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Study of Lantana Camara, Parthenium Hysterophorus and Allium Sativum Linn. For Mosquito Larvicidal Potential and the Formulation of Larvicidal Sachet.

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ABSTRACT

Mosquito born diseases are noxious for human being life and were always a never ending hazard . They have also developed a resistance to this chemical insecticides. Alternatively bio-pesticides are been used. In Indian the Lanata camara , Parthenium hysterophorus are weeds that shows the larvicidal activity along with the Allium sativum linn. The present studies has shown the positive results of larvicidal activity. And the methanol as the choice of extract for the extraction process. Formulation of larvicidal sachets and it's evaluation were done. Leading to sachets formulation which shown high solubility and stability. With the dose dependent activity and showing the effective measures at low concentrations. Which is economical and ecofriendly less toxic then chemical synthetic pesticides, user - friendly, easy availability. Control and prevention of Mosquito larvaes ,using herbs showing larvicidal activity in sachets formulation.

Keywords: Medicinal herbs, larvacidal activity, harmful insecticides, bio-pesticides, environmental safe.

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INTRODUCTION

The mosquito-borne diseases such as Malaria, Dengue fever, Encephalitis, Yellow fever, Chikungunya, Filariasis, West Nile virus and Zika virus are creating major loss of human life in many countries¹. The diseases and illness is caused due to the bacteria, viruses, and parasites transmitted by mosquitoes². There are around 1 million deaths each year without affecting themselves². And about 700 millions people gets infected each year². With this all records Malaria is endemic in 91 countries and about 40 % of world's population at risk². According to the Southeastern Asian Region of WHO, around 1.4 billion people living in 11 countries 1.2 billion are exposed to the Malaria of which most of lives in India. And Southeast Asia contributes 2.5 million cases to the global burden of malaria. Of which India alone contributes 76% of the total cases³. Whereas most of the burden of Malaria cases are reported from African regions (93%). And in very recent years there is reduction of malaria cases as per the World Malaria Report 2019, an estimated 228 million Malaria cases across the world in 2018, reduced from 231 million cases in 2017. Including the sharp reduction in Malaria cases between 2017 and 2018 in India, It witnessed about 28 % fall in malaria cases. Making India one of the only two countries to report a reduction in Malaria cases other nation was Uganda⁴. Chemical pesticides have used to overcome the diseases in India and all around the world. Though their use and misuse creates a considerable waste, contributing to the adverse environmental and health consequences. And inappropriate application of pesticides affects the whole ecosystem⁵. Humans get exposed to the pesticides by different routes such as inhalation, digestion, and dermal contact⁶. Thus leads to acute and chronic health problems. Due to which there is an increase in the cancer, chronic kidney disease, low immunity system, sterility in male and female, endocrine disorders, neurological and behavioural disorders are chronic pesticides poisoning⁷. Moderate effects are mild headaches, flu, skin rashes, blurred vision while severe effects are blindness, paralysis and death⁸. Some of the insecticides like carbamate and pyrethroid are frequently used to control insects. They particularly deal with the aphides and other pests which shows resistance to organophosphorus compounds. Permethrin which is pyrethroid insecticide with high level of activity against lepidopterous pests and also

Table 1: Pesticides used in India

Pesticides: common name	Chemical Name
Phorate	Organophosphate
Carbofuran	Carbamate
Monocrotophos	Organophosphate
Endosulfan	Organochlorine
Carbamyl	Carbamate
Cyhalothrin	Pyrethroid

against Hymenoptera, Diptera and Coleoptera. The compound shows adulticidal, ovicidal and particularly larvicidal activity. Pesticides used in India are shown in^{9 10}. Table 1:-¹¹

Biopesticides, biocontrol strategies¹² are used for reducing the continuous application of insecticide for mosquito-borne diseases which were primary method for mosquito control. Eco – friendly, safe and sustainable method can be developed by using biocontrol agents to target different mosquito species. More than 80 plant species has been used for the successful synthesis of nano mosquitocides, which has particular reference to larvicidal purposes. And used to prepare cheap repellents for people with low human toxicity¹² Recently many efforts have been focused on plants extract or phytochemicals as potential sources of mosquito control agents. A survey of literature indicates that most of the studies on larvicidal effects of plant products on mosquitoes are well known horticultural plants and commonly grown plants. A weed plants that is found in a vast areas on plains as well as on hill regions shows a larvicidal activity¹ *Parthenium hysterophorus* Linn. (*P. hysterophorus*) is commonly and easily available weed in India. Which is also known as congress grass, carrot weed, chatak chandani, whitetop, ragweed, whitehead, bitter weed, ramphool or gajarghas¹. It is a flowering plant from family Asteraceae¹³. The plant is poisonous, pernicious and aggressive weed. They show pharmacological properties against rheumatism, helatic amoebiasis, tumors, muscle relaxant and hypoglycemia. Due to its deleterious properties. For many years limited work is been carried out. However it shows more potential to control mosquito growth. As it shows the larvaecidal activity to kill the 2 and 3 instar of larvae, leaves extract was used showed the effect after 30 mins¹⁴. By formulation and other strategies to reduce mosquito population¹. Along with *Parthenium hysterophorus* another plant that is used as larvicidal is *Lantana camara*. *Lantana camara* Linn. Occurs along the roadsides and degraded lands. It is waste land notorious weed species of flowering plant from family Verbenaceae. *Lantana camara* is commonly called as West Indian *Lantana*, wildsage, red sage, tickberry. Different parts of plants are used for medicinal and non - medicinal purposes, also including its complex are toxic to small ruminants. The effect is associated with the types and relative amounts of some triterpene ester metabolites. In India it is used for folk and traditional medicine systems like antimicrobial, Fungicidal, insecticidal and nematicidal activity including hepatotoxing in animals. Antimicrobial immunosuppressive and antitumor activities are shown by *Lantana camara* the present investigation shows larvicidal effect of *Lantana camara* extract against the 3 and 4 instar larvae of *Aedes aegypti*^{15 16 17} *Allium sativum* linn commonly known as garlic, it is a fresh bulbs of plant *Allium sativum* linn. Family Liliaceae (*Garlic*) (L) (Liliaceae) The essential oil obtained from the bulb contains allicin, diallyl disulfide, allyl propl disulfide and other sulfur compounds.¹⁸

MATERIALS AND METHOD

Plant collection:

The fresh leaves of *Lantana camara*, *Parthenium hysterophorus* was collected from various places in Junnar region, district Pune, India and garlic was bought from the Bhalgaon, Kalyan, India. The plant specimens were confirmed and authenticated by a Botanist Dr. Anil Ahvad sir, Associate Professor in Ramniranjan Jhunjhunwala college, Ghatkopar Mumbai 400086, India. Then the leaves were collected separately and shade dried for 5 – 6 days. The dried leaves were then finely powdered.

Preparation of extract:

The dried leaves and crushed garlic were collected. And were weighed properly and dissolved in 100 ml of methanol (as a choice of solvent) for 72 hours undisturbed and then the extraction was filtered with Whatmann No. 1 filter paper. Concentrated extract were diluted with water¹⁹.

Collection of mosquito larvae:

Mosquito larvae are aquatic and are adapted to a wide range of habitats such as swamps, marshes, free holes, pools, etc. Larvae are settled on the surface of water to get oxygen for respiration. For collection, 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 away. Larvae growth were also done by providing light and optimal conditions in the tray for reproduction. And were covered with a net for trapping grown mosquitoes in it.

Bioassay test:



Three groups of each containing 100 ml of water with 0.20 mg, 0.40 mg and 1 mg of the *Lantana camara*, *Parthenium hysterophorus*, *Allium sativum* extract were prepared. To each group, 10 numbers 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.

Screening of extracts for their larvicidal efficiency against the mosquito larvae:

The bioassay test were performed on the second and third instar larvae stage, according to the standard WHO procedure. The extract solution of different herbals with the different concentrations were prepared and the larvae were placed. The solution was shaken slightly to perform uniform larvicidal action on all the larvae. Group of 10 larvae were placed in 80 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 three replicates.²⁰

Evaluation of larvicidal potential of selected extracts:

The extracts concentration which showed 80% to 100% mortality were considered effective against the larvae and were evaluated for larvicidal potential. Bioassay was performed for each concentration. Three replicates were carried out for each assay.

Phytochemical screening:

Phytochemical screening suggest the numbers of chemical constituents, this procedure shows many²¹. Phytochemicals like carbohydrates, tannins, saponins, flavonoids, Alkaloids, Betacyaning, Quinones, Glycosides, cardiac Glycosides, terpenoids in *Lantana camara*. Phytochemicals like Parthenin, Quercelagetin, Fumaric acid, P-hydroxybenzoin, Vanillic acid, Coffeicacid, P-coumoric acid, P- anisic acid, Chlorogenic acid, ferulic acid. The plant contains alkaloids, terpenoids, saponins and flavonoids²². Chemical compounds found in garlic are € ajoene; (Z) ajoene; allicin, alliin, allixin ; γ - glutamyl-S-2-propenyl cysteine, diallyl disulfide; methyl allyl disulfide; S-allyl-cysteine; 1,2-vinyldiithin.²³

Formulation of larvicidal sachet containing granules:

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

Ingredients	Quantity taken	Role
1.lantana camera	0.30 gm	API
2.Parthenium hysterothorus	0.30 gm	API
3.Allium sativum	0.30 gm	API
4. HPC	4.5 gm	Disintegrant
5. Talc	4.7 gm	Disintegrant +binder
6. Starch	0.5 gm	Glidant

**Evaluation of formulation:****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 mechanically shaken.



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 results shows that mosquito larvae was treated with different concentration viz., 0.2 mg , 0.4 mg and 1 mg per 100 ml of methanolic extract of lantana camera, parthenium hysterophorus leaves and garlic bulb at different time intervals. The mortality rate increased with increase in concentration and exposure of time 24 and 48 hr. The result indicate that the mortality rate is proportional to concentration of the extract. But, it was remarkable to note that the mortality rate was maximum in 1mg /100 mL concentration of the extract in 48 hr in all the three groups of mosquito larvae in all plants extract. The methanolic extract of all plants showed lethal effect on mosquitoes larvae in all concentrations, and mortality rate increased for every 24 hours continuously up to 48 hrs. From table 3 observation we concluded that at higher concentration mostly parthenium showed their effect. It is remarkable to note that plant extract did not cause any appreciable mortality at a lower concentration after which a steep rise in larval mortality was noticed(1mg/100ml). From table 2, 3,4 its noted that as concentration is increases the mortality rate

also increases with the increase in time. Highest mortality was found at concentration 1 mg/100 ml.

Percentage mortality= Number of dead larvae/Number of larvae tested \times 100

For packaging purpose, we used sachets. Tea bags and filter paper (normal) for solubility of larvicidal. We see that naturally tea bags is slow and for fast solubility they need mechanical shaker for release of drug in to the water . Hence we selected the filter paper, which is commonly used un laboratories and for experimental use. Which has small porous that contains the granules and when immersed in water to steep and make a infusion. Thus filter paper easily gets dissolved in water, it required less then 1 min to reach at the bottom of flask containing water. And for accurate results we perform sachets three times and it gives on an average we repeated for 3 times for solubility (less then 1 minute).



Table 1: Biological uses of plants Parthenium hysterphorus

Botanical name	Lantana Camara (figure 1)	Parthenium Hysterphorus (figure 2)	Allium Sativum (figure 3)
Common name	West Indian lantana	Congress grass	Garlic
Family	Verbanaceae	Asteraceae	Liliaceae
Medicinal properties	Larvicidal, anti-oxidant, antifungal	amoebiasis, larvaecidal activity, muscle relaxant	Anticancer, antimicrobial, antioxidant
Plant part used	Leaves	Leaves	Bulbs

Table 2: New Mosquito Larvae Has Taken

Drug	Concentration mg/ml	Volume of solution in 100nl of beaker	Number of larvae taken	Mortality percentage	Time (Hr.)
Lantana Camera	0.20	80	10	8 ± 0.95	24
Lantana camara	0.40	80	10	20 ± 1.41	48
Lantana camara	1.0	80	10	40 ± 1.91	48

Table 3: New Mosquito Larvae has taken

Drug	Concentration mg/ml	Volume of solution in 100nl of beaker	Number of larvae taken	Mortality percentage	Time (Hr.)
Parthenium hysterophorus	0.20	80	10	10 ± 0.66	24
Parthenium hysterophorus	0.40	80	10	20 ± 0.81	48
Parthenium hysterophorus	1.0	80	10	78 ± 1.70	48

Table 4: New Mosquito Larvae Has Taken

Drug	Concentration mg/ml	Volume of solution in 100nl of beaker	Number of larvae taken	Mortality percentage	Time (Hr.)
Allium sativum	0.20	80	10	3 ± 0.50	24
Allium sativum	0.40	80	10	25 ± 0.95	48
Allium sativum	1.0	80	10	50 ± 1.33	48

DISCUSSION:

Mosquito borne diseases are one of the most important and major public health problems form ages, and it is seen more in developing countries. It can be controlled by preventing mosquito bite using repellent, causing larval mortality and killing mosquitos^{24 25 26}. As the solution to control the mosquito diseases, synthetic chemicals were in use to which the mosquito showed the resistance and therefore, the development of new strategies were considered. Bio-pesticides were in use as an alternative and more effective than chemical pesticides as the bio pesticides were less harmful, toxic and caused less environmental pollution then the synthetic chemicals¹⁴. By the Extraction as the term pharmaceutically used, is defined as the technique used for the separation of therapeutically desired active compounds and elimination of undesirable insoluble material by treatment with selective solvents²². Extraction yields of the extracts are strongly dependent of the nature of solvent, the herbs moisture content and the extraction technique. The presence of various constituents with different chemical characteristics and polarities requires the selection of an appropriate solvent. In this study we used the methanolic extract for better extraction process and for effective larvicidal activity²⁷. Various extracts were found effective and studied in a dose dependent manner. The methanol and ethanol flower extract of Lantana camara was found to show the higher rate of larvicidal activity against *Aedes aegypti*, where as in the *Culex quinquefasciatus*

variety, the concentration of extracts were increased for better larvicidal effect¹⁴. He studied that extracts result in maximum activity on 3 and 4 instar larvae¹⁴. Senthilnathan observed that higher larvicidal effect of *Eucalyptus tereticornis* oil (leaf extract) with increased doses on *Anopheles stephensi*. He also observed that first and second instar larvae were most susceptible to all treatment²⁸. Antifeeding effects of crude lantadene from *Lantana camara* on *Plutella xylostella* and *Spodoptera litura* larvae²⁹ and antifeeding and repellent effect of *Lantana camara* on tea mosquito bug³⁰ were reported. The insecticidal³¹ nematicidal³² activities have also reported. Since the last decades, a lot of studies and research were carried on natural plants and herbs as larvicidal against the mosquitos showing the positive results and use as alternatives against synthetic chemicals. The mixtures and extract prepared from different parts of plants are used against mosquito and have found to be very effective. According to a study, it was reported that synthetic chemical insecticides are used routinely and on constantly for Mosquito control but they have usually developed a resistance against this chemical insecticides¹. And additionally the major use of chemicals have disturb and shown hazardous effects on natural environment. Evidences have been found that these materials act as immunosuppressant which means it reduces the immunity of the animals and human beings.¹⁴ In recently the use of eco – friendly, biodegradable insecticides from the plant to control the growth of Mosquito born diseases and eventually it is gaining importance. As this botanical plants shows the activities and are found effective, economical and user – friendly. Kweka er al reported that the repellency of oils extracted from plants has a given a high protection of 83% - 91% against mosquito biting²⁶. Many phytochemicals have observed in earlier studies and shown major role in mosquito control programme^{34,35}. Gopieshkhan and Kannabiran³⁶ have studied and observed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plant extract having mosquito larvicidal activity. Pelah et al.³⁷ has reported the use of commercial saponin from *Quillaja*, *saponaria* bark as a natural larvicidal against *Aedes aegypti* and *Culex pipens*. And all Isolated triterpenoids were found to have an antibacterial activity^{38,39}. Cardiac glycoside was found to have a acaricidal effect against larva and adult stages of the camel tick⁴⁰.

CONCLUSION:

Thus we can conclude that the plants larvicidal constituents are effective against the larvae growth. And with the help of proper methanolic extract the activities gets enhance. In this study we prepared the larvicidal sachets as the sachets shows more stability of drug and gets highly soluble in water. Are easily prepared and available. And study shows the dose dependency at very low concentrations and reveals that the extracts shows the effect against mosquito.

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