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Larvicidal Activity Of Leaves Extracts Of Lemon Grass On Mosquito Larvae

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ABSTRACT

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE). In India, malaria is one of the most important causes of direct or indirect infant, child and adult mortality with approximately two to three million new cases arising every year. The traditional chemical pesticides used to control mosquito have many drawbacks, and natural products are more acceptable in this aspect. The bio-efficacy of Lemon grass aqueous and ethanolic leaves extracts was assessed against the fourth instars larvae of Aedes aegypti, under the laboratory conditions. Thereafter, the phytochemical profiles and the cellular toxicity of the extracts were evaluated. Both extracts shows larvicidal activity against Aedes aegypti Larvae. Ethanolic extract showed better effects as compared to aqueous extract. Overall, our findings indicate that the plant, Lemon grass, may prove to be highly promising alternatives as bioinsecticides. This is an eco-friendly and low-cost approach, may use to control Aedes aegypti.

Keywords: *Lemon grass, Herbal medicine, Phytochemical investigation, Larvicidal activity.*

INTRODUCTION

Japanese encephalitis (JE), filariasis, schistosomiasis, dengue disease, yellow fever, and malaria are all primarily spread by mosquitoes [1]. With two to three million new cases each year, malaria is one of the leading causes of direct or indirect newborn, child, and adult mortality in India. Anopheles subpictus Grassi is found in Borneo, China, Malaysia, the Philippines, Sri Lanka, Java, Indonesia, Afghanistan, and India. In the states of Uttaranchal and Haryana, it is a prominent species [2]. Seventy-seven percent of Southeast Asia's malaria cases are caused by India [3,4]. The Japanese encephalitis (JE) virus is mostly transmitted by Cx. tritaeniorhynchus, which is found in Southeast and South Asia. According to Keiser et al., estimates of the annual global incidence and mortality of JE are 30,000 to 50,000 and 10,000, respectively. [5,6] The arbo-virus that causes dengue fever, which is indigenous to Southeast Asia, the Pacific Island region, Africa, and the Americas, is commonly known

to be carried by Aedes aegypti [7]. In West Africa, Central America, and South America, this mosquito also spreads yellow fever [8]. However, as more cases are reported, dengue fever has grown to become a significant public health concern. This is especially the case for more severe strains of the disease, such as dengue hemorrhagic fever and dengue shock syndrome, or for uncommon symptoms such central nervous system fever. A. aegypti is a cosmotropical species that proliferates in water containers in and around houses [9, 10].

Because chemical insecticides contain neurotoxic and carcinogenic substances and are even absorbed down the food chain, they pose a health risk to humans through bioaccumulation. Furthermore, vectors may develop resistance to the same insecticide as a result of repeated application [11, 12]. Chemical insecticides are also hazardous for non-target animals like bees, butterfly etc., which have ecological relevance. Therefore, in order to reduce mosquito-borne diseases, alternative, environmentally benign methods of reducing mosquito larvae are crucial.

About 55 species make up the genus Cymbopogon, which is native to tropical and semi-tropical regions of Asia and is grown in South and Central America, Africa, and other tropical regions. Similar to citrus flavour, these tufted perennial C4 grasses have several stiff stems that emerge from a short, rhizomatous rhizome [13]. They can be used fresh or dried and powdered. Lemongrass (C. citratus) has known for its harmful effect on mosquito larvae. This impact has to do with the chemical substance called citronella, which is found in the stem and leaves of lemongrass. When it reaches 35%, it becomes the densest chemical compound in the plant. Mosquitoes are directly impacted by citronella through touch. A mosquito exposed to citronella would lose bodily fluids and eventually perish [14, 15]. C. citratus inhibits growth and increases mortality in later developmental stages of A. aegypti, although it has no effect on larvae in their first instar [16, 17]. Methanol was used in this investigation to extract the leaves of lemongrass. To evaluate the extracts' larvicidal potential, fourth instar A. aegypti larvae were used as test subjects. In this work, we produced an ethanolic and aqueous extract of lemon grass leaves and evaluated their larvicidal effectiveness in a lab setting against Aedes aegypti.

MATERIALS AND METHODS

Collection of plant and preparation of extract: Plants leaves of Lemon grass was collected from cultivation field near Bareilly region. The voucher specimen kept in our research laboratory for further reference (PS/MJP/2024/010). The plant leaves were properly cleaned, allowed to dry in the shade at room temperature, and then blended into a powder using an electrical blender. Plant material was produced as an ethanolic extract in a 1:100 (w/v) ratio at room temperature. Whatman number 1 filter paper was used to filter the extracts via a Buchner funnel, and the filtrate was then allowed to air dry [18, 19]. Then, in order to create a stock plant extract with a final concentration of 1000 mgl-1, these metabolite extracts were dissolved in ethanol. The percentage of yield (w/w) was calculated using the following formula [20]:

$$\text{Percentage of yield} = (\text{Weight of dry extract})/(\text{Weight of fresh plant material}) \times 100$$

Phytochemical and Proximate analyses: The phytochemical screening and proximate analysis of the powdered leaves were done according to standard procedures [21, 22, 23].

Larval toxicity test: For the larvicidal bioassay, a colony of A. aegypti larvae raised in a laboratory was utilised. The larvicidal activity was carried out with adjustments in accordance with World Health Organisation recommendation 14. To put it briefly, twenty fourth instar mosquito larvae were kept at room temperature with a 12/12 h light/dark cycle in a conical flask filled with 249 ml of phosphate buffered saline. Different quantities of plant extract from a 1000 mgl-1stock were applied to the mosquito larvae. [24] The following formula was used to compute the number of dead larvae and mortalities after a 24- and 48-hour count, compared to an untreated control [25, 26, 27].

$$\%\ \text{of Mortality} = (\text{Mortality in treatment}/\ \text{Mortality in control}) \times 100$$

The experiment was run three times, and the average of the three runs is given as the outcome.

RESULTS AND DISCUSSION:

Proximate and phytochemical analyses: A pharmacopoeia standard for verifying the authenticity of unprocessed powdered plant material is proximate analysis. It speaks about figuring out what the main ingredients are in feed and other unprocessed plant components. It is employed to determine whether the feed has been tampered with or is within its typical compositional bounds. The proximate parameters of the powdered Cymbopogon citratus leaf, such as its moisture content, ash content, alcohol content, and water extractive values, were determined in this study. The results showed that C. citratus leaf has moisture (12.65 ± 1.28%), ash (8.11 ± 0.44%) and extractive value (13.65 ± 1.43 and 10.11 ± 2.76) (Table 1). The phytochemical constituents of the leaf of Cymbopogon citratus are presented in Table 2. The result revealed the presence of tannins, saponins, flavonoids and other phenolics compounds.

Table 1: Proximate analysis of the leaves of Cymbopogon citratus

S. No.	Parameters	Mean ± SD
1	Moisture content	12.65 ± 1.28
2	Total ash	8.11 ± 0.44
3	Acid insoluble ash	4.00 ± 1.08
4	Water soluble ash	6.13 ± 0.48
5	Alcohol soluble extractive value	13.65 ± 1.43
6	Water soluble extractive value	10.11 ± 2.76

Table 2: Phytochemical Composition of the Leaves of Lemon grass extracts

S. No.	Phytochemical	Ethanol extract	Aqueous extract
1	Alkaloids	-	-
2	Tannins	+	-
3	Carbohydrates	+	+
4	Flavonoids	+	+
5	Essential oil	+	+
6	Saponins	-	+
7	Phenolics compounds	+	-

+: indicates presence of component; –: indicates absence of component

Larval toxicity test: Table 3 displays the mortality rate of A. aegypti following treatment with ethanolic and aqueous extract. In this investigation, larvae treated with aqueous and ethanolic leaf extract for 24 hours demonstrated 38% and 68% mortality, respectively, at a concentration of 100µgml-1 (Figs. 1 and 2). It rose to 61% and 70%, respectively, after 48 hours. Therefore, there is no relevance when using both extracts of leaf extract alone. This outcome unequivocally shows that lemon grass ethanol leaf extract has the capacity to operate as a larvicidal agent against A. aegypti larvae and has also shown a synergistic effect.

Table 3: Results Of The Mortality Of Iv Instar A. Aegypti Larvae After The Treatment Of Plant Extract At Different Concentrations And In Different Period

S. No.	Extract	Concentration (μgml^{-1})	% of Mortality (24 h)	% of Mortality (48 h)

1	Aqueous	25	10	22
		50	25	35
		75	32	47
		100	38	61
2	Ethanolic	25	10	35
		50	40	49
		75	38	75
		100	68	70

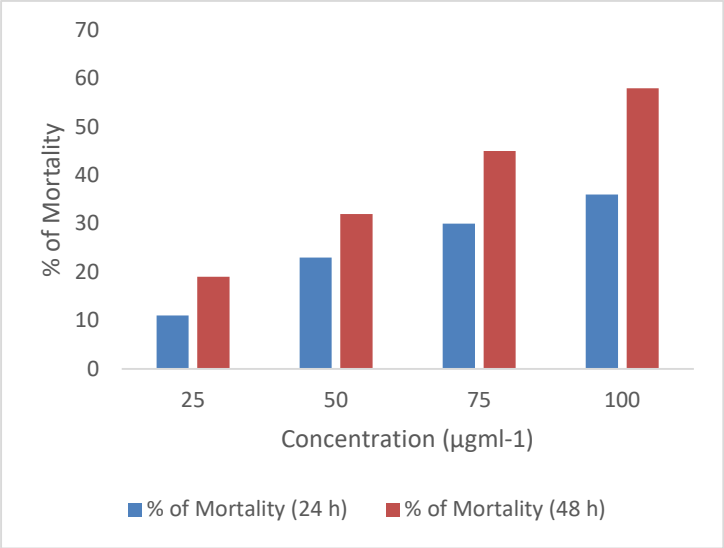

Figure 1. Larvicidal Activity Of Lemon Grass Leaf Aqueous Extract. Mortality After 24h Treatment and Mortality After 48h Treatment.

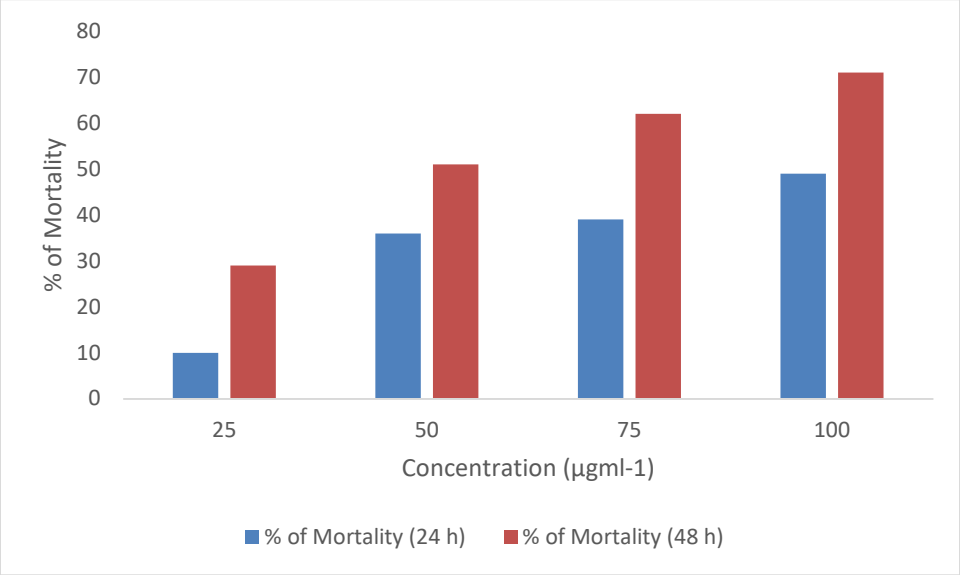

Figure 2. Larvicidal Activity Of Lemon Grass Leaf Ethanolic Extract. Mortality After 24h Treatment and Mortality After 48h Treatment.

CONCLUSION

The stages of a mosquito's life cycle are eggs, larvae, pupae, and adults. Since they can walk on water at this stage, making it easier to deal with them in their natural habitat, the larval stage is a more appealing target for death. Additionally, certain fish eat these larvae. In the end, using traditional chemical pesticides in water sources puts both human health and the ecosystem at danger. In this sense, insecticides made from plants are more acceptable. The dengue vector could be managed with the current approach. This strategy may lessen the likelihood that the mosquito population may develop physiological resistance. Prior to that, it is important to assess the two plant extracts' larvicidal effectiveness and mode of action in the field. Besides, further investigation is required regarding the effect on non-target organism, which is extremely important.

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