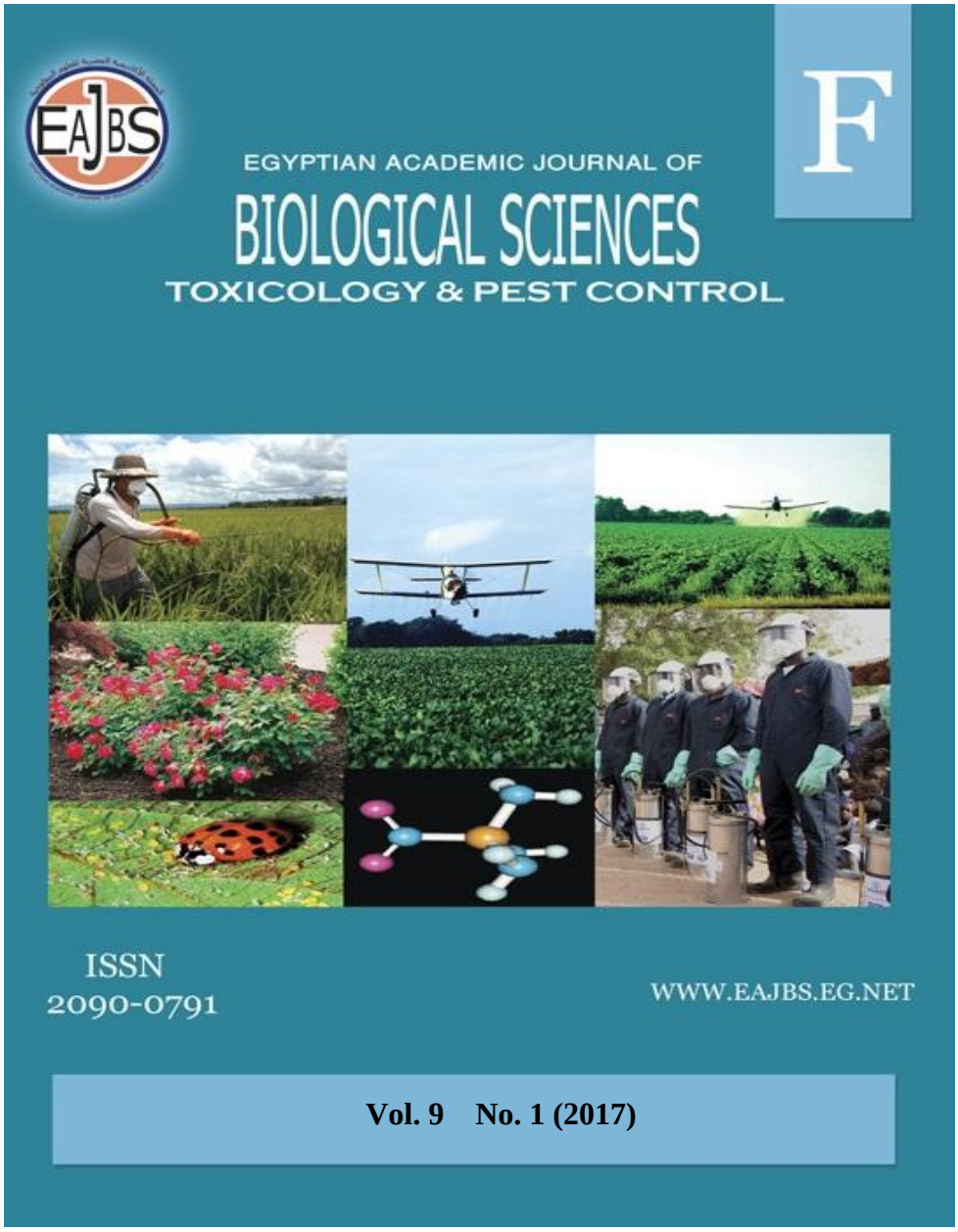


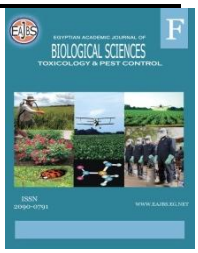
The journal of Toxicology and pest control is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the interaction between insects and their environment.

The goal of the journal is to advance the scientific understanding of mechanisms of toxicity. Emphasis will be placed on toxic effects observed at relevant exposures, which have direct impact on safety evaluation and risk assessment. The journal therefore welcomes papers on biology ranging from molecular and cell biology, biochemistry and physiology to ecology and environment, also systematics, microbiology, toxicology, hydrobiology, radiobiology and biotechnology.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 9(1): 1 – 7 (2017)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest control
ISSN: 2090 - 0791
www.eajbs.eg.net



Efficacy of Some Plant Extracts on *Lucilia Sericata* (Meigen) (Diptera: Calliphoridae)

K.S. Khater
Zoology Department, Faculty of Science, Zagazig University, Zagazig, Sharkia, Egypt.
E-Mail: kkhater@taibahu.edu.sa

ARTICLE INFO
Article History
Received: 17/12/2016
Accepted: 19/1/2017

Key words:
Anethum graveolens
Coriandrum sativum
Ailanthus altissima
Lucilia sericata
Phosphatases
protease

ABSTRACT

The insecticidal effectiveness of three acetone extracts from Tree of Heaven *(Ailanthus altissima*), Dill *(Anethum graveolens*) and Coriander *(Coriandrum sativum*) against third larval instar of *Lucilia sericata* was investigated. The extracts were highly toxic, with median lethal concentrations (LC_{50S}) of 1.37%, 0.31% and 0.87% for tree of heaven, dill and Coriander, respectively. The effects of median lethal concentration of these extracts on pupation and adult emergence percentage were also determined. The pupation and adult emergence percentage were markedly decreased after treatments. Treatments with LC_{50} disturb activity of acid and alkaline phosphatases, and protease enzymes.

INTRODUCTION

Myiasis is an important medical and veterinary problem. Larvae of *Lucilia sericata* are obligatory ectoparasites. *Lucilia sericata* is commonly referred to as sheep blowfly, since sheep are its primary host in places like Australia. The fly lay eggs in the sheep wool. The larvae feed on the skin surface causing massive cutaneous lesions and secondary bacterial infections. This causes a huge economic impact, not only does it cost money to treat infected wounds, but also, measures must be taken to control *L. sericata*.

According to Tellam and Bowles (1997) the blowflies control is accompanied by organophosphate or by insect growth regulators (IGR_S), (Levot and Sales, 2004). These methods of control are becoming ineffective because of resistance to insecticides (Whyard *et al*., 1994) and residues left in the environment. To solve the economic and environmental problems, the search for new materials from plants as an alternative source for controlling of insects is being conducted all over the world (Hasheminia *et al*., 2011).

Ailanthus altissima (Tree of Heaven), is a plant in the family Simaroubaceae. Methanol extract of *A. altissima* leaves has insecticidal activities against *Aedes aegypti* (Tsao *et al.*, 2002).

Anethum graveolens L. (Dill), is a plant in the family Apiaceae. Previous studies revealed the insecticidal activity of *A. graveolens.* Mohamed and Ali (2013) stated that methanol extract of *A. graveolens* has toxic effect against *Tribolium confusum.*

Coriandrum sativum (Coriander) is an important member of the family Apiaceae. It has insecticidal activity against different insect pests. The *C. sativum* petroleum ether extract showed a larvicidal effect against larvae of *Aedes aegypti* (Harve and Kamath, 2004).

The present study is aim to evaluate the effect of acetone extracts from *Ailanthus altissima, Anethum graveolens* and *Coriandrum sativum* plants on some biological and biochemical aspects of third larval instar of *Lucilia sericata*.

MATERIALS AND METHODS

Insects

Colonies of *Lucilia sericata* (*L. sericata*) adult were collected from Zagazig Sharkia Governorate, Egypt, using fly netting. According to El-Khateeb *et al.* (2003), flies were reared in the laboratory.

Plant extracts

Ailanthus altissima (A. altissima) leaves and seeds of both *Anethum graveolens (A. graveolens)* and *Coriandrum sativum* (*C. sativum*) were purchased from the market. Leaves and seeds powders of the three selected plants were pulverized and exhaustively extracted acetone under reflux. The acetone extracts were filtered and evaporated under reduced pressure. The extracts were kept in the refrigerator (4^oC) until use.

Testing technique

Third instar larvae of *L. sericata* were exposed to acetone extracts of plants under investigation at five different concentrations, 0.25%, 0.5%, 1.0%, 3.0% and 5.0%. The experiments were replicated four times for each concentration. Twenty-five larvae were used for each replicate. Two milliliters of the tested materials were added to 25 gm of meat in glass jars (6 cm x 9cm). In the control experiments, acetone only added to the meat. Larvae were transferred to jars containing treated meat after complete evaporation of acetone. Larval mortality was recorded 24h after treatment.

Biochemical analysis Determination of activity of acid and alkaline phosphatases

Acid and alkaline phosphatases activities were determined according to the method of Laufer and Schin (1971).

Determination of protease activity

The activity of protease was estimated according to the method of Ishaaya, *et al.* (1971).

Statistical analysis

The larval mortality average data were subjected to Probit analysis (Finney, 1971) to estimate the median lethal concentrations (LC_{50s}), with their 95% fiducial limits. A one way–ANOVA using (Statistica, 1997) performed to determine differences between larval mortality, pupal stage, adult emergence, acid phosphatases, alkaline phosphatases and Protease activity of each treatment and control.

RESULTS

Toxicological studies

The larval mortality after treatment increased as the extract concentration increased (Fig. 1). There is no mortality detected in the control experiments. The sensitivity of larvae of *L. sericata* to plant extracts was expressed by LC_{50} values of 1.37%, 0.31% and 0.87% for *A. altissima, A. graveolens* and *C. sativum*, respectively. The LC_{50} values demonstrate the efficacy of the plant extracts. The acetone extract of *A. graveolens* was 2.80 and 4.41 times more effective than *C. sativum* and *A. altissima*, respectively (Table 1).

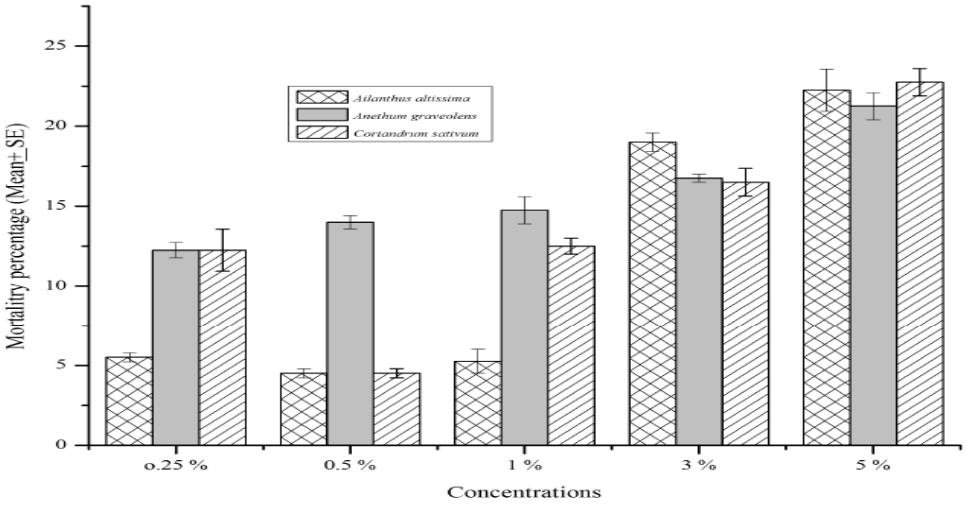


Fig. 1: Mortality percentage of *Lucilia sericata* larvae after feeding on diet treated with *Ailanthus altissima*, *Anethum graveolens* and *Coriandrum sativum* acetone extracts at different concentrations.

Table 1: LC_{50}, slope function and relative efficacy values of *Ailanthus altissima*, *Anethum graveolens* and *Coriandrum sativum* acetone extract against third larval instar of *Lucilia sericata*.

Treatments	LC_{50} (L.F.L.-U.F. L)	Slope	X^2 (df =4)	Relative efficacy
Ailanthus altissima	1.37 (0.73 – 2.81)	1.75±0.14	24.71	4.41
Anethum graveolens	0.31 (0.13 -0.55)	0.65±0.12	5.91	1.00
Coriandrum sativum	0.87 (0.40- 1.64)	1.33±0.13	16.98	2.80
Control	0	-	-	-

Larval treatments altered the biological aspects as pupation and percentage of adult emergence relative to control (Table 2). The pupation percentage reduced to-18.48%, -60% and -25.5% as a result of treatments with *A. altissima*, *A. graveolens* and *C. sativum*, respectively. The adult emergence also affected after treatments with *A. altissima* (-24.5%), *A. graveolens* (-84.6%) and *C. sativum* (-51.0%) as compared with control (Table 2).

Table 2: Effect of *Ailanthus altissima*, *Anethum graveolens* and *Coriandrum sativum* acetone extracts on pupal stage and adult emergence of *Lucilia sericata*.

Treatments	Pupal Stage		Adult Emergence	
	Mean± SE	Change %	Mean± SE	Change %
Alianthus altissima	$20±0.46^a$	-18.4%	$16.0± 1.47^b$	-24.5 %
Anethum graveolens	$9.5±0.95^c$	-60%	$3.75±1.10^d$	-84.6 %
Coriandrum sativum	$18.2±2.78^a$	-25.5%	$12.0±2.34^c$	-51.02%
Control	24.5±0.50	-	23.0± 0.70	-

Effect of extracts on acid and alkaline phosphatase activities

According to table (3) acid phosphatase (ACP) activity in body homogenate affected depending on the extract. Treatments with median lethal concentrations of *A. altissima*, *A. graveolens and C. sativum* extracts lead to clear reduction in the enzyme activity in larvae by -37.29%, -48.16% and -8.10%, respectively.

The present work also carried out to detect the effect of selected plant

extracts on alkaline phosphatase (ALP) of third larval instar of *L. sericata*.

Results in the table (3), ALP activity increased after treatment with all extracts. The most promoting action was recorded by *A. altissima* extract (39.50± 0.37) µgm phenol/min/gm body weight followed by *C. sativum* extract (26.11± 0.15) µgm phenol/min/gm body weight, and *A. graveolens* extract (9.49±0.19) compared to 7.77± 0.08 µgm phenol/min/gm body weight in control.

Table 3: Effect of treatments with acetone extracts of *Ailanthus altissima*, *Anethum graveolens* and *Coriandrum sativum* on acid, alkaline phosphatase, and protease activities of *Lucilia sericata larvae*.

Treatments	Acid phosphatase (µg phenol/min./g body weight)		Alkaline phosphatase (µg phenol/min./g body weight)		Protease (mg tyrosine/min./g body weight)	
	Mean± SE	Change %	Mean± SE	Change %	Mean± SE	Change %
Ailanthus altissima	23.60± 0.21^{c}	- 37.29	39.50± 0.37^{d}	+ 412.52	163.52± 3.00^{c}	- 11.14
Anethum graveolens	19.51± 0.37^{c}	- 48.16	9.49±0.19 ^{c}	+ 23.22	189.70± 3.91^{c}	+ 3.07
Coriandrum sativum	23.29 ±0.28^{c}	- 38.10	26.11± 0.15^{d}	+ 238.78	248.90±4.83^{c}	+ 35.24
Control	37.64 ±0.60	-	7.70± 0.08	-	184.04± 4.04	-

(+, -) increase or decrease from control

(^{c}) High significant difference ($p< 0.01$) and (^{d}) very high significant difference ($p< 0.001$)

Effect of extracts on protease activity

The data in table (3), a general enhancing action on activity of protease in larvae treated with *A. graveolens* and *C. sativum* extracts (189.70± 3.92 and 248.90± 4.84) mg tyrosine/ min/gm body weight compared to control (184.04± 4.05) mg tyrosine/ min/gm body weight. On contrary, *A. altissima* extract show clear reduction in enzyme activity (163.53± 3.00) mg tyrosine/ min/gm body weight.

DISCUSSION

The three applied extracts were effective against larvae of *L. sericata*, similar results have been recorded following treatment of *L. sericata* with certain commercial neem extracts, (El-Khateeb *et al*., 2003). Extracts of *Chenopodium ambrosiodes* and *Thymus vulgaris* have insecticidal effect on *L. sericata* (Morsy *et al*., 1998b).

In the present study, treatments with median lethal concentrations of plant extracts under investigation affect number of pupae, pupation percentage, number of adults and adult emergence percentage. Such results have been observed against *L. sericata* exposed to some plant oils (Khater and Khater, 2009) as well as with neem extracts and pomegranate (*Punica granatum*), (El-Khateeb *et al*., 2003).

The activity of acid phosphatase is usually correlated to histolysis (Spates and Wright, 1975). The activity of ACP after treatment with extracts of the selected plants showed a drastic reduction, especially with *A. graveolens* extract. These results are similar to results recorded the inhibitory effects of Jojoba oil on *Musca domestica* (Ghoneim *et al*., 2008). Senthil-Nathan *et al.* (2005) investigated, a reduction in ACP activity lead to reduction in libration of phosphorus for energy metabolism and decrease metabolism.

Alkaline phosphatase is located in cells that are most active in the synthesis of fibrous proteins that develop with histolysis of larval tissues (Bassal and Ismail, 1985). So many similar results recorded increase in ALP activity by several botanicals on different insects, Hasheminia *et al.* (2011) and Khater (2009). Increasing ALP activity in *L. sericata* larvae treated with plant extracts under investigation may indicate the contribution of this enzyme in process of detoxification against the toxins present

in the extracts as reported by Shekari *et al.* (2008).

Proteases cleave the peptide bonds in the insect food to release amino acids. The decrease in protease activity with *A. altissima* treatment may be due to the presence of protease inhibitors in this extract. This result similar to the finding of Remya *et al*. (2013). These protease inhibitors may bind with in the alimentary canal proteases of insects, interfering their digestion and reducing development in some insect species (Joesphrajkumar *et al*., 2006). On the contrary, the increase in enzyme activity with *A. graveolens* and *C. sativum* extracts treatment is in accordance with similar effects on other insects (Devanand and Usha Rani, 2011). Many insects adapted to protease inhibitor in their food, by either increasing effected proteases or by expressing novel proteases insensitive to the ingested protein inhibitors (Brousseau *et al.*,1999).

In this research, neither isolated nor identified constituents was applied on *L. sericata*, but crude extract has been applied. Thus, the active ingredient(s) needs to be explored by the future investigation.

REFERENCES

Bassal, T.M. and Ismail, I.E. (1985). Acid and alkaline phosphatases of normal and juvenilized *Spodoptera littoralis* (Lepidoptera: Noctuidae) during metamorphosis. Proc. Zool. Soc. A.R. Egypt., 9: 249-256.

Brousseau, R., Masson, L. and Hegedus, D. (1999). Insecticidal transgenic plants: Are they irresistible? CAB International, UK, 22 p.

Devanand, P. and Usha Rani, P. (2011). Insect growth regulatory activity of the crude and purified fractions from *Solanum melongena* L., *Lycopersicume sculentum* Mill. and *Capsicum annuum* L. J. Biopest., 4(2): 118-130.

El-Khateeb, R. M., Abdel-Shafy, S. and Zayed, A. A. (2003). Insecticidal effects of neem seed and vegetable oils on larval and pupal stages of sheep blowfly, *Lucilia sericata* (Diptera: Calliphoridae), J. Egypt. Veter. Medi. Associ., 63:255-268.

Finney, D.J. (1971). Probit Analysis, 3rd edition, Cambridge University Press, Cambridge, UK.

Ghoneim, K.S., Abdel-Ghaffar, A.A. and Tanani, M.A. (2008). Changes of acid phosphatase activity by the plant extracts, Margosan-Oand Jojoba, during the pupal stage of *Musca domestica* (Diptera: Muscidae). J. Egypt. Acad. Sci. Environ. Develop., 9(1): 47-55.

Harve, G. and Kamath, V. (2004). Larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents against *Aedes aegypti*. Indian J. Exp. Biol., 42: 1216-1219.

Hasheminia, S.M, JalaliSendi, J, TalebiJahromi, K. and Moharramipour, S. (2011). The effects of *Artemisia annua* L. and *Achillea millefolium* L. crude leaf extracts on the toxicity, development, feeding efficiency and chemical activities of small cabbage *Pieris rapae* L. (Lepidoptera: Pieridae). Pest. Biochem. Physiol., 99 (3): 244–249.

Ishaaya, I., Moore, I. and Joseph, D. (1971). Protease and amylase activity in larvae of the Egyptian cotton leafworm, *S. littoralis*. J. Insect Physiol., 17: 945- 953.

Josephrajkumar, A., Chakrabarty, R. and Thomas, G. (2006). Midgut proteases of the cardamom shoot and capsule borer *Conogethes punctiferalis* (Lepidoptera: Pyralidae) and their interaction

with aprotinin. Bull. Entomol. Res.,96: 91-98.

Khater, K.S. (2009). Toxicological and biological studies on the fly, *Synthesomyia nudiseta* (Wulp)(Muscidae: Diptera). Ph D. Thesis, Zagazig Univ. Egypt.

Khater, H.F. and Khater, D.F. (2009). The Insecticidal activity of four medicinal plants against the blowfly, *Lucilia sericata* (Diptera: Calliphoridae). Int. J. Dermatol., 48: 492-497.

Laufer, H. and Schin, K.S. (1971). Quantitative studies of hydrolytic enzymes activity in the salivary gland of *Chironomus tentans* (Chironomidae: Diptera) during metamorphosis. Can. Entomol., 103: 457-454.

Levot, G. and Sales, N. (2004). Insect growth regulator cross-resistance studies in field–and laboratory–selected strain of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). Aust. J. Entomol.,43:374-377.

Mohammed, H.H. and Ali, W.K. (2013). Toxic effect of some plant extracts on the mortality of flour beetle *Tribolium confusum* (Duval) (Coleoptera: Tenebrionidae). Entomol. Ornithol. Herpetol., 2(3): 115. http://dx.doi.org/10.4172/2161-0983.1000115.

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

Remya, P.P., Sameena, U., Parambath, B.P. and Meethal, K.V. (2013). Identification of plant extracts containing protease inhibitors against the gut proteases of *Spodoptera mauritia* Boisdual (Lepidoptera: Noctuidae). Acta Biol. Indica, 2 (2): 451-455.

Saeed, S.A., Naqvi, S.N.H. and Akhtar, K. (1987). Toxicity of NFC (neem extract) against *Musca domestica* L. and their effects on esterase activity. Zool. Pakis., 1(1): 25-39.

Senthil Nathan, S. and Kalaivani, K. (2005). Efficacy of nucleopolyhedro virus (NPV) and azadirachtin on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Biol. Control, 34: 93-98.

Shekari, M., Sendi, J.J., Etebari, K., Zibaee, A. and Shadparvar, A. (2008). Effects of *Artemisia annua* L. (Asteracae) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogalerucus luteola* Mull. (Coleoptera: Chrysomelidae). Pest. Biochem. Physiol., 91:66-74.

Smith, K.E., Wall, R. and Howard, J. J. (2000). In vitro insecticidal effect of fipronil and beta-cyfluthin on larvae of the blowfly, *Lucilia sericata*, Vet. Parasitol., 88:261-268.

Spates, G.E. and Wright, J.E. (1975). Effect of juvenile hormone analogue on phosphatase activity in pupae of the stable fly, *Stomoxys calcitrans*.J. Insect Physiol., 21: 1789-179

Sridhara, S. and Bahat, J.V. (1963). Alkaline and acid phosphatases of the silk worm *Bombyx mori*. J. Insect Physiol., 9: 693-701.

Statisticastatsoft Inc. (1997). Statistica release 5.1.1 Tulsa, ok, USA

Tellam, R.L. and Bowles, V. M. (1997). Control of blowfly strike in sheep: Current Strategies and future prospect. Inter. J. Parasitol., 27 :261-273.

Tsao, R., Romanchuk, F.E., Peterson, C.J. and Coats, J.R. (2002). Plant growth regulatory effect and insecticidal activity of the extracts of the Tree of Heaven (*Ailanthus*

altissima L.). B M C., Ecol., 2 (1). http://www.biomedcentral.Com/1472-6785/2/1.

Whyard, S., Russel, R.J. and Walker, V.K. (1994). Insecticide resistance and malathioncarboxyl esterase in the sheep blowfly, *Lucilia cuprina*. Biochem. Gene., 32 :9- 24.

ARABIC SUMMERY

كفاءة بعض المستخلصات النباتية ضد ذبابة لوسيليا سيريكاتا (ميجن) (رتبة ثنائية الاجنحة: كاليفوريدى)

كريمة شكري خاطر
جامعة الزقازيق- كلية العلوم- قسم ع علم الحيوان (شعبة الحشرات) الزقازيق الشرقية- مصر

تمت دراسة فاعلية الإبادة الحشرية لثلاثة مستخلصات اسيتونيه مستخرجة من نباتات شجرة السماء والشبت والكزبرة ضد العمر اليرقي الثالث لذبابة الليوسيليا سيريكاتا وظهر ان هذه المستخلصات شديدة السمية وكان التركيز نصف المميت لنباتات شجرة السماء والشبت والكزبرة هو ١,٣٧% و٠,٣١% و٠,٨٧% على التوالي.

وقد تم تحديد تأثير التركيز نصف المميت على كل من نسبة التعذر ونسبة خروج الطور البالغ. واظهرت النتائج ان هناك تأثير مثبط للتعذر ولخروج الطور البالغ.

وبمعاملة العمر الثالث لليرقة بالتركيز النصف مميت ظهر اختلال في نشاط كل من انزيم الفوسفاتيز الحامضي والفوسفاتيز القاعدي وانزيم البروتييز.