

Short Communication

Evaluation of Some Plant Fruit Extracts for the Control of West Nile Virus Vector *Culex pipiens* (Diptera: Culicidae)

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Abstract

Background: The extracts of different parts of plants were found very effective against various pests. The aim of this research was to determine the insecticidal activity of fruit methanol extracts obtained from *Melia azedarach* (Meliaceae), *Phoenix theophrasti* (Arecaceae), *Styphnolobium japonicum* (Fabaceae) and *Pyracantha coccinea* (Rosaceae) against the larvae of *Culex pipiens* (Diptera: Culicidae).

Methods: The fruits of test plants were collected from the Campus of Akdeniz University, Antalya, Turkey in 2013. A series of concentrations of the extracts ranging from 62.5–1000 ppm were tested against second instar larvae.

Results: Only the extracts of *Me. azedarach* and *Ph. theophrasti* showed significant larvicidal activity against *Cx. pipiens* and the LC₅₀ values of these extracts were found to be 169.48 and 220.60 ppm, respectively. This is the first research investigating the insecticidal or larvicidal activity of *Ph. theophrasti*, *St. japonicum* and *Py. coccinea* extracts on mosquitoes.

Conclusion: The methanol extract of fruits of *Me. azedarach* and *Ph. theophrasti* showed significantly higher larvicidal activity against *Cx. pipiens*.

Keywords: Larvicidal, *Melia azedarach*, *Phoenix theophrasti*, *Pyracantha coccinea*, *Styphnolobium japonicum*

Introduction

Mosquitoes are important organisms known being vectors of many fatal or neurologic diseases such as malaria, dengue and West Nile Virus (WNV) infections. Fortunately, most people infected with some of these diseases have no or develop a fever with other symptoms. However, mosquitoes know no borders and more than one million people worldwide die from mosquito borne diseases every year (Becker 2008). Some insecticide groups can be applied to control adults (adulticides) or larvae (larvicides) of mosquitoes. Insecticides used for larviciding include, chitin synthesis inhibitors, diflubenzuron, novaluron and triflumuron, juvenile hormone analogs pyriproxyfen and methoprene, bacterial products (*Bacillus thuringiensis* subsp *israelensis* and *B. sphaericus*), spinosyns (spinosad) (Cetin et al. 2005, 2006, 2007). These larvicides were

highly effective against different mosquito species in the genus of *Anopheles*, *Culex* and *Aedes* but in some conditions some of them have negative effects on non-target organisms (Lawler et al. 1999, Ser and Cetin 2015). In addition, many researchers observed resistance toward larvicides in field populations (Sharma et al. 2003, Wirth 2010).

Researches about botanical insecticides and acaricides have grown dramatically in recent years and essential oils and extracts of aromatic plants were found effective against different instars of arthropod pest species (Koc et al. 2012). Most plant essential oils and extracts obtained from flowers, fruits and leaves are complex mixtures that contain active constituents such as alcohols, aldehydes, esters, ketones, phenols and terpenes (Gu et al. 2009). The use of botanical compounds

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extracted from aromatic plants may be an alternative to conventionally used insecticides to control of mosquitoes (Sukumar et al. 1991).

Therefore, in this study the toxic effects of fruit extracts of four plant species; *Melia azedarach* L (Meliaceae), *Phoenix theophrasti* Greuter (Arecaceae), *Styphnolobium japonicum* (L) (Fabaceae) and *Pyracantha coccinea* Max Joseph Roemer (Rosaceae) were investigated against larvae of *Culex pipiens* L (Diptera: Culicidae). This mosquito is found worldwide and is vector of different pathogenic organisms that cause serious diseases (i.e. WNV and filariasis). In Turkey, *Cx. pipiens* is abundant in many cities and developed resistance to some insecticide groups.

Materials and Methods

Plant materials and their extractions

Tested plants, *Me. azedarach*, *Ph. theophrasti*, *St. japonicum* and *Py. coccinea* were taxonomically identified by the second author. The fruits of them were collected from the Campus of Akdeniz University, Antalya, Turkey in 2013. Fruit samples were deposited in the Insecticide Test Laboratory of Biology Department, Faculty of Science, Akdeniz University. Fruits of each species were separated from their stalks. After that, fruit materials were dried at 25 °C about 2 weeks and ground to fine powder using blender. Extractions of the samples were carried out by using methanol for 2 days at 25 °C. Then filtered using a Whatman No.1 filter paper and dried under vacuum rotary evaporator.

Target mosquito species

Culex pipiens used in the studies originated from Arapsuyu, Antalya, and was collected from a pool in August 2011. The larvae were reared at 12 h dark: 12 h light photoperiod, 60±10% relative humidity, and 26±2 °C temperature in an insectary in the Biology Department, Akdeniz University. The second instars larvae were used for bioassays.

Experiments of larvicidal activities

Larvicidal activity of the methanol extracts of *Me. azedarach*, *Ph. theophrasti*, *St. japonicum* and *Py. coccinea* against *Cx. pipiens* was assessed (Oz et al. 2013). For experiments, first 2 gr of each extracts were dissolved in 25 ml distilled water. Then a series five concentrations (62.5, 125, 250, 500 and 1000 ppm) were prepared and controls in 500 ml tap water in containers. After approximately 5 min, 10 larvae taken on an egg tray with water were transferred gently to the test medium by tapping. Four replicates of each concentrations and controls were run at a time. Mortality was recorded after 24-, 48- 72- and 96-h of exposure, during which pellet fish food was given to the larvae. All experiments were conducted at 26±2 °C and 60±10% relative humidity with 12 h dark: 12 h light photoperiod. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period) or showing the characteristic diving reaction when the water was disturbed. Larvae were also observed for discoloration, unnatural positions, uncoordination or rigor.

Statistical analyses

Corrected means of percentage mortalities were calculated using Abbott's formula. The percentage values were transformed to ensure normality and variance homogeneity using an arcsine transformation. The data was subjected to analysis of variance (ANOVA) and the means compared with Duncan's multiple range tests ($P < 0.05$). The LC_{50} and LC_{90} values were calculated from percent mortality data by using probit analysis (Finney 1971).

Results

The percent mortality values of four plant fruit methanol extracts against second

instar larvae of *Cx. pipiens* are shown in Table 1. In our experiments, low mortalities were observed in the extracts of *St. japonicum* (14.45%) and *Py. coccinea* (6.42%) after 96 h exposure. These results are not significantly different from the controls ($P < 0.05$) (Table 1).

The highest mortalities were achieved by *Me. azedarach* and *Ph. theoprasti*. The *Me. azedarach* extract caused 100% mortality at the concentration of 1000 ppm after 96 h exposure. *Ph. theoprasti* also demonstrated sig-

nificant larvicidal activity and in the same concentration, mortality rate was 83.49%. There was a remarkable increase in mortality (19.95 to 83.49%) when concentrations were increased (62.5 to 1000 ppm) ($P < 0.05$). For 125 ppm of the extracts of *Me. azedarach* and *Ph. theoprasti* more than 39% larvae died only after 96 h of exposure (Table 1).

The LC_{50} values against the second instar larvae of *Cx. pipiens* were 220.60 ppm for *Ph. theoprasti* extract, 169.48 ppm for the *Me. azedarach* extract (Table 2) (Fig. 1).

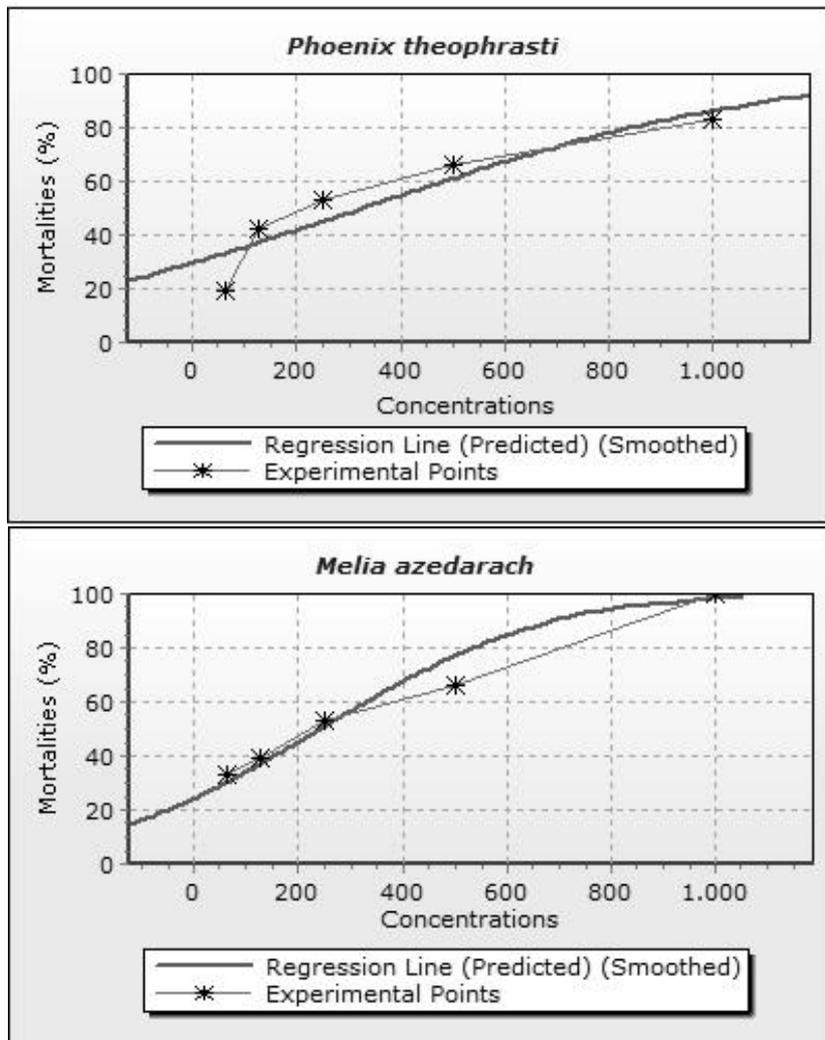


Fig. 1. Concentration-response lines for most effective test extracts

Table 1. Larvicidal activity of the fruit extracts of *Phoenix theophrasti*, *Melia azedarach*, *Styphnolobium japonicum* and *Pyracantha coccinea* (Percent mortalities \pm Standard Errors)

| Times After Exposure (hours) | Concentrations (ppm) | | | | | Control |
|--------------------------------|----------------------------|----------------------------|---------------------------|--------------------------|--------------------------|--------------------------|
| | 62.5 | 125 | 250 | 500 | 1000 | |
| <i>Phoenix theophrasti</i> | | | | | | |
| 24 | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 5.0 \pm 2.89 b, A | 0.83 \pm 0.83 a, A |
| 48 | 0 \pm 0 a, A | 2.5 \pm 2.5 a, A | 2.5 \pm 2.5 a, A | 5.0 \pm 5.0 a, A | 5.0 \pm 2.89 a, A | 1.67 \pm 1.12 a, A |
| 72 | 7.5 \pm 4.79 a, AB | 27.5 \pm 11.09 b, AB | 27.5 \pm 10.31 b, B | 35.0 \pm 6.45 b, B | 40.0 \pm 4.08 b, B | 3.33 \pm 1.88 a, A |
| 96 | 19.95 \pm 7.14 a, B | 42.2 \pm 12.21 b, B | 53.21 \pm 2.75 bc, C | 66.97 \pm 4.49 c, C | 83.49 \pm 3.18 d, C | 9.17 \pm 2.6 a, B |
| <i>Melia azedarach</i> | | | | | | |
| 24 | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0.83 \pm 0.83 a, A |
| 48 | 7.5 \pm 4.79 a, A | 0 \pm 0 a, A | 7.5 \pm 4.79 a, A | 20.0 \pm 4.08 b, AB | 20.0 \pm 7.07 b, A | 1.67 \pm 1.12 a, A |
| 72 | 27.5 \pm 14.36 ab, A | 25 \pm 11.9 ab, AB | 32.5 \pm 6.29 ab, B | 40 \pm 12.25 b, B | 80 \pm 7.07 c, B | 3.33 \pm 1.88 a, A |
| 96 | 33.94 \pm 11.89 ab, A | 39.45 \pm 17.12 bc, B | 53.21 \pm 9.4 bc, C | 66.97 \pm 7.79 c, C | 100 \pm 0 d, C | 9.17 \pm 2.6 a, B |
| <i>Styphnolobium japonicum</i> | | | | | | |
| 24 | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0.83 \pm 0.83 a, A |
| 48 | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 1.67 \pm 1.12 a, A |
| 72 | 0 \pm 0 a, A | 2.5 \pm 2.5 ab, A | 0 \pm 0 a, A | 5.0 \pm 2.89 ab, A | 10.0 \pm 5.77 b, A | 3.33 \pm 1.88 ab, A |
| 96 | 2.98 \pm 2.98 a, A | 3.21 \pm 2.91 a, A | 5.96 \pm 3.44 a, B | 3.21 \pm 2.91 a, A | 14.45 \pm 7.08 a, A | 9.17 \pm 2.6 a, B |
| <i>Pyracantha coccinea</i> | | | | | | |
| 24 | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0.83 \pm 0.83 a, A |
| 48 | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 0 \pm 0 a, A | 2.5 \pm 2.5 a, A | 1.67 \pm 1.12 a, A |
| 72 | 5.0 \pm 2.89 a, B | 2.5 \pm 2.5 a, A | 5.0 \pm 2.89 a, A | 5.0 \pm 2.89 a, A | 10.0 \pm 4.08 a, A | 3.33 \pm 1.88 a, A |
| 96 | 0.46 \pm 0.26 a, A | 0.46 \pm 0.26 a, A | 6.19 \pm 3.31 a, A | 6.42 \pm 3.18 a, A | 6.42 \pm 5.51 a, A | 9.17 \pm 2.6 a, B |

: Means within a line followed by the same lower case letter are not significantly different Duncan's multiple range test ($P < 0.05$).

: Means within a column followed by the same capital letter are not significantly different Duncan's multiple range test ($P < 0.05$).

Table 2. The Lethal Concentration 50 and Lethal Concentration 90 values (ppm) of fruit extracts of *Phoenix theophrasti*, *Melia azedarach*, *Styphnolobium japonicum* and *Pyracantha coccinea* on *Culex pipiens* second-instar larvae in the laboratory conditions

| Plant species | LC ₅₀ | LC ₉₀ | x ² | P-value |
|------------------------|------------------|------------------|----------------|---------|
| <i>Ph. theophrasti</i> | 220.60 | 1813.67 | 1.42 | 0.69 |
| <i>Me. azedarach</i> | 169.48 | 1098.82 | 6.84 | 0.07 |
| <i>St. japonicum</i> | 39054.36 | 1792194.42 | 3.50 | 0.32 |
| <i>Py. coccinea</i> | 32110.12 | 808652.32 | 6.19 | 0.10 |

Discussion

The calculated LC₅₀'s were higher or lower to other comparable studies on the toxicity of *Me. azedarach* to mosquitoes. These differences may be attributed to many factors such as, method of extraction (extraction solvent, extraction time, extraction temperature), parts of plant (flowers, leaves, fruits), vegetation period, origin of the pest species. The higher LC₅₀ values were determined for *St. japonicum* and *Py. coccinea* extracts, since significant mortalities were not determined even at the highest exposure concentration (1000 ppm) (Table 2).

The Meliaceae family has been known as a source for pesticides and the efficacy of neem products on different pest species was reported (Singh et al. 2006, Ndione et al. 2007). *Melia azedarach* has insecticidal effects on different mosquito genus including *Aedes* and *Culex* (Wandscheer et al. 2004, Ndione et al. 2007). The LC₅₀ value of aqueous extracts of fruits was 2035 ppm for *Cu. quinquefasciatus* Say third and fourth instar larvae (Ilahi et al. 2012). During our research the lowest dose of fruit extract (62.5 ppm) caused 33.94% mortality and the highest concentration (1000 ppm) caused 100% mortality. Mode of action of *Me. azedarach* extract on mosquito larvae may explain that extract causing serious damage to the gastro intestinal system cells. RM Al-Mehmadi and Al-Khalaf (2010) showed that changes in the midgut, included separation of the epithelial cells from the basement membrane with dam-

age of the peritrophic membrane causes that the mixing of the gut contents with the hemolymph caused the mortality. Fruit extracts of this species have also different effects on insects such as changes of behavior, anti-feedant, inhibit the chitin or hormone synthesis, reduced fecundity etc. (Schmidt et al. 1998, Gajmer et al. 2002).

According to our literature survey, no researches have been carried out so far on the insecticidal properties of *Ph. theophrasti*, *St. japonicum* and *Py. coccinea* against pest organisms. To our knowledge, this is the first research showed the insecticidal or larvicidal activity of *Ph. theophrasti* against *Cx. pipiens*. Fruit extracts of *Ph. theophrasti* caused high mortalities as much as *Me. azedarach* extract except the highest concentration (P < 0.05). A series of experiments aimed at clarifying the mode of action of *Ph. theophrasti* should be done on mosquito larvae.

The various solvent extracts (e.g. acetone, methanol, chloroform) of different parts (flowers, leaves, fruits) of many plant species were found very effective against various pests (Kalyanasundaram and Das 1985, Govindarajan and Sivakumar 2014). Most of them contain active components of which have toxic effects on insect biology (Cecilia et al. 2014).

Conclusion

The methanol extract of fruits of *Me. azedarach* and *Ph. theophrasti* showed sig-

nificantly higher larvicidal activity against *Cx. pipiens*. Our results suggest that the fruit extracts of *Ph. theophrasti* have the potential to be used as an alternative product for the control of mosquitoes. Extracts of both plants may be useful to control larvae of mosquitoes in their breeding sites. However further studies should be done to identify the active ingredients of the extracts responsible for insecticidal activity.

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