Original Article

Bio-Pesticides: New Tool for the Control of Aedes (Stegomyia) albopictus (Culicidae: Diptera) in Pakistan

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Abstract
Background: Application of plant extracts as mosquito control strategy was practiced from centuries. These are easily available, non-toxic, biodegradable and exhibit broad-spectrum target specific activities against larval stages of mosquitoes.

Methods: Different potential parts of locally grown plants, seeds of nutmeg (Myristica fragrans), peel of musambi (Citrus sinensis), leaves of babuna (Matricaria chamomilla), mint (Mentha spicata) and ginger rhizome (Zingiber officinale) selected and evaluated for their larvicidal properties against Aedes (Stegomyia) albopictus. Oils were extracted through steam distillation process and extracts were evaluated as per WHO 2005 guidelines for testing of insecticides against larvae of mosquitoes.

Results: Among the five plant extracts, C. sinensis had the lowest LC50 (400.81ppm) while M. fragrans had the highest LC50 value (710.30ppm) respectively after 24h of exposure. In terms of % age mortality, a series of concentrations (300–800ppm) gave high % mortality in case of C. sinensis while M. fragrans gave low % age mortality.

Conclusion: All the five plant species have larvicidal effects to certain extent and C. sinensis had great potential. Further small-scale field trials with the extracts of the most promising one (C. sinensis) shall be conducted to determine operational feasibility.

Keywords: Mosquitoes, Plant extracts, Larvicide

Introduction

Dengue, malaria, filariasis, yellow fever and Japanese encephalitis are the most important diseases transmitted by mosquitoes (Rozendaal 1997). Fifty million cases of dengue occur globally every year (WHO 2009). Dengue has now emerged in many countries, especially in Pakistan where occurred outbreaks affected the socio-economic development in the Region (Savioi and Velayudhan 2014). Dengue epidemic in Pakistan (2011) is being observed where, more than 22,778 cases confirmed and 353 deaths reported (Anonymous 2011). In 2013, a Punjab and Khyber-Pakhtunkhwa (KPK) provinces hit by second epidemic, in northern areas of Pakistan the human mobilization from dengue endemic regions, geographic expansion of dengue fever vector due to importation, climatic change, all are the factors, which resulted in the emergence of dengue in northern areas (Ali 2013). In district Swat (KPK) 6,000 dengue cases with 47 deaths were reported (Khan and Khan 2013).

Worldwide mosquito control depends on the application of synthetic insecticides as a part of Integrated Vector Control (IVM) Programmes (Becker et al. 2010). Toxic effects and resistance to synthetic insecticides are barriers in controlling mosquitoes. Therefore, it is necessary to develop safe alternative insecticides, which required minimum care (Mittal and Subbarao 2003).

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Plant based insecticides may be the best option for mosquito control as they have biologically active chemicals that are easily decomposed into products which are not toxic to other species (Sanjay and Tiku 2009) and potentially suitable for use in control of mosquito larvae (Yang et al. 2004).

In fact, many researchers have reported the effectiveness of plant extracts or essential oils against mosquito larvae (Rahuman et al. 2008). Research on insecticidal properties of botanicals inferred that they are bio-degradable, environmentally safe and target specific (Gowindarajan et al. 2008). (Muthukrishnan 2012) and (Bilal et al. 2012) evaluated the larvicidal effects of extracts from Cinnamomum cassia, Citrus sinensis Linnaeus var musambi, Tribulus terrestris, Eucalyptus camaldulensis, Piper nigrera, Ricinus communis, Allium sativum, Linum usitatissimum and Citrus sinensis L var sucarri against Aedes albopictus. A number of other researchers which have used plant products for the mosquito control like (Komalamisra et al. 2005) reported the ether extracts of Trigonostemon reidioides, Rhinacanthus nasutus, Derris elliptica, Homalomena aromatica, Stemona tuberosa, Acorus calamus and Piper nigrum (Siddiqui et al. 2004), Artemisia annua, Sonchus oleraceus and Chenopodium album (Sharma et al. 2006) Solanum xanthocarpum (Mohan et al. 2005) Argemone mexicana (Sakthivadivel and Thilagavathy 2003).

In the view of increased interest in development of plant-based insecticides as an alternative to synthetic insecticide, this study was planned and conducted to assess the larvicidal potential of five medicinal plants against the medically important mosquito (Aedes (Stegomyia) albopictus).

Materials and Methods

Collection of Plants

Nutmeg (M. fragrans Houtt) seeds, musambi (C. sinensis (L) Osbeck) peel, babuna (M. chamomilla L) leaves, Mint (M. spicata L) leaves and ginger (Z. officinale Roscoe) rhizome were collected from botanical garden of University of Agriculture Faisalabad (31.4339° N, 73.0649° E) and local market of Faisalabad (31.4181° N, 73.0776° E).

Extraction of oil

The seeds, peel, leaves and rhizomes were washed, then dried and later pulverized in an electric grinder (Anex Germany). The pulverized material was placed in thimble and kept in extraction tube of Soxhelt apparatus with extractor ID 38mm, extractor volume 85ml and flask volume 250ml (Vogel 1978) for the extraction of oil by steam distillation method using ether as solvent (250ml/20g sample). The cycle time for one sample was 4–5h. Solvent was evaporated at room temperature, leaving oil, collected in flask. Stock solution was prepared by adding 1ml of oil from each plant in 99ml of ether and considered as 1% stock solution from which series of concentrations (%) were prepared (Akram et al. 2010).

Collection and Rearing of Mosquitoes

Mosquito larvae were collected from potential breeding sites of Aedes around Islamabad (33.7167° N, 73.0667° E) with a standard pipette while adults were collected by battery-operated aspirator. Larvae were reared for mass population in the insectary operated by providing 10% sucrose solution while female mosquitoes were also fed on the blood of albino rats (Imam et al. 2014). Larvae of Ae. (Stg.) albopictus were identified using identification key of Leopoldo (Leopoldo 2004).
Larvicidal Bioassay

The extracted oils were used in six different concentrations along control with three replicates for each treatment, each replicate containing 200 ml of the oil solution in 250 ml Pyrex glass beakers. A batch of fifteen 3rd instar larvae of the Ae. (Stg.) albopictus were exposed in each beaker containing oil solution (WHO 2005), while control was treated with ether only. Mortality of larvae was counted after 24 hours. The experiment performed under lab conditions at 28±2 °C and 75±5% relative humidity.

Statistical Analysis

Abbot’s formula (Capinera 2008) was used for correction of mortality and the data so obtained was analyzed by probit analysis (WHO 2005) by using MANITAB-15 software for dose mortality regression line and % age mortality graph were prepared using Microsoft office 2007.

Results

The crude ether extracts of nutmeg (M. fragnars) seeds, musambi (C. sinensis) peel, babuna (M. chamomilla) leaves, mint (M. spicata) leaves and ginger (Z. officinale) rhizome had been evaluated as potential source of insecticides. Results on the larvicidal activities of extracts were reported in the present study (Table 1) confirms their potential for the control of Ae. (Stg.) albopictus larval population. All extracts showed moderate larvicidal effects however, the highest larval mortality was found in musambi peel with 400.81 ppm LC50 value, followed by babuna (438.60 ppm), ginger (502.55 ppm) while mint and nutmeg had the highest LC50 value (596.94 ppm and 710.30 ppm) respectively after 24h of exposure.

In terms of % mortality musambi had the high mortality (64.25%) followed by babuna and ginger (58.51 and 56.48%) respectively, while nutmeg and mint had the lowest % mortality (33.51 and 50.92%) respectively after 24h of exposure as shown in Fig. 1. The percentage of mortality was directly proportional to concentration of the extract (Table 1). After exposure to the test concentrations, the treated larvae exhibited restlessness, tremors, sluggishness and convulsions followed by paralysis at the bottom of the bowl.

Table 1. Larvicidal activity different plant extracts at different concentrations against 3rd instar larvae of Aedes (Stg.) albopictus

| Plants      | Con. | % mortali | LC50 (ppm) | 95% FL (LFL–UFL) | 2     | p     |
|-------------|------|-----------|------------|------------------|-------|-------|
| Musambi     | 300  | 32        | 400.81     | 359.82–435.22    | 4.62  | 0.32 |
| C. sinensis | 400  | 46        |            |                  |       |       |
|             | 500  | 54        |            |                  |       |       |
|             | 600  | 61        |            |                  |       |       |
|             | 700  | 70        |            |                  |       |       |
|             | 800  | 82        |            |                  |       |       |
| Nutmeg      | 300  | 10        | 710.30     | 654.35–793.80    | 4.60  | 0.33 |
| M. fragnars | 400  | 16        |            |                  |       |       |
|             | 500  | 25        |            |                  |       |       |
|             | 600  | 29        |            |                  |       |       |
|             | 700  | 43        |            |                  |       |       |
|             | 800  | 58        |            |                  |       |       |
Table 1. Continued…

| Plant     | Concentration (mg/ml) | LC50 (mg/ml) | LC50 (mg/ml) | R² |
|-----------|-----------------------|--------------|--------------|----|
| Babuna    | 300                   | 27           | 438.60       | 0.18 | 0.99 |
| M. chamomilla | 400               | 41           | 394.18–477.52 |     |
|           | 500                   | 51           |              |     |
|           | 600                   | 61           |              |     |
|           | 700                   | 66           |              |     |
|           | 800                   | 70           |              |     |
| Mint      | 300                   | 31           | 596.94       | 1.11 | 0.89 |
| M. spicata | 400               | 38           | 521.21–719.96|     |
|           | 500                   | 42           |              |     |
|           | 600                   | 47           |              |     |
|           | 700                   | 55           |              |     |
|           | 800                   | 62           |              |     |
| Ginger    | 300                   | 35           | 502.55       | 1.05 | 0.90 |
| Z. officinale | 400          | 39           | 437.55–570.33|     |
|           | 500                   | 47           |              |     |
|           | 600                   | 56           |              |     |
|           | 700                   | 61           |              |     |
|           | 800                   | 67           |              |     |

*LC50 ie, lethal concentration (%age) to kill 50% population of the subjected organism

Fig. 1. Percentage mortality of different plant extracts against *Aedes (Stg.) albopictus* larvae after 24 hours of exposure.

**Discussion**

Recently *Ae. (Stg.) aegypti* along with *Ae. (Stg.) albopictus* played havoc in different parts of Pakistan. Different control measures have been adopted with the major focus on chemical control. Resultantly, occurrence of insecticide resistance in mosquitoes and other public health pests have been reported (Khan et al. 2011, 2013). Their residues in the environment and effects on humans and non-target organism are major problems due to which investigators are now directing their attentions towards the development of plant...
Various compounds, including phenolics, terpenoids, and alkaloids, exist in plants (Wink 1993, Swin 1977, Kim et al. 2001) and may jointly or independently contribute to the generation of larvicidal activities of mosquito (Assabugi et al. 1997, Hostettmann and Potterat 1997).

Outcome of five different plant oils, which were, used against the 3rd instar larvae of Ae. (Stg.) albopictus are shown in Table 1. Results were satisfactory and showed efficacy. Larval mortality increased with increase in dose of plant oil and at higher doses, it gave more than 80% mortality without any pupal or adult emergence. While in control, there was less than 5% mortality after 24h. Citrus sinensis peel extract gave reasonably good results (400.81ppm) against the larvae of Ae. (Stg.) albopictus when compared with other tested plant oils, like findings of Warikoo et al. (2012) they concluded that C. sinensis leaf extracts had 446.84ppm LC₅₀ value against the larvae of Aedes aegypti as Citrus plants contain limonoids which work both as toxicant and feeding deterents (Akram et al. 2010) and also has insecticidal effects (Bilal et al. 2012). Thus, the larvicidal activity of Citrus sinensis is due to limonoids, in addition to alkaloids, saponins, steroids, flavonoids and tannins. While on the other side, previous workers (Michaelakis et al. 2009, Din et al. 2011) reported LC₅₀ values for some citrus peel and seed oils against the larvae of Ae. albopictus and Cx. pipiens different than the obtained values in the present investigation.

In our findings, M. fragrans had least effectiveness and gave 50% mortality after 24h of exposure is not in agreement with the studies of Senthilkumar et al. (2009). They tested M. fragrans, Eucalyptus globulus, Artemisia annua, Cymbopogan citratus, Justicia gendarussa, Annona squamosa and Centella asiatica and found that all gave 80–100% mortality against larvae of Anopheles stephensi. It is well documented that toxicity values of a substance may be largely varied due to several factors (Busvine 1971) attributed to the test conditions (temperature, light, humidity, exposure period and solvent); tested species (age, stage and susceptibility) and tested plant material (season, location, extraction method and used part) and there may be different constituents in a botanical extract may interact with each other’s, leading to synergistic or antagonistic effects (Mansour et al. 2000, 2003).

Conclusion

Out of the 5 plants extracts, C. sinensis has good larvicidal potential against larvae of Ae. (Stg.) albopictus in terms of LC₅₀ and % age mortality. Therefore, we suggest that C. sinensis extracts as well as other plant extracts should be investigated and compared with other plant extracts, which already been tested against mosquitoes.

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