10% sugar, 2% milk solution as a source of food.

Treatment of newly emerged 3rd larval instar:

Studies were conducted in a rearing chamber at 27± 2°C and 60 ± 10% R.H. . Extracted groups (calactin, calotoxin and calotropin) were applied topically on the dorsal surface of newly moulted early 3rd larval instar (twenty five larvae) with dose of 80 µg/larva by using Hamilton serange as water extract . After application, larvae were put in small plastic cups, 7 cm in diameter, and covered with larval medium . This experiment was replicated three times. The larvae of control groups were treated with 1 µL of the solvent only and replicated two times. Samples were taken after 24 and 48 hrs. after treatment of early and late 3rd larval instars to determine total carbohydrates, proteins and lipids contents.

a) Preparation of samples for the determination of total proteins and total carbohydrates: A known weight of larvae was homogenate in two milliliters of 10% saline solution using a homogenizer for about two minutes. The tubes were centrifuged and the supernatant was used to determine total proteins or total carbohydrates or stored in freezer until used.

b) Preparation of samples for the determination of total lipids: A known weight of larvae were homogenate in two milliliters of 1 : 1 chloroform – methanol using a homogenizer for about two minutes. The tubes were centrifuged and the supernatant was taken and heated until evaporation of the solvents. The precipitate was redissolved in 2 ml ethanol and used to determine total lipids or stored in freezer until used.

A) Determination of total proteins:

Protein reacts with folin ciocalteau reagent to give a blue coloured complex. The color so formed is due to the reaction of alkaline copper with protein. The intensity of color was measured by using colorimeter at 750 nm. according to (Lowery *et al*., 1951)

Reagents :

a) Alkaline sodium carbonate solution, 2% Na_2 Co_3 in 0.1 N NaOH (Solution I):

20 gm sodium carbonate were dissolved in 1 liter of distilled water, then 4 gm sodium hydroxide were added. This solution can be kept for about 2 months in brown bottle at room temperatures.

b) Copper sulphate sodium potassium tartarate solution, 0.5% $CuSO_4$ – $5H_2O$ in 1% Na, K tartarate (solution II):

5 gm copper sulphate were dissolved in 1 liter water, then 10 gm sodium potassium tartarate were added. This solution was freshly prepared every two days.

c) Alkaline solution (solution III):

Prepared on day of use by mixing 50 ml of solution I with 1 ml of solution II.

d) Folin ciocalteau reagent, stock solution:

Before use, one volume of syock solution of Folin reagent (SIGMA) was diluted with an equal volume of distilled water. Stock solution was kept at – 20 C°.

e) Standard protein albumin solution (0.5 mg/ml distilled water):

50 mg bovine albumin were dissolved in 100 ml distilled water. This solution can be kept for about one week at – 20 C°.

Procedure:

0.2 ml of diluted whole body extract was added to 5 ml of the alkaline solution (solution III). In the blank experiment distilled water was replaced by the whole body extract while in the standard experiment the standard protein albumin solution was used. The solution was mixed well and allowed to stand at room temperature for 10 minutes. Half ml of the diluted folin - ciocalteau reagent was added 10 minutes. Half ml of the diluted folin – ciocalteau reagent was added and mixed rapidly after 30 minutes at room temperature, the intensity of the violete colour obtained was compared spectrophotometrically against the standard at 750 nm.

Calculation :

$$\frac{\text{absorbanc of sample}}{\text{dabsorbance of standard}} \times \text{concentration of standard} \times \frac{1}{\text{volume of sample used}} \times \frac{100}{\text{wt. of sample homogenized}}$$

= mg. Protein / gm of fresh body

B) Determination of total carbohydrate:

The total carbohydrate content of the whole body was determined according to (Singh and Sinha, 1977) .

Reagents:

a- Anthrone reagent was prepared by the addition of 72 ml analar sulphuric acid (B.D.H) to 28 ml distilled water. While this mixture is still warm, 50 mg of anthrone was added with vigorous shaking.

b- Standard solution was prepared by the addition of 50 gm glucose to 100 ml distilled water

Procedure:

Aqueous sample and diluted with 1 ml distilled water then treated with 5 ml freshly prepared anthrone reagent. Blank was prepared by adding 5 ml of anthrone reagent to 1.2 ml H2O. Standard was prepared by adding 0.2 ml of Standard solution to 1 ml distilled water and then treated with 5 ml anthrone reagent. All test tubes of blank, unknown and standard were placed in boiling water bath for 10 minutes. Then left to cool for 15 minutes at room temperature in a dark place. Reagents were made at 620 nm. using colourimeter.

Calculation :

$$\frac{\text{absorbanc of sample}}{\text{dabsorbance of standard}} \times \text{concentration of standard} \times \frac{1}{\text{volume of sample used}} \times \frac{100}{\text{wt. of sample homogenized}}$$

= mg. Carbohydrate / gm of fresh body

C) Determination of total lipid:

According to (Knight *et al.,* 1972), the total lipids were determined by colorimetric method. Lipids react with phosphor vanilline reagent to give rose or pink color, the intensity of color was measured by using colorimeter at 520 nm.

Reagents:

a- Vanilline reagent (0.04M):

Dissolve 6.1 of vanilline in H2O and dilute to 1 Liter. This solution is stable for about two months in a brown bottle at room temperature.

b- Phosphovanilline reagent:

Add 350 ml of the vanilline reagent and 50 ml of water to flask. Add with constant stirring, 600 ml of conc. Sulphuric acid (85%). This solution is also stable for about 2 months in brown bottle at room temperature.

c- Standard solution:

In a 100 ml flask 0.5 ml of a good grade of Olive oil was added. The flask weighted to obtain the exact weight of the oil. The concentration was adjusted to exactly 500 mg /100 ml with absolute ethanol. The solution is kept for about one month.

Procedure:

In separate tubes add 0.5 ml of H_2O (blank), 0.5 ml of sample and 0.5 ml of the standard solution. To each tube add 5 ml of concentrated sulphuric acid. Mix well on a vortex mixer. Place all tubes in boiling water bath for 10 minutes. Remove and cool in water to room temperature. To each tube add 10 ml of phosphovanilline reagent and mix well. Incubate at 37 C° in water bath for 15 minutes. Cool and read standard against blank at 540 nm.

Calculation :

$$\frac{\text{absorbanc of sample}}{\text{dabsorbance of standard}} \times \text{concentration of standard} \times$$

$$\frac{1}{\text{volume of sample used}} \times \frac{100}{\text{wt. of sample homogenized}}$$

= mg. Lipid / gm of fresh body

Results and discussion

The effective groups (clalactin, calotoxin and caloropin) of *Caltropis procera* were topically applied to early 3rd larval instar of *M. domestica* to estimate the physiological aberration induced as result of treatment with these active toxic groups at the highest dose of 80 µg / larva.

Table (1). showed the mean total carbohydrate in mg/gm fresh body weight in early 3rd larval instar, after24 and 48 hrs from application. Data showed that the mean total carbohydrate content increases remarkably during the larval period in the control groups, it was 8.2 ± 0.57 mg/gm after 24 hrs and reached to 16.3 ± 0.68 mg/gm after 48 hrs. The obtained results revealed that the total carbohydrate content estimated in the treated early 3rd larval instar, with calotropin group was 21.44 ± 0.96, 25.77 ± 1.23 mg/gm after 24 and 48 hrs, respectively. This means that the toxic group causes increase in the total carbohydrate content than control by 198% and 58.09% respectively. This differences were statistically very highly significant. Our results, are in agreement with (Abou-Ela *et al.,* 1989) who stated that hormonal treatment of different larval instar of *Spodoptera littoralis* showed a relative increase in the haemolymph total and reducing sugars, this due to a decrease in the uptake in the different physiological process especially chitin synthesis. Similar results were obtained also by different investigators, (Laila and Moursy, 1997 and Morsy *et al.,* 2001). The larvicidal properties of leaf extract of *C. procera* were tested against mosquitoes by (singh *et al*., 2005), the results of this study suggest the utility of milkweed as potential technology for insect control. Contradictory results were obtained by (Taha *et al.,* 1989) who found a significant decrease in glycogene content in the different nymphal instar of *Shistocerca gregaria* after treatment with the acetone extract of *Venca rosea*; *Colcasia antiguorum* and *Melia azedarach*. Again our results were in agreement with that obtained by many other authors, (Morsy *et al*., 2000; Reddy *et al*., 2002; Scott *et al.,* 2003; Seth *et al.,* 2004; Cespedes *et al*., 2005; Pavela ,2005; Abdel Halim and Morsy, 2005; Al-Mathal and Fouad, 2006; Shoukry, 2006; Khatter and Abuldahb, 2010 and khatter, 2010).

Data in table (2) showed that the mean total lipids increases gradually in quantity during the larval period in control groups from 40.92 ± 1.96 mg/gm to 52.05 ± 0.97 mg/gm after 24 and 48 hrs, respectively. The mean total lipid content in early 3rd larval instar treated with calctin was 52.63 ± 2.45 and 87.8 ± 2.1 mg/gm after 24 and 48 hrs. respectively. These values were significantly increased than controls by 28.6% and 68.68% after 24 and 48 hrs. respectively. From the mentioned results, it is evident that the toxic groups of the *C. procera* used causes significantly increasing in the total lipid of early larval instarof *Musca domestica*. In the present study an increase in lipid contents was explained that the toxic groups increased the conversion rate of carbohydrates to lipids, the tested compounds mainly affected the fat body led to strong accumulation of carbohydrates in tissues.

The obtained data in Table (3) indicates that, the total protein of normal larvae is higher than that of treated one through the larval periods. Treatment with three toxic groups (calactin, calotropin and calotoxin) induces highly significant reduction in the total protein content of the treated larvae after 24 hrs of treatment .

Protein values in the larvae treated with the toxic groups were, 52.8 ± 0.82, 54.23 ± 0.85, 55.66 ± 0.88 and 56.56 ± 0.42 mg/gm fresh body weight respectively. The levels were lower than controls by 18.11%, 15.89%, 13.67% and 12.28% for the mentioned effective groups, respectively. The plant groups were arranged ascendingly according to their efficiency in decreasing the main total protein as follows calotoxin; calotropin; calactin. The present data agreed with that of (Shonouda *et al.,* 2003, Pineda *et al.,* 2004, Seth *et al.,* 2004 Nathan *et al*., 2005). Also, the data agreed with that of (Pavela, 2005) who tested thirty-four essential oils against larvae of *S. littoralis*, found that these oils were highly toxic of the 3rd larval instars of *S. littoralis* after topical application. The high degree of biodegradation exhibited by most phyto-chemicals is what makes them eco-friendly and attractive as replacements of synthetic chemicals in the first place. Although the evaluation of phyto-chemicals is yet in its infancy and much research aims to further characterize promising agents and discover new agents in insect control programs. The present work showed a strong efficiency of the botanical extracted groups which could be used, (Stringer *et al.,* 2008) found that successful trapping of female *Thyanopusia orichalcea* (F.) in either a lure-and-kill or mass trapping system may offer an effective way to manage its population size and (Kostic *et al.,* 2008) were tested the toxicity and anti-feedant activity of *Osmium basilicum* against second instars gypsy-moth larvae in the laboratory bioassay, they found that all tested solutions showed low to moderate larvicidal effect in test residual toxicity. Again, (Gamal and Abuldahab 2012) concluded that botanicals tested individually or in combinations cause a toxic effect against mosquitoes, by this way botanicals may be used in integrated pest control manage, In conclusion, the larvicidal action of tested groups could be due to growth inhibition effects.

It is more pronounced against early instar and there is a delayed physiological processes. The toxicity of the tested groups of *C. procera* plant may be used as a part of integrated pest control of *M. domestica.*

Table (1): Effect of the three effective toxic groups, (calatin, calotropin and caboxin) of latex *Calotropis procera* plant on the mean total carbohydrate content of early 3rd larval instar of *Musca domestica* after 24 and 48 hrs after treatment.

Active plant groups	Observations							
	After 24 hrs				After 48 hrs			
	Range	Mean ± S. E. (mg carb. / gm fresh body	*P* value	Change %	Range	Mean ± S. E. (mg carb. / gm fresh body	*P* value	Change %
Control	7.14 9.52	8.20 ± 0.57	---	---	15.33 17.95	16.3 ± 0.68	---	---
Calctin	15.41 8.24	14.49 ± 1.79	P < 0.05	+ 76.7	27.38 34.28	31.79 ± 1.81	P < 0.001	+ 95.03
Calotropin	23.80 20.14	21.44 ± 0.96	P < 0.001	+ 198.0	30.73 25.76	25.77 ± 1.23	P < 0.001	58.09
Calotoxin	11.90 18.89	15.55 ± 1.65	P < 0.01	+ 89.6	24.80 18.03	21.15 ±0.61	P < 0.05	29.75

P > 0.05 : non significant *P* < 0.05 : significant *P* < 0.01 : highly significant *P* < 0.001 : very highly significant

Table (2): Effect of the three effective toxic groups, (calatin, calotropin and caboxin) of latex *Calotropis procera* plant on the mean total lipids content of early 3rd larval instar of *Musca domestica* after 24 and 48 hrs after treatment.

Active plant groups After 48 hrs Change %	Observations							
	After 24 hrs				After 48 hrs			
	Range	Mean ± S. E. (mg carb. / gm fresh body	*P* value	Change %	Range	Mean ± S. E. (mg carb. / gm fresh body	*P* value	Change %
Control	36.11 43.33	40.92 ± 1.96	---	---	53.33 49.66	52.05 ± 0.97	---	---
Calctin	49.58 58.63	52.63 ± 2.45	*P* < 0.05	+ 28.60	90.42 82.65	87.80 ± 2.1	*P* < 0.001	+ 68.68
Calotropin	60.44 52.86	57.21± 1.85	*P* < 0.01	+ 39.80	81.25 75.00	78.7 ± 1.55	*P* < 0.001	+ 51.20
Calotoxin	49.24 57.27	47.71 ± 2.24	*P* < 0.01	+ 33.69	56.16 65.00	16.00 ± 2.26	*P* < 0.05	+ 71.19

P > 0.05 : non significant *P* < 0.05 : significant *P* < 0.01 : highly significant *P* < 0.001 : very highly significant

Table (3): Effect of the three effective toxic groups, (calatin, calotropin and caboxin) of latex *Calotropis procera* plant on the mean total protein content of early 3rd larval instar of *Musca domestica* after 24 and 48 hrs after treatment

Active plant groups	Observations							
	After 24 hrs				After 48 hrs			
	Range	Mean ± S. E. (mg carb. / gm fresh body	*P* value	Change %	Range	Mean ± S. E. (mg carb. / gm fresh body	*P* value	Change %
Control	44.64 49.10	46.37 ± 1.13	---	----	61.35 57.97	60.26 ± 0.95	---	---
Calctin	39.33 30.87	35.74 ± 2.06	*P* < 0.01	-22.12	44.57 39.43	41. 43 ± 1.29	*P* < 0.001	-31.35
Calotropin	40.57 41.44	41.15 ± 0.24	*P* < 0.01	-11.26	46.37 41.64	44.11 ± 1.12	*P* < 0.001	-26.9
Calotoxin	40.70 39.50	40.34 ± 0.35	*P* < 0.01	-13.00	38.51 42.78	46.79 ± 1.64	*P* < 0.01	-13.03

P > 0.05 : non significant *P* < 0.05 : significant *P* < 0.01 : highly significant *P* < 0.001 : very highly significant

Corresponding author
Najat A. Khatter
King Abdulaziz University, Faculty of Science for Girls- Biology Department Jeddah, Saudi Arabia
najat.khatter4@gmail.com

References

Abd El-Hamid, H.S. (1993): Effect of some botanical extracts on *Musca domestica*. M. Sc. Thesis, Zagazig University, Egypt.

Abdel Halim, A.S. and Morsy, T.A. (2005): The insecticidal activity of Eucalyptus globules oil on the development of *Musca domestica* larvae. J. Egypt. Soc. Parasitol., 35(2): 631-636.

Abou El-Ela, R.G,; Abd El-Meguid, A.; Radwan, Z. and Youssef, H. (1989): Effect of juvenile hormone analogue on certain biochemical processes of *Spodoptera littoralis* (Noctuidae: Lepidoptera) during metamorphosis. Ain Shams Sci. Bull., Vol., 27: 215-230.

Ahmed, S.M.; Chander, H. and Pereira, J. (1981): Insecticidal potential and biological activity of Indian indigenous plants against *Musca domestica.* Int. Pest Control., 23(6): 174-175.

Al-Mathal, E.M.; Fouad, M.A. (2006): Effect of *Commiphora molmol on* adults, egg masses and egg deposition of *Biomphalaria Arabica* under laboratory conditions. J. Egypt. Soc. Parasitol., 36(1): 305-314.

Al-Moajel, N.H. (1993): Studies on the efficiency of the natural products of some plants in controlling *Trogoderma gran*arium. Ph.D. Thesis, Girls College of Education, Riyadh, Saudi Arabia.

Bruce Chwatt and leonard. Jan. (1985): Essential Malariology English language Book Society Alden press, Oxford II edn., p. 299.

Cespedes, C.L.; Salazar, J.R.; Martine, Z.M. and Aranda, E. (2005): Insect groeth regulatory effects of some extracts and sterols *Myrtillocactus geometrizans* (Cactaceae) against *Spodoptera* frugipera and *Tenebrio molitor*. Phytochemist., 66 (20): 2481-2493.

Chavan, S.R. and Nikam, S.T. (1982-a): Larvicidal activity of *Lasiosiphon riocephulus*. Bull. Haffkin Ins., 10: 42-44.

Chavan, S.R. and Nikam, S.T. (1982-b): Investigation of *Lantana camara* leaves for larvicidal activity. Bull. Haffkin Ins., 10: 21-22.

Chopra R.N. (1933): I ndigenous drugs of India. The Art Press, Calcutta, 310.

Court, D. H. G. Hassal, C. H. and Jones, T. L. (1964): Cardenolides. Part VI- Uscharidin, Calotropin and Calotoxin. J. Chem Soc., 2187-2194.

El-Nagger, M.E.A. and Mosallam, S.S. (1987): Insecticidal properties of some isolates from *Duranta repents*. J. Egypt. Soc. Parasitol., 17(1): 243-250.

El-Shzly, M.M.; Nassar, M.I. and El-Sherief, H.A. (1996): Toxic effect of ethanolic extract of *Nerium olender* leaves against different developmental stages of *Muscina stabulans* (Diptera- Muscidae). J. Egypt. Soc. Parasitol., 26(2): 461-473.

Gamal, Z. A. and Abuldahab, F. F. (2012). Combined effect of Biopesticides on the digestive enzymatic profiles of *Aedes aegypti* (Diptera: Culicdae). J. Egypt. Soc. Parasitology., 42(3): 1-13.

Gbewonyo, W. S. and Candy, D.J. (1992): Separating insecticidal components from an extract of roots of the African black pepper. Toxi, 30 (9): 1037-1042.

Green, M.M; Singer, J.M.;Sutherland, D.J. and Hibben, C.R. (1991): Larvicidal activity of *Tagetes minuta* toward *Aedes aegypti*. J. Am. Mosq. Control Assoc., 7: 282-286.

Jacobson, M. (1985): Insecticides from plants, a review of the literature 1941-1953. U.S. Dept. Agric. Handb., 154.

Khatte, N.A. (2010-b): Insecticidal activity of certain plant extracts against potato tuber moth *Phthorimaea operculella* (Zeller). Egypt. J. Exp. Biol. (Zool.), 6(2): 347-355.

Khatte, N.A. (2010-a): Effects of some botanical extracts on the midgut , integument and the fat body of the cotton leaf worm, *Spodoptera littoralis* (Lepidoptera : Noctuidae). J. Egypt. Soc. Parasitol., 40(2): 413-418.

Khatter, N.A. and Abuldahab, F.F. (2010): Effects of *Ricinus communis*, *Brassica nigra* and mineral oil Kemesol on some biochemical aspects of larval stage of *Spodoptera littoralis* (Biosd) (Lepidoptera: Noctuidae). J. Egypt. Soc. Parasitol., 40(1): 151-158.

Kinght, J.A.; Anderson S. and Jams M.R. (1972): Chemical basis of the sulphovanillin reaction for estimating total lipid. J. Clin. Chem., 18(3): 199.

Kostic, M.; Popovic, Z.; Brkic, D.; Milanovic, S.; Siveev, I. and Stankovie, S. (2008): Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* (Lepidoptera: Limantriidae). Bioresou. Technol., 99(16): 7897-901).

Kubo, L.; Mastsumoto, T., Kllocke, J.A. and Kami, Kawa, T. (1984): Molluscicidal and insecticidal activities of isolated from *Fagara macrophylla*. Experienctia, 40: 340-341.

Laila, E. and Morsy. T.A. (1997): Insecticidal activity of *Calotropis procera* extracts on the flesh fly, *Sarcophaga haemorrhoidalis* Fallen. J. Egypt. Soc. Parasitol., 27(2): 505-514.

Lelwallen, L.L. (1954): Biological and toxicological studies of the little house fly. Econ. Entomol., 47: 1137-1141.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.I. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Miiller, J.A. and Chamberlain, W.F. (1989): Azadirachtin as a larvicide against the horn fly, stable fly and house fly. J. Econ. Entomol, 82 (5): 1375-1378.

Mittal, P.K. and Subbarao, S.K. (2003): Prospects of using herbal products in the control of mosquito vectors. ICMR Bullin., 33(1): 1-10.

Morsy. T.A.; Abou El-Ela, R.G,; Nassar, M.M.; Khalaf, S.A.A. and Mazyad, S.A.M. (2000): Evaluation of the in-vitro pediculicidal action of four known insecticides and three medicinal plant extracts. J. Egypt. Soc. Parasitol., 30(3): 699-708

Morsy. T.A.; Mohammad, A.A and Kamelia A.M. (2001): Control of *Musca domestica* third instar larvae by the latex of *Calotropis procera* (Family: Asclepiadaceae). J. Egypt. Soc. Parasitol., 31(1): 107-110.

Morsy. T.A.; Shoukry, A.; Mazyad, S.A.M. and Makled, K.A. (1998): The effect of the volatile oils of *Chenopodium ambrosioides* and *Thymus vulgaris* against the larvae of *Lucilia sericata* (Meigen). J. Egypt. Soc. Parasitol., 28(2): 503-510.

Naqvi, S.N.H. (1987): Biological evaluation of fresh neem extracts and some neem components, with reference to abnormalities and esterase activity in insect. Natural pesticides from the neem tree (*Azadirachta indica* A. Juss) and other tropical plants. In: H. Schmuitere and K.R.S. Ascher (eds.) Proccedings of the 3rd International Neem Conference. Nairobi, Kenya. 10-15 July, 1986.

Nassar, M.M. (1995): The potential of some juvenoids, precocenes and botanical extracts for the control of Muscina stabulans. Ph. D. Cairo University, Egypt.

Nathan, S.; Kalaivani, K.; Murugan, K. and Chung, G. (2005): Efficacyof neem limonoids on *Cnaphalocrocis medinalllis* (Gunenee) (Lepidoptera: Pyralidae) the rice leaffolder. Crop Protect., 33: 187-195.

Omar, A.A. (1987): Some toxic plants in Gassim region. King Saud University, Faculty of Agriculture. Information Bull., 8: 21.

Pavela, R. (2005): Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. Fitotera-pia, 76 (7/8): 691-696.

Perich, M.J.; Wells, C.; Bertesch, W. and Tredway, K.E. (1994): Toxicity of extracts from three Tagetes against adults and larvae of yellow fever mosquito and *Anopheles stephensi*. J. Med. Entomol., 31: 833-837.

Pineda, S.; Budia, F,; Schneider, M.; Gobbi, A. and Vinuela, E. (2004): Effects of two biorational insecticides, spinosad and methoxyfenozide, on *Spodoptera littoralis* (Lepidoptera : Noctuidae) under laboratory conditions. J. Econ. Entomol., 79 (6): 1906-11.

Quadri, S.S.H. and Rao, Bh. B. (1977): Effect of combining some indigenous plant seed extracts against household insects. Pesticides (Bombay), 11(12): 21-25.

Quadri, S.S.H. and Rao, Bh. B. (1980): Effect of oleoresin in combination with neem seed and garlic clove extracts against household and stored products pests. Pesticides (Bombay), 14(3): 11-17

Reddy, g.y.; Quero, C. and Quero, A. (2002): Activity of octylthiotriflu ropropin-Z-One, a potent esterase inhibitor on growth, development and intra specific communication in *Spodoptera littoralis* and *Sesamia nonagrioides*. J. Agric. Food Chem., 50 (24): 7062-8.

Scott, I.M.; Jensen, H.; Scott, J.G.; Isman, M.B. and Arnason, J.T. (2003): Botanical insecticides for controlling agricultural pests: *Piper amides* and the Colorado potato beetle *Lentinotarsa decemlineata* Say. (Coleoptera: Chryomelidae). Arch. Insect Biochem. Physiol., 54(4): 212-225.

Seth, R.K.; Kaur, J.J.; Rao, D.K. and Revnolds, (2004): Effects of larval exposure to sub-lethal concentrations of the ecdysteroid agonists RH-5849 and tebufenozid (RH-5992) on male reproductive physiology in *Spodoptera littoralis.* J. Insect Physiol. 50 (6): 505-517.

Shonouda, M.L.; Farrag, R.M. and Salman, O.M. (2000): Efficacy of the botanical extract (myrrh), chemical insecticides and their combination on the cotton leaf worm, *Spodoptera littoralis* (Lepidoptera : Noctuidae). J. Environ. Sci. Hlth., B. 35(3): 347-356.

Singh, N.B. and Sinha, R.N. (1977): Carbohydrates, lipids and protein in the developmental stages of *Sitophilus oryzae* and *Sitophilus granaries*. Sco. Am., 70: 107-111.

Singh, R.K.; Mittal, P.K. and Dhiman R.C. (2003): Laboratory study on larvicidal properties of leaf extract of *Calotropis procera* (Family: Asclepiadaceae) against mosquito larvae. J. Commun. Dis., 37(2): 109-113.

Stringer, L.D.; El-Sayed, A.; Cole, L.M.; Manning, L.A. and Sukling, D. (2008): Floral attractants for the female soybean looper, *Thysanoplusia orichalcea* (Lepidoptera : Noctuidae). Pest manage. Sci., 64(12): 1218-1220.

WHO (1986): Resistance of Vectors and Reservoirs of Diseases to pesticides, WHO Expert Committtee, 10th Report, World Health Organization, Geneva.

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