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Hira Amir
Government Post Graduate College for Women Samanabad, Lahore, Pakistan.

Beenish Zia Butt
University of the Punjab, Lahore, Pakistan.

Seemal Ejaz Vehra
Government Post Graduate College for Women Samanabad, Lahore, Pakistan.

Correspondence
Hira Amir
Government Post Graduate College for Women Samanabad, Lahore, Pakistan.

Evaluation of larvicidal activity of *Parthenium hysterophorus* against *Aedes aegypti*

Hira Amir, Beenish Zia Butt and Seemal Ejaz Vehra

Abstract
The present study was conducted to find out the larvicidal effect of *Parthenium hysterophorus* against 2nd and 3rd in star *Aedes aegypti* larvae. Leaves and stem extracts were used, extracts divided into two parts, the first part (stored stock extract) stored for 45 days and then its activity was checked against larvae while the second part (fresh stock extract) was used immediately. Different concentrations of fresh and stored stock extracts were made and tested against *A. aegypti*. Results showed that all the extracts (with different concentrations) were found to be most useful against larvae of *A. aegypti*. Percentage mortality of larvae from leaves stored stock solution was calculated to be 80% while percentage mortality through leaves fresh stock solution was calculated as 100%. The fresh stock solution of leaves prepared in 200g/500ml distilled water was most effective against the larvae and caused 100% mortality. The larvae died within 30 minutes in this fresh solution of leaves. So, it is determined that *P. hysterophorus* leaves can be used as larvicidal against *Aedes aegypti*. It is concluded that as the use of synthetic chemicals are associated with many problems and mosquitoes can also cause resistance against a lot of chemicals therefore, the development of new strategies is necessary. Bio-pesticides are more useful than chemical pesticides because they are less harmful, toxic and cause less environmental pollution. The use of plants as larvicidals agents are very useful and can be used as substitute against pesticides.

Keywords: Mosquitoes, *Parthenium hysterophorus*, bio-pesticides, percentage mortality, larvicidal agents, *Aedes aegypti*

1. Introduction
A recent estimate revealed that more than 50 million people are at risk of dengue virus exposure worldwide. Annually, dengue hemorrhagic fever causes 12,000 deaths [1]. Dengue fever is basically a viral disease caused by infection with 1 out of 4 serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) of dengue virus [2]. Dengue fever was distributed worldwide in the tropics and subtropics during 18th and 19th centuries mainly due to the expansion of commerce and shipping industry. *Aedes aegypti* is the principal mosquito vector [3].
The bite of *A. aegypti* mosquito transmits the dengue virus in human body. Mosquitoes acquire dengue viruses by feeding on the blood of the infected person. Once a mosquito is infected it gains the capacity of transmitting virus to susceptible individuals for its whole life. The infected female mosquitoes may transmit the virus to next generations of mosquitoes through its eggs known as transversal transmission. Humans are the main host of virus, although studies have revealed that in some parts of world monkeys may also become infected and may serve as a source of virus for uninfected mosquitoes. The virus circulates in the blood of infected person from 2 to 7 days and causes fever at the same time [4]. Dengue fever, or "break bone fever" usually takes 3 to 14 days (commonly 3-7 days) to show severity of high fever after bite of an infected mosquito. Frontal headache, rash, hemorrhagic manifestations, retro-orbital pain, low white blood cell count, nausea, anorexia and loss of appetite are the basic symptoms of dengue fever. Acute symptoms usually remain for 1 week, but weakness may persist for several weeks [2].
Use of synthetic insecticides to control mosquitoes is not well accepted as these are not save for humans, have damaging effects on environment, non-biodegradable and expensive [5]. The exploitation of native plants and plants based products to control the mosquito population is gaining much importance in the recent century. The larvicidal action of many local plants has been reported in different parts of the world [6].
Conventionally, plants and their products were used to destroy mosquitoes and other infectious

agents. Secondary metabolites of plants possess insecticidal activity (antibacterial, antifungal and larvicidal potential) that protects themselves against herbivorous insects [7]. *Parthenium hysterophorus* L. commonly known as carrot weed, star weed, white top and congress grass belongs to family Asteraceae. It is native to the subtropics of South and North America. *P. hysterophorus* is an annual herb [8]. The weed had toxic effects, it is an aggressive, and evil weed besides this it have great pharmacological properties against hepatic amoebiasis, tumors and rheumatism etc. The weed has also been stated to have muscle relaxant and Hypoglycemia [9]. Larvicidal efficiency of *P. hysterophorus* leaves, stem and roots was reported. The extracts of each part of plant were prepared with four solvents (acetone, diethyl ether, hexane and petroleum ether. It was further observed that the hexane extracts from root or stem were 13%-28% more efficient than petroleum ether extracts. The investigations demonstrated the potential of *P. hysterophorus* roots and stems against *Ae. aegypti* larvae [10]. It is considered that *Parthenium* possesses the larvicidal, ovicidal, oviposition and pupicidal properties against *A. aegypti* because of the combined effect of parthenin and phenolic acid (ansic acid, caffeic acid, chlorogenic acid, p-ansic acid, vanillic acid, and parahydroxy benzoic acid) [10, 12]. Objective of the present study: 1. Assessment of larvicidal potential of various concentrations prepared from the leaves and stem of *Parthenium hysterophorus* against 3rd and 4th instars of *A. aegypti*. 2. Determination of the larvicidal potential of an exotic plant, *Parthenium hysterophorus* against *A. aegypti* to suppress its population. 3. Determination of the optimum dozes of *P. hysterophorus* or its larvicidal activity against *A. aegypti*.

2. Materials and methods

2.1: Collection of larvae

Samples of larvae of *Ae. aegypti* collected from the Institute of Pakistan Health (IPH) Lahore, Pakistan. Mosquitoes were collected in bottles covered with gauze.

2.2: Plant collection

Parthenium hysterophorus plants were collected from the surrounding areas of Sabzazar (Latitude-31.51857120, Longitude-74.25890604 and Altitude-188) Lahore, Pakistan for larvicidal bioassays.

2.3: Preparation of stored stock solution

Collected plants parts stems and leaves were separated and thoroughly washed with tap water. Each part of plants was weighed separately using weighing balance. Plant parts were crushed separately and obtained weight of each plant part (150 gm of stem and 200 gm of leaves) were soaked in 1000 ml of water using separate beakers and left for 48 hours at room temperature. After 48 hours, the crude extract was filtered by using filter paper. Stock solution was poured in 4 separate bottles. Each bottle was air tight and stored in refrigerator for 45 days.

2.4: Preparation of fresh stock solution

Plant parts were crushed separately and obtained weight of each plant part (150 gm of stem and 200 gm of leaves) were soaked in to 500 ml of water in separate beakers for 24 hours at room temperature (28 ^{o}C). After 24 hours, the crude extract was filtered by using filter paper. Fresh solutions were poured in beakers. Percentage of each stock solution was calculated using formula;

Weight/Volume % = Mass of solute/Volume of solution x 100

Percentage of the fresh stock solution of leaves and stem was calculated as 40% and 30% respectively while for stored solution percentage of the stored stock solution of leaves and stem was calculated as 20% and 15% accordingly.

2.5: Screening of extracts for their larvicidal efficacy against *Ae. Aegypti*

Different concentrations (20%, 40%, 70% and 100%) of fresh stock solution of leaves and stem were made by mixing (20 mL, 40 mL, 70 mL and 100 mL) of fresh stock solution to the (80 mL, 60 mL, 30 mL and 0 mL) of distilled water. Similarly different concentrations (20%, 40%, 70% and 100%) of stored stock solution of leaves and stem were made by mixing (20 mL, 40 mL, 70 mL and 100 mL) of stored stock solution to the ((80 mL, 60 mL, 30 mL and 0 mL) of distilled water. Larvae of the *A. aegypti*, in batches of 5 were carefully transferred in each bottle containing different concentrations of plant extract with the help of dropper. The larvae were taken out with care, and no larvae was hurt or killed during transfer. The bottles were covered with gauze. The larvicidal bioassay was performed at room temperature. Percentage mortality of larvae in each bottle was observed after every 15 minutes and overall mortality rate was calculated after 24 hours using the formula;

Percentage mortality= Number of dead larvae/ Number of larvae tested × 100

3. Results

The larvicidal activity of different concentrations prepared from stored and fresh solutions stem and leaves of *P. hysterophorus* was tested against larvae of *A. aegypti*. The results clearly showed that both the extracts (with different concentrations), found effective against larvae of *A. aegypti* with 100% mortality rate.

3.1: Effect of fresh stock solution on *Aedes aegypti* larvae

Fresh stock solution of leaves and stem were used to make different concentrations of the leaf and stem extract. All the applied concentrations of leaf and stem extracts were found effective against larvae of *A. aegypti* with various mortality rates. Larvicidal affectivity of the applied concentrations of was tested by placing 5 *A. Aegypti* 2nd and 3rd instar larvae in a 200 ml glass beaker containing different concentrations. Percentage mortality of larvae was observed after 24 hours. Larvicidal activity of *P. hysterophorus* leaf extract against *A. aegypti* showed the mortality rate 40%, 60%, 100%, and 100% against 20 mL, 40 mL, 70 mL, and 100 mL respectively while the concentrations of the fresh stock solution showed 20%, 20%, 40%, and 80% against 20 mL, 40 mL, 70 mL, and 100 mL concentration accordingly as shown in (Figure 1). Results clearly showed that the fresh stock solution of leaves proved to be more significant. Fresh stock solution of leaves found to be most effectual in causing 100% mortality of larvae as compared to fresh stock solution of stem that caused 80% mortality at 100% concentration.

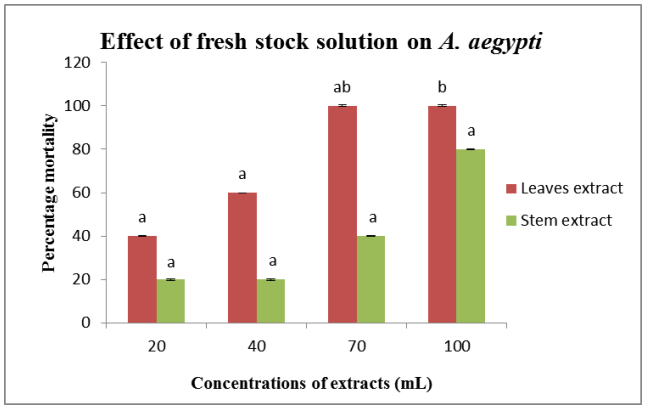

Fig 1: Effect of different concentrations of *Parthenium hysterophorus* leaves and stems extract on the mortality rate of *Aedes aegypti* larvae. Each value is a mean of three replicates with standard deviation. One way ANOVA with Duncan's multiple test range is applied to compare means by using SPSS software.

3.2: Effect of stored stock solution on *Aedes aegypti* larvae

Stored stock solutions of leaves and stem were used to prepare various concentrations of the leaf and stem extract. All the used concentrations of the leaf and stem extracts were found effective against larvae of *A. aegypti* with different mortality rates. Larvicidal potential of the applied concentrations of was assessed by placing five *A. Aegypti* 2nd and 3rd instar larvae in a 200 ml glass beaker containing different concentrations. Percentage mortality of larvae was calculated after 24 hours. Larvicidal activity of *P. hysterophorus* leaf extract against *A. aegypti* showed the mortality rate 20%, 40%, 60%, and 80% against 20 mL, 40 mL, 70 mL, and 100 mL respectively while the concentrations of the stem stored stock solution showed 0%, 20%, 40%, and 60% mortality against 20 mL, 40 mL, 70 mL, and 100 mL concentration respectively as shown in (Figure 2). Results clearly showed that the stored stock solution of leaves proved to be more significant against larvae. Stored stock solution of leaves found to be most powerful in causing 80% mortality of larvae as compared to stored stock solution of stem that caused 60% mortality at 100% concentration.

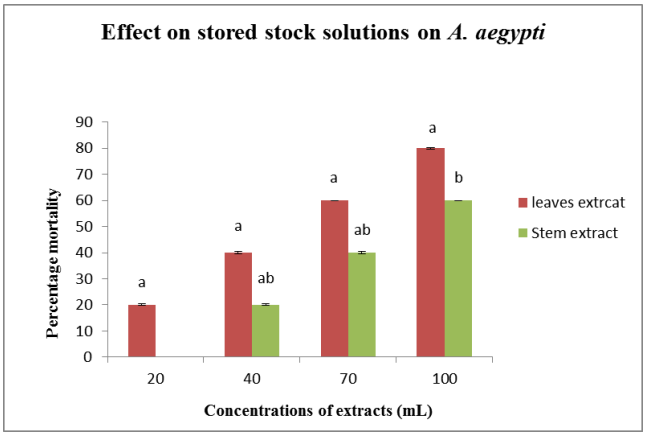

Fig 2: Effect of different concentrations of *Parthenium hysterophorus* leaves and stems extract on the mortality rate of *Aedes aegypti* larvae. Each value is a mean of three replicates with standard deviation. One way ANOVA with Duncan's multiple test range is applied to compare means by using SPSS software.

4. Discussion

The use of synthetic chemicals to control mosquitoes is associated with many problems as mosquitoes have developing resistance against many chemicals therefore, the development of new strategies is considered. Bio-pesticides are more useful than chemical pesticides because they are less harmful, toxic and cause less environmental pollution as they do not contain synthetic chemicals. During the last decades, a lot of studies on natural plants as larvicidals against mosquitoes indicates positive results and can be used as alternatives against pesticides. Plants have medicinal and pesticidal properties as well as they are rich in bioactive chemicals. The mixtures prepared from different parts of plants are used against mosquitoes and have found to be effective [10].

According to a study it was reported that synthetic chemical insecticides are used routinely for mosquito control but they usually develop resistance against the insecticides which reduces the effectiveness of these chemicals to control the population of mosquitoes. Additionally, synthetic chemicals disturb the natural environment. Evidences have been found that these materials act as immunosuppressant which reduces the immunity of animals and human beings. *Parthenium* plant extract can be used as a larvicidal agent against *Aedes aegypti.* As the *Parthenium* is an exotic plant and it is usually uprooted to save other species so it's a good way to use this plant as a larvicidal agent against *Aedes aegypti* to control its population and in this way population of *Parthenium* can also be managed. *Parthenium* has a lot of Allelopathic advantages and used to cure many diseases. The plant contains alkaloids, terpenoids, saponins and flavonoids [11].

Larvicidal and pupicidal activity of *Parthenium* was reported against *Ae. aegypti* because of the combined effect of parthenin and phenolic acid (ansic acid, caffeic acid, chlorogenic acid, p-ansic acid, vanillic acid, and parahydroxy benzoic acid) [12]. *Parthenium* may possess the ovicidal and oviposition deterrent property against *A. Aegypti* [10]. Fast growth rate, adaptive nature, high reproductive nature and interference by allelopathy gives a lot of importance to *Parthenium* [9]. The insecticidal effect of plant extracts may attribute to one or more of the following properties including repellency, stomach poisoning effect where insects feed on ad mixed rains and pick up lethal doses of treatment particles, and these powders might reduce insect movement and also cause death through occlusion of their spiracles, thereby, preventing respiration via trachea [13]. According to a study, chemical analysis of *Parthenium* has indicated that all the plant parts including trichomes and pollen contain toxins called sesquiterpene lactones [14]. The major component of these toxins is parthenin and other phenolic acids of caffeic acid, vanillic acid, anisic acid, chlorogenic acid, parahydroxy benzoic acid and p-anisic acid [15].

Therefore, present study explored its potential for use against *Aedes aegypti* larvae. The present research revealed that *Parthenium* is effective against *Aedes* larvae. The leaves and stem of *Parthenium* were used against the vector and the results showed that the leaves are most potent in causing high mortality rate. The solution prepared from the leaves and stem in distilled water and used by making different concentrations given in different concentration. The fresh solution of leaves proved to be most efficient against the larvae and have 100% mortality rate. The larvae died within 30 minutes in the 100

mL fresh solution of leaves. Therefore, *Parthenium* leaves can be used as larvicidal against *Aedes aegypti* larvae.

5. References

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