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### Study On Parthenium Hysterphorous for Mosquitoes Larvicidal Potential and The Formulation Of Larvicidal Sachet

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#### ABSTRACT

In this study crude leaf extract of Parthenium, were tested for their larvicidal activity. The different solvents were used namely ethanol, methanol, water (aqueous) for the preparation of crude extracts from the plant leaves. The larval mortality after 24 hours of exposure were observed separately at concentration like 0.2mg, 0.4mg and 1m extract. The different concentration solvent extract of the plants showed good larvicidal activity. The highest potency was recorded by methanol extract of Parthenium. From These results it was observed that the leaf extract of parthenium contain toxic compounds to mosquito larvae and therefore suggest that parthenium extract has potential in the control of the malaria control and prevention of larvae for social health and can be developed and used.

**Keywords:** Mosquitoes, Parthenium hysterphorus, bio-pesticides, solvent, crude leaf extract, concentration, mortality, larvicidal activity.

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## INTRODUCTION

Parthenium is the most widely used botanical insecticide. Its derived from the flowers of a plant in the genus *Heterophorus*, which belong to the family Compositae. This genus contains many species of which only a few produce insecticidal substances which have been exploited at one time or other. Mosquitoes act as vector for most of the life threatening diseases such as malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, west Nile virus infection and other more <sup>1</sup>. Efforts are made in the prevention and control of malaria. *Anopheles gambiae* s.s. and *Anopheles arabiensis* (Diptera: Culicidae) are the most widely distributed and best known as malaria transmitters and sub-Saharan Africa suffers by far greatest malaria burden worldwide (Bryne, 2007) <sup>2</sup>. The vectors in the affected regions by application of appropriate use of chemical insecticides in the field and indoor spraying of insecticides. It is observed that reiterated application of chemical insecticides has the negative effect of developing resistance in malaria vectors <sup>1,3</sup>. Besides this chemical insecticides have the properties of environmental persistence, are also unbiodegradable and toxic. A good number of them tend to bioaccumulate in tissues of organisms consequently becoming carcinogenic, teratogenic, mutagenic and allergenic <sup>4</sup>. For this reason there is great need for the alternative strategies for the malaria vector control of which plant extracts seem to take lead as good products for vector control, especially in view of the fact that these natural products are biodegradable, non-toxic and do not bioaccumulate in fat tissues of organisms. They may not develop resistance in vectors, however, if they do will be slow<sup>5</sup>. Mosquitoes can transmit more diseases than any other group of arthropods and affect million of people throughout the world. WHO has declared the mosquitoes as “public enemy number one” <sup>6</sup>. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population. They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, etc., in almost all tropical and subtropical countries and many other parts of the world. To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher

rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale<sup>7,8</sup>. Most of the world's supply of parthenium comes from America, which produces the most potent flowers (Moore and Levy, 1975)<sup>9</sup>. The vector borne diseases, along with development of insecticide resistance in vector population, poor human acceptance of indoor house spraying and environmental concerns against the use of insecticides led to a rethinking in vector control strategies<sup>10</sup>. As a result, emphasis was given on the application of alternative methods in mosquito control as part of the Integrated Mosquito Management (IMM)<sup>11</sup>. Integrated Mosquito Management (IMM) is a decision-making process for the management of mosquito populations, involving a combination of methods and strategies for long-term maintenance of low levels of vectors. The purpose of IMM is to protect public health from diseases transmitted by mosquitoes, maintain healthy environment through proper use and disposal of pesticides and improve the overavailability of life through practical and effective pest control strategies. The larvicidal action of many local plants has been reported in different parts of the world<sup>12</sup>. Use of synthetic insecticides to control mosquitoes is not well accepted as these are not safe for humans, have damaging effects on environment, non-biodegradable and expensive<sup>13</sup>. Conventionally, plants and their products were used to destroy mosquitoes and other infectious Mosquitoes. Secondary metabolites of plants possess insecticidal activity (antibacterial, antifungal and larvicidal potential) that protects themselves against herbivorous insects<sup>14</sup>.

## MATERIALS AND METHOD

### **Plant Collection and extraction:**

Fresh and disease free leaves of *Parthenium hysterophorous* were collected from various places in Junner region of district of Pune. The leaves were collected separately and shade dried for 5 days. Finely powdered leaves were then dissolved with 100ml distilled methanol and was kept undisturbed for 72 hours after extraction with Whatman No.1 filter paper<sup>15</sup>.

### **Collection of mosquito larvae:**

Mosquito larvae are aquatic and adapted to a wide range of habitats including swamps, marshes, free hole, pools, etc. Larvae are settled on the surface of water to get oxygen for respiration. The larvae were drained from the water surface, using a long handled tea-strainer causing least disturbance to the larval population in order to avoid their scattering.

**Bioassay test:**

Three groups of each containing 100 ml of methanol with 0.20 mg, 0.40 mg and 1 mg of the parthenium hysterophorus extract were prepared. To each group, 10 number of mosquito larvae were added and the beakers were covered with net at room temperature ( $25 \pm 5^\circ\text{C}$ ) in the laboratory. Mortality of the mosquito larvae was recorded for 24 hr and 48 hr intervals.

**Larval susceptibility tests:**

The larval susceptibility tests were carried according to standard WHO procedure [16]. The extract solutions of different concentrations were prepared and the larvae were placed in each test solution to observe the larvicidal property as per the following procedure. Group of 10 larvae were placed in 100 mL of the extract solution. The larvae in each solution were then left for 24 hr and numbers of dead larvae were counted after 24 hr of exposure, and the percentage mortality was reported from the average of four replicates. The larvicidal bioassay was performed at room temperature. Percentage mortality of larvae in each beaker was observed after every 15 minutes and overall mortality rate was calculated after 24 hours using the formula; Percentage mortality.

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae tested}} \times 100$$



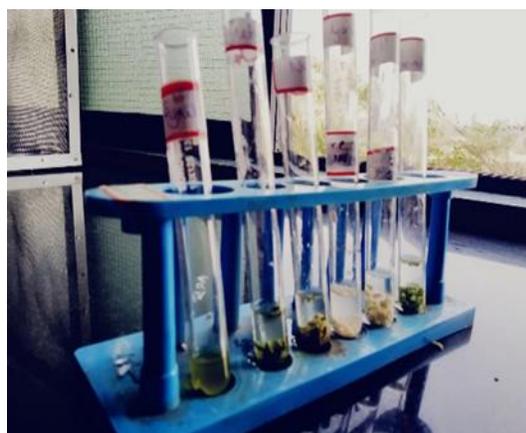
**Phytochemical screening:**

Phytochemical screening suggesting the numbers of chemical constituents. Phytochemicals like Parthenin, Quercelagetin, Fumaric acid, P-hydroxybenzoin, Vanillic acid, Coffeic acid, P-coumoric acid, Panisic acid, Chlorogenic acid, ferulic acid. The plant contains alkaloids, terpenoids, saponins and flavonoids <sup>17</sup>.

**Formulation of larvicidal sachet containing granules:**

The extract of parthenium was dried to form a thick wet mass. After drying, the total mass of parthenium become 0.30 gram because of evaporation of solvent. For the preparation of granules other ingredient are mixed together as excipient(see table below) to form a wet mass. After that screening of wet mass using a suitable sieve number like 6-12 are taken. Then drying of moist granules in hot air oven for 15-20 min. Result in a improper shape of granules are getted then again sieving is done through (sieve number 14) to get proper shape of granules.

Ingredients	Quantity taken	Role
1. Pyrethrum	0.30 gm	API
2. HPC	4.5 gm	Disintegrant
3. Talc	4.7 gm	Disintegrant +binder
4. Starch	0.5 gm	Glidant

**Evaluation of Formulation:****A. Filter paper solubility:**

Filter paper having a small, porous typically packed containing the granules which is immersed in water to steep and make an infusion. They are easily dissolved in water; it's required less than 1 minute to reach the bottom of flask containing water. For accurate result we performed sachet three times and filter paper gave on average same result for solubility(less than 1 minute).

**B. Solubility of sachet:**

Take a 5 sachet which containing granules put into a 100 ml of water. after 24 hours, it appreciable released the drug into the water . Released of content is so fast when its mechanical shaken.



### C. Solubility test for granules:

Take 0.1 gram of granules and it is soluble in 10ml of water with help of vigorous shaking. After 1-2 min the granules are completely soluble in water.

## RESULTS AND DISCUSSION

The plant extract of parthenium against larvae showed significant larvicidal activity at 1 mg level of significance. Methanol leaf extract of parthenium was the most effective mosquito larvicidal which presented the highest percentage mortality. Larvicidal and most important step in the development of an insecticide of botanical source. There are probabilities that the active principle contained in these plant extracts, especially the methanol extracted fractions will be further more potent as mosquito larvicides as compared with their crude forms. The active constituents for pyrethrum is pyrethrins. The identification of these active components is part of further search for an efficient, eco-friendly, biodegradable insecticide of plant origin and is under consideration in the laboratory. From many more research paper, journals and article paper its concluded that the active constituent is responsible for the larvicidal action. Table 1 show that parthenium plant used in present study, with their common name, family, medicinal property and parts used for bioassay. The plant part were selected based on traditional use and previous report of presence of bioactive compounds. The extract is complex mixture which contains many active component. Plants are well known to produce secondary metabolites, which act as defense mechanisms. This characteristic reveals that the natural insecticides play a pivotal role in vector control, and their use represents an excellent alternative to synthetic insecticides. The mechanism of action of secondary plant metabolites against *A. aegypti* larvae is still poorly understood, in particular at the molecular level. Most of these secondary metabolites demonstrated certain interference in the central nervous

system via cutaneous or respiratory absorption, leading to death by intoxication, via the inhibition of acetyl cholinesterase (AChE), which is similar to organophosphates and carbamate insecticides<sup>18</sup>. Some other mechanisms of action observed for the insecticidal compounds when in contact with the predators involve action on the GABA system, leading to seizures and death; inhibition of mitochondrial activity; action as repellent preventing oviposition; action in the digestive system inhibiting the appetite, among others<sup>19</sup>. mosquito larvae was treated with different concentration viz., 0.2 mg , 0.4 mg and 1 mg per 100 ml of methanolic extract of parthenium hysterophorus leaves at different time intervals. The mortality rate increased with increase in concentration and time of exposure is 24 and 48 hrs. The result indicate that the mortality rate is proportional to concentration of the extract. But, its was remarkable to note that the mortality rate was maximum when 1mg /100 mL concentration of the extract in just 48 hrs in all the Three groups of mosquito larvae. In the present study the mortality rate of the larvae was studied at different concentration with methanolic extract of parthenium hysterophorus leaves. The methanolic extract of parthenium hysterophorus leaves showed lethal effect on mosquito's larvae in all concentrations, and mortality rate increased for every 24 hours continuously up to 48 hrs. But, it was remarkable to note that mortality was maximum in 1mg/100 mL concentration of methanolic extract of Parthenium hysterophorus leaves.

**Table 1: Biological used of parthenium:**

Botanical Name	Common Name	Family	Medicinal property	Plant part used
Parthenium hysterophorus	Parthenium	Compositiae	Insecticidal, Wound healing	Leave

**Table 2: Extraction solution of parthenium**

Drug	Concentration mg/ml	Volume solution beaker	Number of larvae taken	Mortality in percentage	Time (Hours)
Parthenium hysterophorus	0.20	100	10	10%	24
Parthenium hysterophorus	0.40	100	10	50%	48
Parthenium hysterophorus	1.0	100	10	100%	48

A gradient of increasing mortality with increasing concentration was observed in all treatments. The higher activity of methanol extract of parthenium may be due to the presence of bioactive components. Further study show that semi-polar solvents had ability to dissolve polar and non polar compounds<sup>20</sup>. In our case Methanol is a polar solvent, But somewhere its found that methanol can dissolve some non polar molecules as well. Methanol is polar solvent so it can be dissolved polar compounds in the methanol crude extracts of plants. Therefore the presence of polar active compounds is more soluble in the methanol extract makes it of higher larvicidal

activity. Testing the plant crude extracts against mosquitoes can lead to identifying potential bioactive compounds that can be used as larvicides to control mosquitoes <sup>21</sup>. It is reported that mosquitoes programmes can be easily carried out targeting larval stages as they are confined to water bodies which are mainly manmade and can be located <sup>22</sup>.

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#### CONCLUSION:

The use of plant extract in vector control is an alternative control method for minimizing the effects of persistence, bioaccumulation and toxicity caused by chemical compounds used as insecticides in the environment, results obtained suggest that the promising effect of larvicides. Consequently, methanol extract of parthenium possess higher larvicidal activity than other solvent extracts.

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