



Effect of biologically active compounds of *Cucumis melo* on Mosquito Species *Aedes aegypti*.

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Abstract: Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases such as filariasis, dengue, malaria and chickungunya fever. Some species transmit major disease like, *Aedes aegypti*, which is a vector of dengue disease distributed throughout tropical as well as subtropical zones. The continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non-target organisms including human health. Application of active toxic agents from plant extracts as an alternative mosquito control strategy was available from ancient times. To determine biologically active compounds β -sitosterol and Saponin from the petroleum ether fruit extract of *C. melo* which is used against the larvae of *Aedes aegypti*. The Larval mortality was observed after 24h exposure and highest larval mortality was found in petroleum ether fruit and leaf extract of *C. melo*.

Keywords: *Aedes aegypti*, *cucumis melo*, petroleum ether, larvicides

I. INTRODUCTION

Mosquitoes are also known as bioterrorist, mosquito transfer number of viral and parasitic diseases such as filariasis, dengue malaria and chickungunya fever (Jebanesan, 2007). Japanese encephalitis diseases cause millions of deaths per year. Some species transmit major Various diseases are to humans and other animals by more than hundred species of mosquitoes. In India, mosquito, *Anopheles stephensi* is the major malaria disease vectors. (Wernsdorfer and Wernsdorfer 2003). *Aedes aegypti*, which is a vector of dengue disease distributed throughout tropical as well as subtropical zones. Occurrence of dengue fever disease has increased from 1970 till now in India. Transmission of dengue was greater than 50% at the region of estimated risk (Hales et al., 2002).

Most people dislike mosquitoes and are aware of the diseases and discomfort that they cause. Chemical controls are typically very effective against mosquitoes. However if the same chemicals are used against many generations of mosquitoes over a large area, the mosquitoes have genes that make them less sensitive to the toxin (Lawler and Lanzaro 2005). Insect repellents are useful for prevention and control the eruption of insect-borne diseases such as bubonic plague, Lyme disease, Dengue fever, malaria, and West Nile fever. Pest animals are usually helping as vectors for some disease such as insect's flea arachnid tick, and mosquito; and the fly, (Mishra, et al., 1995).

The most commonly used insect repellents are synthetic chemicals that mostly have contained DEET (N, N-diethyl-3-methylbenzamide) in their formulations. Although DEET is an effective repellent against a broad spectrum of insects, however there are disadvantages of DEET. There have also been

concerns over toxicity of DEET (Liu, et al., 1987). A variety of botanical substances have been evaluated for their repellency against mosquitoes (Sukumar, et al., 1991). In ancient medicine Lemon citrus (*Citrus limon* Burm) and Melissa (*Melissa officinalis* L.) have long been used as natural insect repellents in Iran (personal observation) and the world (Sukumar, et al., 1991 and Rojas and Scorza 1991).

The continuous application of synthetic insecticides causes development of resistance in vector species, biological magnification of toxic substances through the food chain and adverse effects on environmental quality and non-target organisms including human health

Plants are rich source of bioactive organic chemicals and present an advantage over synthetic pesticides as these are easily biodegradable, less prone to development of resistance, and less toxic. In present study we have selected plant *cucumis melo* species of cucurbitaceae family because of the bitter taste of the fruits of *C. melo* whose major biologically active compounds, All parts, such as root, stem leaf and fruit which possess a multitude of phytochemical of secondary metabolites, which communicate a record variety of medicinal uses of the plant.

REVIEW OF LITERATURE

Most of the plants contain those chemicals, which are mostly used for take precaution during the attack of phytophagous insects. Further these chemicals are divided into various categories, with feeding deterrent, repellent, growth regulator, etc. Mostly they are grouped in 5 chemical categories (Pichersky and Gershenzon, 2002) nitrogen compounds, terpenoid, phenols and growth regulators (Harrewijnet al., 1995; Ditzenet al., 2008; Pitts et al., 2004 and Hallemet al., 2006).

Primary functions of these chemicals are defensive in nature against insects. Different types of chemicals are available and shows best results against mosquitoes and different types of biting insects, particularly these mechanisms are unstable and released as a results of herbivores (Pichersky and Gershenzon, 2002).

Many of these chemical compounds are used as a repellent to haematophagous insects and it could be an evolutionary relict from a plant feeding ancestor. Many of these chemical compounds are also evolved as repellents for phytophagous insects (Pichersky and Gershenzon 2002).

Many plants are available, which are used for the preparation of medicine. These naturally prepared ayurvedic medicines from can be used for the healing of a variety of diseases caused to humans. Mullai, and his co-workers in 2007 prepared benzine extract mixed with the leaf of *Citrullus vulgaris*, and this extract was applied to check the larvicidal activity against two mosquito *Aedes aegypti* and *A. stephensi*, the results found that highest larvicidal activity was observed more against *A. stephensi* than *Aedes aegypti*

MATERIALS AND METHODS

Plant material:

The plant of *Cucumis melo* L. (Cucurbitaceae). Collected from the Tal. Phaltan, Dist. Satara, State Maharashtra, India and identified from the Botanical Survey of India, Pune, M.S., India.

MOSQUITO:

The eggs of mosquito's species *Aedeis aegypti* was collected and identified from the National Chemical Laboratory, Pune, M.S., India. The eggs culture was developed and maintain in department of Zoology, Prof. Ramkrishna More College, Akurdi, Pune, M.S. India.

These cultures were used for further Bioassay.

Preparation of Fruit Extract of *Cucumis melo*:

The collected fruits are peeled to separate the epicarp and immediately dried under the shade. The dried fruits (500gm) were made into fine powdered mechanically using commercial stainless steel blender and extracted with petroleum ether (1500ml at 60 to 80°C) in a soxhlet apparatus

separately until exhaustion. The extract was concentrated under reduced pressure 22-26 mm Hg at 45°C and the residue obtained was stored at 4°C.

Leaf extract of *Cucumis melo*:

The healthy and disease free leaves were separated from freshly collected plants and cleaned with the help of distilled water so as to eliminate soil and dust particles. After cleaning with water the leaves were chopped into small pieces in a grinder. The leaf powdered (500gm) can be prepared by using stainless steel blender and extracted petroleum ether (1500ml at 60 to 80°C) in a soxhlet apparatus separately in anticipation of fatigue. The extract can be concentrated under reduced pressure 22-26 mm Hg at 45°C and the residue obtained can be stored at 4°C.

LARVICIDAL BIOASSAY:

Early fourth stage larvae of *Aedes aegypti* were used for the bioassay test. Experiment was conducted in a glass jar for 24 hrs at (28±2°C). A total of 30 larvae were exposed in three concentrations at triplicate form of 10 larvae each. This bioassay was divided into three concentrations of 5ml, 10ml and 15ml, of crude extract in glass jar containing water and made the volume up to 500ml in each jar. After 24 hr the numbers of dead larvae and the percentage mortality was reported comparing with the control. The experimental media, in which 100% mortality of larvae occurs alone, were selected for isolation and purification of crude extracts. Among the crude extracts tested for larvicidal activity, petroleum ether fruit extract of *C. melo* showed maximum activity and it was selected for the purpose of isolation and purification of compounds for further methods.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC):

An HPTLC technique was followed for the qualitative analysis and the confirmation of biologically active compounds present in *Cucumis melo* (Passera, *et al.*, 1964).

STATISTICAL ANALYSIS:

Data were analyzed by one-way ANOVA: Duncan Multiple Range Test (DMRT) using SPSS software Data were expressed Mean + SE (n=3). Values followed by the same letter (a,b,c,d& e) were not significantly different at 5% level.

RESULTS

In the present investigation detect the biologically active compound present in fruit and leaf of *C. melo* which is effect on mosquito *Aedes aegypti* by the Larvicidal Bioassay method. In which Petroleum Ether Fruit Extract (PEFE) and Petroleum Ether Leaf Extract was prepared. After 24 hr the numbers of dead larvae and the percentage mortality was reported comparing with the control. In this method effect of Petroleum Ether Fruit and leaf Extract of *C. melo* against early fourth instar larvae of *Aedes aegypti* was recorded (Table 1). As compare to 5ml, 10ml, 15ml, 20ml, 25 ml& control, the mortality percentage was greater in 25ml 83.33% in Fruit extract and 88.33% in Leaf extract. In control the mortality percentage was nil.

With the help of HPTLC technique β -sitosterol and Saponin was identified and confirmed from fruit and leaf of *C. melo*.

DISCUSSION AND CONCLUSION

Dighe., *et al* 2011 reported β -sitosterol which is principally phytosterol and which appears to have important immunomodulatory and anti-inflammatory activities in human and animal physiology. He reported β -sitosterol from seeds of *Celastrus paniculatus* in sample solution and it was confirmed by comparing its Rf value with that of standard β -sitosterol (0.49). The mobile phase used in the present research work for quantification of β -sitosterol from methanolic dried seed Powder extract of *Celastrus paniculatus* Willd. Is also relatively simpler as compared to the mobile phase used in the above reported method (Shailajan., *et al* 2010).

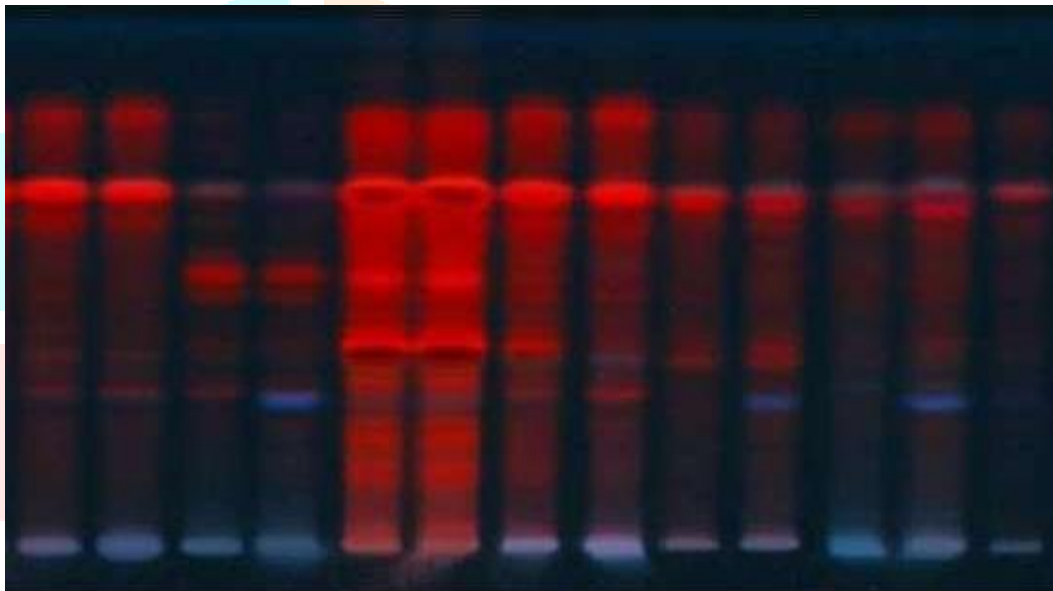
These studies and results indicated that, the mosquito repellent and mosquitocidal activity exists in the studied plant part(s) are because of these phytotoxic chemicals These plant can be used as natural mosquito repellent. It can be used as a repellent in the household to kill mice, mosquitoes, etc.

Table 1: Effect of *C. melo* fruit and Leaf extract against early fourth instar larvae *Aedes aegypti*

| Sr. No. | Extract concentration in 500ml vol. of water | Petroleum ether Fruit Extract (PEFE) | | Petroleum ether Leaf Extract (PELE) | |
|---------|----------------------------------------------|--------------------------------------|-------------|-------------------------------------|-------------|
| | | Mortality of Larvae Mean + SE | % Mortality | Mortality of Larvae Mean + SE | % Mortality |
| 1 | 5 ml | 4+ 0.01ab | 18.33% | 3+ 0.01ab | 13.33% |
| 2 | 10 ml | 8+ 0.02cd | 38.33% | 7+ 0.02cd | 33.33% |
| 3 | 15 ml | 13+ 0.01c | 63.33% | 12+ 0.01c | 58.33% |
| 4 | 20 ml | 15+0.03a | 73.33% | 14+0.03a | 68.33% |
| 5 | 25 ml | 17+0.02d | 83.33% | 18+0.02d | 88.33% |
| 6 | Control | Nil | Nil | Nil | Nil |

Data were analyzed by one-way ANOVA: Duncan Multiple Range Test (DMRT) using SPSS software. Data of mortality were expressed by Mean + SE (n=3). Values followed by the same letter (a,b,c,d& e) were not significantly different at 5% level.

1) DETECTION OF β -SITOSTEROL WITH THE HELP OF HPTLC METHOD.

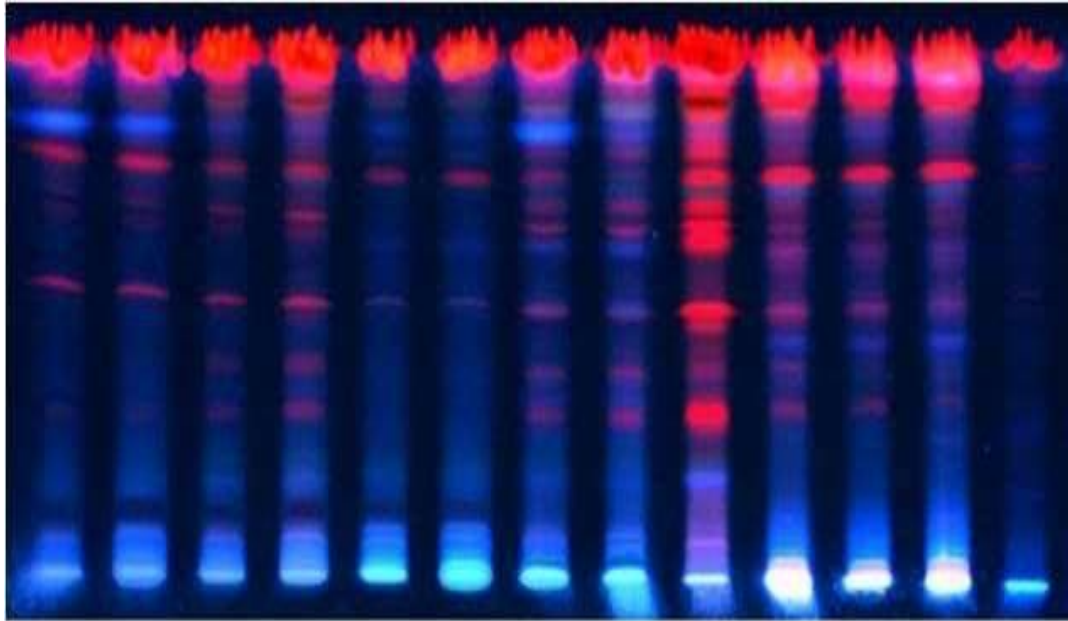


A1 A2 A3 B1 B2 B3 C1 C2 C3 D1 D2 D3 Std

B2 – Leaf Extract of *Cucumis melo*

B3 – Fruit Extract of *Cucumis melo* Std - β -Sitosterol

2) DETECTION OF SAPONIN WITH THE HELP OF HPTLC METHOD



A1 A2 A3 B1 B2 B3 C1 C2 C3 D1 D2 D3 Std
 B1 – Leaf Extract of *Cucumis melo*
 B2 – Fruit Extract of *Cucumis melo* Std – Saponin

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