# Effect of Certain Plant Extracts on Mortality, Development and Haemogram of *Culex pipiens* L. Mosquitoes Larvae

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## ABSTRACT

**The effect of methanolic plant extracts from *Solanum nigrum*, *Acokanthera spectabilis* and *Heliotropium aegyptiacum* were investigated on the larval mortality and development of *Culex pipiens* L. Also, the effects of the $LC_{50}$ of the three plant extracts on the larval haemogram were studied. Plant extracts exhibited variable bioactivities. The greatest activity was observed for *S. nigrum* which showed $LC_{50}$ values of 130.8 ppm after 48 hr of exposure, respectively. Percentage of larval mortality was 67.5% in the treatment with extracts of *S. nigrum* and 66.5% in case of *A. spectabilis* at 500ppm. Egg hatchability was not significantly reduced in all *S. nigrum* concentrations. All concentrations of the plant extracts from *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* caused significant hindrance to the subsequent larval development and reduced both pupation and adult emergence. Drastic retardation of development was shown by *S. nigrum* extracts, where only 18.1% and 8.5% of the larval managed to reach pupal and adult stages, respectively, when reared in 100 ppm of the extract. However, *A. spectabilis* and *H. aegyptiacum* were more effective at higher concentrations. Moreover, the effects of the $LC_{50}$ of tested plant extracts caused a reduction in the number of haemocytes and also markedly decreased the haemocytes surface areas. Application of such plant extracts to mosquito breeding site may have great practical importance in relation to non-synthetic chemical control of this serious disease vectors.**

## INTRODUCTION

The house mosquito, *Culex pipiens* L. (Diptera: Culicidae) is one of the most harmful insects affecting humans and farm animals and transferring several pathogens as Cache-Valley (CV) and West Nile Virus that cause infertility and congenital malformations in ruminants (Edwards *et al.*, 1998 and Smartt and Erickson, 2008). This insect showed great resistance to the used synthetic insecticides by many mechanisms (Raymond *et al.*, 2001, Dary *et al.*, 1990). It also appeared to be sensitive to several plant extracts and products. Myrrh (oleo-gum-resin) obtained from the stem of *Commiphora molmol* proved to have insecticidal activity affecting fat, muscles, gut and nervous tissues of the larvae (Massoud and Labib, 2000). Methanolic extracts of aerial parts of the medicinal plant Argel, *Solenostemma argel* (Del.) Hayne, incorporated into rearing media of *Culex pipiens* L. reduced oviposition, hatchability and larval viability (Al-Doghairi *et al.*, 2004). Cetin *et al.*, (2006) reported high larvicidal activity of *Teucrium divaricatum* Sieber, *Mentha longifola* (L.) Huds., *M. pulegium* L., *Melissa officinalis* L. and *Saliva sclarea* L. oils against *C. pipiens*. Zhu *et al.* (2006) reported that the oils of thyme, catnip, amyris, eucalyptus and cinnamon revealed larvicidal activity besides repellent effect on this pest. Several plant species extracts killed and altered developmental periods, pupation rates and adult emergences of this insect (Khater and Shalaby, 2008). Several pure active potential sources of larvicidal substances against mosquito were isolated as beta-thujaplicin from *Chamaecyparis obtuse* leaves (Jang *et al.*, 2005), 7-hydroxycoumarin from *Stellera chamaejasme* root powder (Xiaorong and Taiping, 2008) and pure synthetic natural naphthoquinones (alkannin, shikonin and shikalkin) and three acetylated derivatives of shikonin (Michaelakis *et al.*, 2009). Control of *C. pipiens* is becoming increasingly difficult because the over production of detoxifying mechanisms of chemicals insecticides by this insect (Severini, *et al.*, 1993). The botanical insecticides are generally pest specific and are relatively harmless to non-target organisms including humans. They are also bio-degradable and harmless to environment. The phytochemicals derived plant resourses can act as larvicides, insect growth regulators, repellent and ovipositional attractant (Das *et al.*, 2003, Venkatachalam and Jebanesan, 2001). One plant species may possess substances with a wide range of activities, like extracts from *Azadiracta indica* which showed antifeedent, antioviposition, repellent and growth-regulating activity (Schmutterrer, 1995). Insecticidal activity of many plants against several insect pests has been demonstrated (Carlini and Grossi-de-sa, 2002, Kundu, *et al.*, 2007 and Boussada, *et al.*, 2008).

In this study the effects of methanolic extracts of aerial parts from three plant species *Solanum nigrum*, *Acokanthera spectabilis* and *Heliotropium aegyptiacum* on egg hatchability, larval development and different haemocyte counts of *C. pipiens* mosquitoes, have been investigated.

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## MATERIALS AND METHODS

**Insects:** A *Culex pipiens* L. (Diptera: Culicidae) colony maintained in the laboratory for more than 10 years was used. Mosquitoes were held at 27±1C°, 70±5% RH and a photo period of 14:10 (light:dark). Adults were provided with a 10% sucrose solution as food source. A pigeon was introduced twice a week to the adults for blood feeding. Larvae were reared in dechlorinated water under the same temperature and light conditions and were fed daily with baby fish food. The experiment was carried out at the faculty of agriculture, Alexandria University, Egypt.

**Plant extracts:** Fresh fruits (1100 gm) of *Solanum nigrum*, *Acokanthera spectabilis* and *Heliotropium aegyptiacum* for each were collected from different places (Abbis, Wady el-Natrun) in Egypt. The collected samples were washed from dust and freshly blended with acetone (1.0 L) times for a month in the dark at room temperature. Fruits were air-dried for 5 days, ground to fine parts and extracted with 500ml of methanol at ambient temperature. A gentle warming to 35-40 C° was sometimes found necessary. The mixture was stirred for 30 minutes by magnetic stirrer and left 24 hr. Then, it was concentrateded in a rotary evaporator under vacuum of solvent in a water bath at 55 C° according to Chitra *et al.*, 199). The resulted filtrate was further concentrated to 0.25 L for each plant fruit.

**Test procedure:** Stock solutions of three plant methanolic extracts were prepared by dissolving the extracted in warm distilled water (at 0.5g / 100ml water) containing 0.5% Triton X100 as an emulsifier to ensure complete solubility of the extract in water. Different concentrations of 100, 200, 300, 400 and 500ppm were prepared from stock solutions. Ten 2nd instar larvae were transferred from the culture into plastic cups (8 cm diameter, 10 cm depth) each containing 30 ml of desired concentration. Treatments were triplicate and control had only distilled water. Larvae were fed daily and kept under laboratory conditions. 500 eggs were treated in water by the same previous concentrations of the plant extracts, 5 replicates (100 eggs for each). Egg hatchability was determined at 2 days after treatment. Larval mortalities were counted at 7 days after treatment. Percentage of successful pupation and adult emergence were determined by monitoring on daily basis until all adults in the control have emerged.

**Haemocyte studies:** The hemolymph of treated larvae with the $LC_{50}$ of each tested plant extract were separately smeared to thin film between two glass slides .The blood smears were air–dried, stained with Wright's blood stain (Essawy, 1990) for 1 min and distained for 2 min. with 70% ethyl alcohol.

The larval hemocytes were examined using light microscope. Blood samples were obtained from 5 larvae of each extract and control . Each blood sample was replicated three times. The haemocyte types were examined and identified under oil immersion (100X) using a stereomicroscope. Different Haemocyte Count (DHC) was carried out in random scans of blood films (100 haemocytes from each film). The identification of haemocyte types were performed according to (Arnold and Hinks, 1983) . In addition, the surface areas of each haemocyte type were measured by a micrometric slide in all treatments plus the control.

**Statistical analysis:** Data were analyzed using maximum likelihood procedures and values of $LC_{50}$ were calculated according to finney (1971). Data were corrected for control mortality (Abbott, 1925). Data of egg hatchability were analyzed by analysis of variance. If significant differences ($p<0.05$) occurred, means were separated by Duncan's multiple range test.

## RESULTS AND DISCUSSION

The mortality of *C. pipiens* larvae treated with three plant methanolic extracts and their $LC_{50}$ values and 95% confidence limits at 48 hr after treatment are shown in Table 1. Results showed that the most toxic extract was *S. nigrum* followed by *A. spectabilis* and *H. aegyptiacum* at the concentration of 500 ppm, the larval mortality was 77.5% after 48hr of exposure in the case of *S. nigrum*, while it was 64.7% after 48 hr in the case of *A. spectabilis*, whereas the larval mortality was 21.7% after 48 hr in the case of *H. aegyptiacum*. $LC_{50}$s and 95% confidence limits (CL) for each plant are given in Table 1. Data showed a significant differences. $LC_{50}$ for 2nd instar larvae were 130.8, 403.5 and 470.9 for *S. nigrum*, *A. spectabilis* and *H. aegyptiacum*, respectively, after 48 hr of exposure. *S. nigrum* was significantly more toxic at all exposure times than *A. spectabilis* and *H. aegyptiacum*.

Egg hatchability was not significantly reduced ($p<0.05$) by treatment with all extracts compared with control (Table 2). Whereas the larval mortality was significantly reduced. *S. nigrum* extract at 500 ppm revealed the most severe effect on larval mortality which compared with *A. spectabilis* and *H. aegyptiacum* . At concentration of 500 ppm, *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* caused reduction in larval mortality of 67.5, 66.5 and 63.6%, respectively. The effect of the three plant extracts on growth and development of *C. pipiens* larvae to adulthood are given in Table 2. There were considerable reduction in the percentage of larvae undergoing successful pupation in all treatments compared with control. No further larval development took place after the 2nd instar in *S. nigrum* at 500 ppm.

**Table 1. Mortality percentage of 2nd larvae of *Culex pipiens* larvae in media containing methanolic plant extracts at different exposure periods**

| Plant extract | Conc (ppm) | Mortality% after 48hr of exposure | Slope±S.E. | $LC_{50}$ (95%CL)* |
|---|---|---|---|---|
| *Solanum nigrum* | 100 | 46.1 | 1.2±0.8 | 130.8(95.8-190.1) |
| | 200 | 54.6 | | |
| | 300 | 57.2 | | |
| | 400 | 65.9 | | |
| | 500 | 77.5 | | |
| *Acokanthera spectabilis* | 100 | 24.9 | 1.3±1.1 | 403.5(360-442) |
| | 200 | 30.1 | | |
| | 300 | 35.3 | | |
| | 400 | 52.9 | | |
| | 500 | 64.7 | | |
| *Heliotrobium aegyptiacum* | 100 | 10.1 | 1.5±2.3 | 470.9 |
| | 200 | 12.2 | | |
| | 300 | 14.9 | | |
| | 400 | 16.5 | | |
| | 500 | 21.7 | | |
| Control | 0 | 5.8 | - | - |

*$LC_{50}$ values and 95% confidence limits of 2nd instar larvae of *C. pipiens* larvae

**Table 2. Egg hatchability, Larval mortality, Successful pupation and adult emergence of *Culex pipiens* larvae reared in media containing methanolic plant extracts**

| Plant extract | Conc (ppm) | Egg hatchability at 2 days after treatment | Larval mortality (%) at 7 days after treatment | Pupation (%) | Adult emergence (%) |
|---|---|---|---|---|---|
| *Solanum nigrum* | 100 | $97.8^{a}$ ±1.8 | $16.6^{b}$±2.3 | $18.1^{b}$±1.2 | $8.5^{b}$±0.9 |
| | 200 | $97.6^{a}$ ±2.1 | $28.2^{c}$±1.9 | $15.3^{b}$±0.9 | $6.1^{b}$±1.5 |
| | 300 | $96.9^{a}$ ±1.9 | $46.9^{d}$±0.8 | $6.8^{c}$±1.5 | $4.2^{c}$±2.1 |
| | 400 | $96.7^{a}$ ±1.6 | $55.9^{d}$±2.1 | $5.3^{c}$±2.2 | $1.8^{d}$±1.4 |
| | 500 | $97.^{a}$ 2±3.1 | $67.5^{e}$±1.8 | $0.0^{e}$ | $0.0^{e}$ |
| *Acokanthera spectabilis* | 100 | $98.2^{a}$ ±2.1 | $13.2^{b}$±1.3 | $37.9^{b}$±0.8 | $12.8^{b}$±1.1 |
| | 200 | $97.9±^{a}$ 1.3 | $25.7^{c}$±1.6 | $20.1^{c}$±2.8 | $7.5^{c}$±2.2 |
| | 300 | $97.6^{a}$ ±1.6 | $42.9^{d}$±1.1 | $13.9^{c}$±1.7 | $4.9^{c}$±2.9 |
| | 400 | $97.5^{a}$ ±2.1 | $54.1^{d}$±1.7 | $7.2^{d}$±2.1 | $1.5^{d}$±1.2 |
| | 500 | $96.9^{a}$ ±0.9 | $66.5^{e}$±2.0 | $0.0^{e}$ | $0.0^{e}$ |
| *Heliotropium aegyptiacum* | 100 | $97.2^{a}$ ±1.7 | $11.8^{b}$±0.7 | $64.9^{b}$±1.1 | $36.9^{b}$±2.6 |
| | 200 | $97.5^{a}$ ±2.8 | $23.4^{c}$±1.3 | $55.9^{b}$±2.3 | $17.9^{c}$±3.1 |
| | 300 | $98.6^{a}$ ±3.1 | $39.8^{d}$±3.0 | $56.9^{b}$±0.9 | $16.9^{c}$±1.1 |
| | 400 | $96.8^{a}$ ±2.4 | $52.6^{d}$±1.1 | $29.5^{c}$±1.2 | $8.1^{d}$±0.8 |
| | 500 | $97^{a}$ ±1.3 | $63.6^{e}$±1.9 | $9.8^{d}$±2.2 | $3.2^{e}$±2.9 |
| $98.2^{a}$ | $100.0^{a}$ | $4.1^{a}$ | $98.0^{a}$ | 0 | Control |

Means followed by the same letter are not significantly different at 5% level, Duncan multiple test.

On the other hand all plant extracts had an evident inhibitory effect even at 100 ppm, whereas the successful pupation were only 18.1, 37,9 and 64.9% for *S. nigrum*, *A. spectabilis* and *H. aegyptiacum*, respectively. Complete suppression for adult emergence was evident in case of *S. nigrum*, *A. spectabilis* at 500 ppm. *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* at 100 ppm caused 8.5, 12.8 and 36.9% adult emergence, respectively.

Considerable biological activity related to toxicity and hindrance of growth and development of the larvae of *C. pipiens* has been observed in this study. *S. nigrum* was found to cause higher rate of mortality compared to other plant extracts. Srinivasan *et al.* (2013) found that *Solanum sarrachoides* was effective against green peach aphid. *A. spectabilis* and *H. aegyptiacum* exhibited a relatively mild acute effect on mosquito larvae especially in its lower concentrations. However, after 8 days of exposure the toxicity was almost high above 200 ppm. The larvicidal activity of some plant extracts, essential oils and phytochemicals against *C. pipiens* have been demonstrated (Traboulsi *et al.*, 2005; Abdelgaleil 2006; Michaelakis *et al.*, 2007; Radwan *et al.*, 2008).

Figure 1 showed the effect of the $LC_{50}$ of the tested plant extracts on the mean number of haemocytes in the haemolymph of 2nd instar larvae of *C. pipiens*.Four haemocyte types indentified as Prohaemocytes (Pr), Plasmatocytes (Pl), Granulocytes (Gr) and Oenocytoides were monitored. With regarded to *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* treatments, a reduction in the percentage of the prohaemocyte was 74.6, 62 and 56.6, in respect less than control. The number of (Gr) was markedly decreased especially after treatment with *S. nigrum* and *A. spectabilis* with reduction percentages of 48.8 and 43.6, respectively. The same trend was observed in the number of plasmatocyte which were reduced by 55 and 34.5 %, in respect .An increase was observed in the number of (Oe) about 45.16 and 61.3 %, in respect over the control. Furthermore, treatment with the $LC_{50}$ of tested plant extracts caused a decrease in the haemocyte surface areas.

The obtained results in (Fig., 2) proved that after the treatment with the $LC_{50}$ of *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* mithanolic extracts, the haemocyte surface areas were markedly decreased when compared to the control. The results indicated that treatments with *H. aegyptiacum* extract (Fig.3b) caused a decrease in all haemocytes surface area by about 33%in prohaemocyte, 11% in granulocyte, 13%in plasmatocyte and 7% in oenocytoides less than control. The same trend was observed in *A. spectabilis* extract treatment (Fig.3c)by about 42% in prohaemocytes, 21%in granulocyte, 20%in plasmatocytes and 19%in oenocytoides less than control. Also, treatment with *S. nigrum* extract caused a marked decrease in the haemocytes surface areas about 48%in prohaemocyte, 29% in granulocyte, 27%in plasmatocyte and 21 % in oenocytoides (Fig.3d) less than control. (Fig.3a)

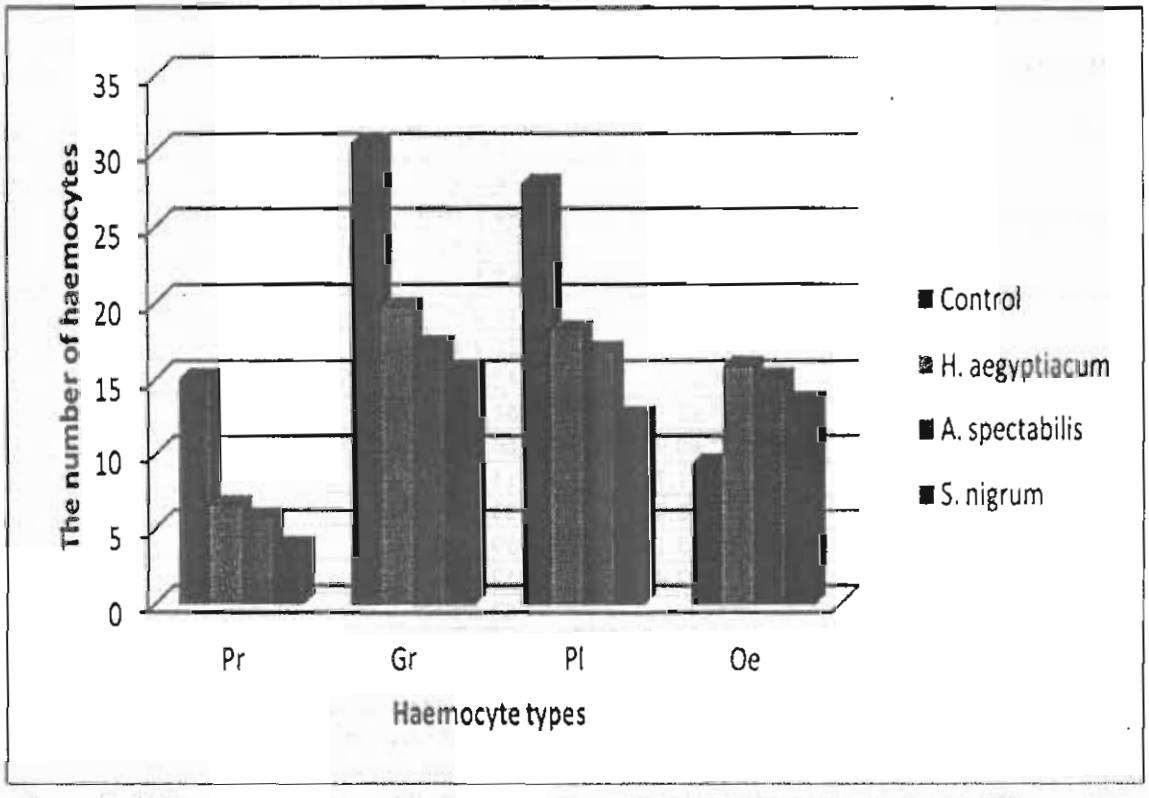


**Figure1. Effects of methanolic plant extracts from *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* on the differential haemocyte count in *C. pipiens* larvae**

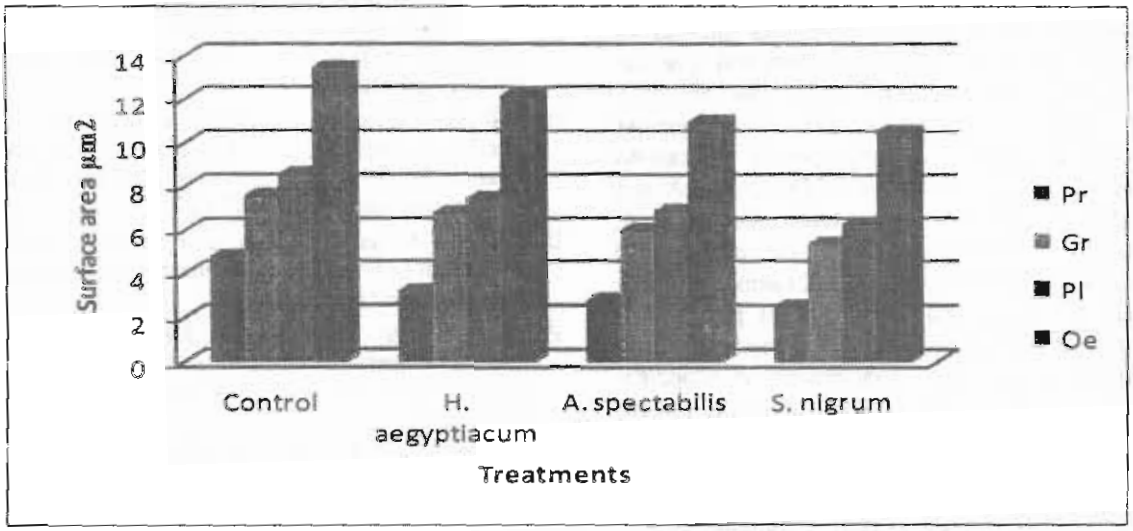


**Figure2. Effects of methanolic plant extracts from *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* on the haemocyte surface area in *C. pipiens* larvae**

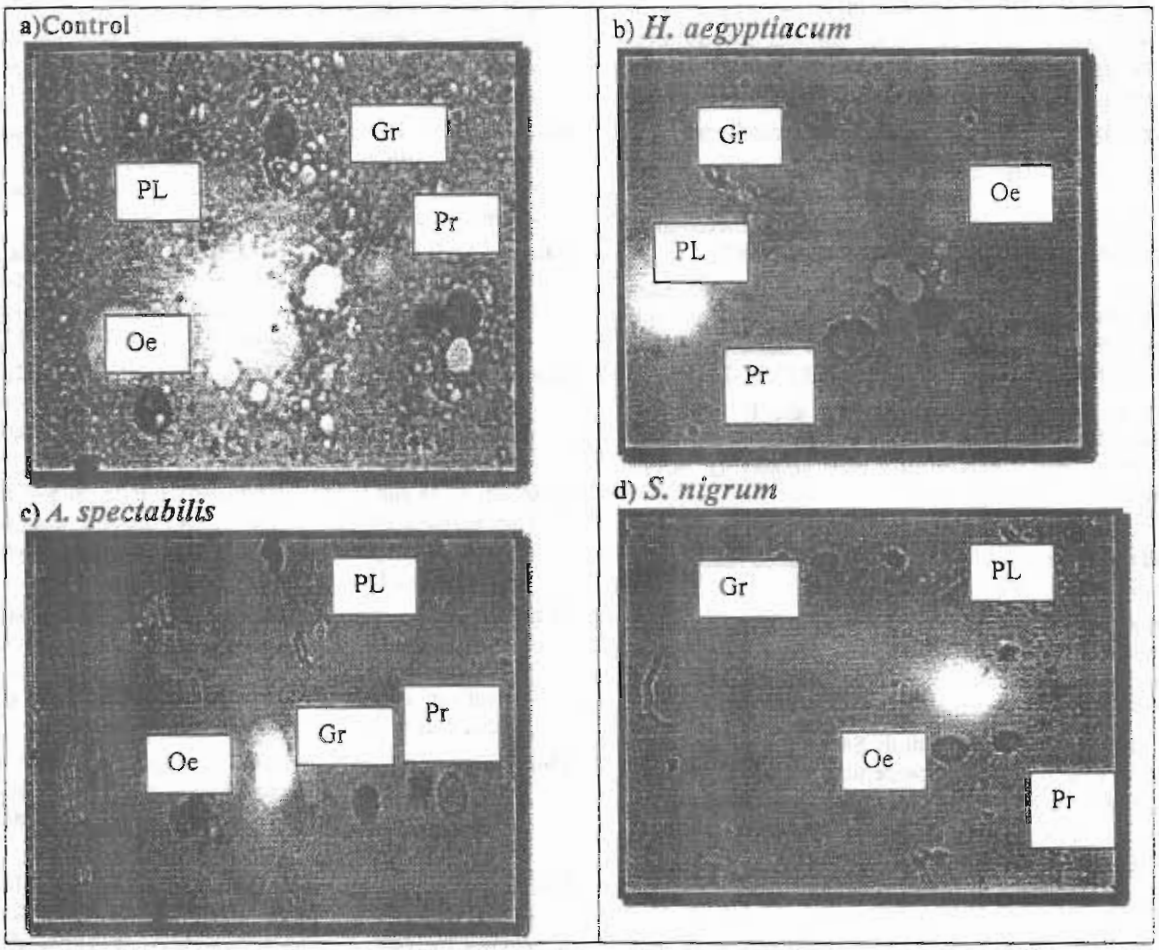


**Figure 3.The effect of methanolic plant extracts from *S. nigrum*, *A. spectabilis* and *H. aegyptiacum* on the *C. pipiens* larval haemogram**

Similar results were reported by Sharma *et al.* (2008) who noticed that the major effect of *Acorus calamus* oil treatment was observed on plasmatocytes and granular haemocytes of *Spodoptera litura* larvae. Gad and El – DaKheel (2009) reported that *Cinnamomum osmophloeum and Matricharia chamomella* oils caused significant decrease in the number of Pr, Gr and Pl and increase the number of Oenocytoide. On contrast, treatment with *C. osmophloeum and M. chamomella* oils caused a significant increase in all haemocyte types surface areas. Saxena and Tikku (1990) proved that plumbagin treatment caused damage in the haemocytes of *Dysdercus koenigii* and suppression of filopodial elongations of plasmatocytes and granulocytes. Pendey *et al.* (2007) found that treatment 5$^{th}$ instar larvae of *Danais chrysippus* caused a marked decrease in the number of haemocytes as well as high variation in relative percentage of different haemocyte types.

The results obtained in this study demonstrate the importance of toxic, growth and development-retarding influence of the extracted plant materials specially *S. nigrum* and *A. spectabilis* on *C. pipiens* mosquitoes. Moreover, application of these materials is not likely to leave harmful residues in the environment since they are naturally materials occurring among the local flora.

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## الملخص العربي

# تأثير بعض المستخلصات النباتية على موت وتطور وخلايا دم يرقات بعوض الكيولكس بيبيتز

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تم دراسة النشاط الإبادي للمستخلص الميثانولي لكل من عنب الديب، الأكوكانثيرا ورقيب الشمس المصري وذلك ضد يرقات وتطور بعوضة الكيوليكس بيبيتز. أظهرت المستخلصات النباتية المختلفة تأثيرات بيولوجية مختلفة وكان الأكثر تأثيرا هو مستخلص نبات عنب الديب والذي أظهر فعل حاد بعد يومين وثمانية أيام من المعاملة حيث كان التركيز المميت لـ٥٠% من اليرقات ١٣٠،٨جزء في المليون على التوالي بعد ٤٨. كانت نسبة الموت في اليرقات ٦٧,٥% عند المعاملة بمستخلص عنب الديب و٦٦,٥% عند المعاملة بمستخلص الأكوكانثيرا عند التركيز ٥٠٠ جزء في المليون. إنخفض فقس البيض بصورة غير واضحة في حالة المعاملة بمستخلص عنب الديب. كل التركيزات المستخدمة من مستخلصات كلا من عنب الديب، الأكوكانثيرا ورقيب الشمس المصري عائقا واضحا في تطور اليرقات وكذلك قللت من تعذير اليرقات وتحولها إلى حشرات كاملة. وقد أظهر مستخلص عنب الديب أكبر تأثير حيث كانت نسب التعذير ١٨,١% بينما نسبة خروج الحشرات الكاملة كانت ٨,٥% وذلك عند التركيز ١٠٠جزء في المليون. وقد وجد أيضا أن مستخلصات كلا من الأكوكانثيرا ورقيب الشمس المصري كانت فعالة عند التركيزات الأكثر ١٠٠جزء في المليون. وعلاوة علي ذلك وجد أن المستخلصات عنب الديب، الأكوكانثيرا ورقيب الشمس المصري لها تأثير على أعداد خلايا الدم اليرقات كما أنها أدت إلي إنخفاض في مساحة سطح الخلايا بدرجة ملحوظة.

ومن هنا نجد أن المعاملة بهذه المستخلصات في بيئة نمو اليرقات تعطي نتائج أفضل من إستخدام المبيدات الكيميائية التي تسبب ضررا للبيئة والإنسان والحيوان وكذلك قد تبدي الحشرات مقاومة لها.