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Sagata Mondal
Post Graduate Department of Zoology, Vidyasagar College, Salt Lake campus, C L Block, Kolkata, West Bengal, India

Sujan Ghosh
Post Graduate Department of Zoology, Vidyasagar College, Salt Lake campus, C L Block, Kolkata, West Bengal, India

Sourav Maity
Post Graduate Department of Zoology, Vidyasagar College, Salt Lake campus, C L Block, Kolkata, West Bengal, India

Goutam Ghosal
Post Graduate Department of Zoology, Vidyasagar College, Salt Lake campus, C L Block, Kolkata, West Bengal, India

Airin Sultana
Post Graduate Department of Zoology, Vidyasagar College, Salt Lake campus, C L Block, Kolkata, West Bengal, India

Corresponding Author:
Sagata Mondal
Post Graduate Department of Zoology, Vidyasagar College, Salt Lake campus, C L Block, Kolkata, West Bengal, India

Comparative study on larvicidal potentials of three medicinal plants on larvae of *Culex quinquefasciatus* Say, 1823 mosquitoes

Sagata Mondal, Sujan Ghosh, Sourav Maity, Goutam Ghosal and Airin Sultana

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Abstract
Mosquitoes acts as principal vectors and transmits several diseases that takes away human life's population globally. They are the vector of many vector-borne diseases affecting human beings and animals. The use of chemical larvicides not only exerts health hazards to the human beings as well as the to the environment but also increases the resistance power of the mosquito species. Thus different botanicals with potential larvicidal values and causes no harm to the living beings and to the environment should be used to diminish the population of these mosquitoes. This study recorded the value of LC_{50} of the ethanol extract of *Lantana camara* was 234.43 ppm, 131.82 ppm and 89.12 ppm at 24hrs, 48hrs. and 72hrs. time intervals respectively; 426.57 ppm, 186.20 ppm and 89.12 ppm at 24hrs, 48hrs. and 72hrs. time intervals respectively in case of *Catharanthus roseus*; 398.10 ppm, 295.12 ppm and 199.52 ppm at 24hrs, 48hrs. and 72hrs. time intervals respectively in case of *Ricinus communis*. Thus, the ethanol leaf extracts of *Lantana camara* found to be more effective over *Catharanthus roseus* and *Ricinus communis* as an important larvicidal agent against the mosquito larvae of *Culex quinquefasciatus*.

Keywords: Medicinal plants, larvicidal activity, ethanol extracts, *Culex quinquefasciatus*, larvae, LC_{50} value

Introduction
The genus *Culex* (Diptera: Culicidae) are the most common and dominant in terms of species variety and it is able to breed in various types of stagnant waters viz. drains, pools, ditches, tree holes, paddy fields, etc. *Culex* have the ability to lay eggs in rafts both in fresh and dirty and polluted water, which makes them most abundant in comparison to other mosquitoes throughout the World. They are the most notorious biters and spreads viral encephalitis, filariasis to human beings and also transmits several viral diseases to many different birds, horses and other animals. These diseases leads to the mortality of human beings and causes huge loss to the World economy [1, 2, 3, 4, 5]. *Culex quinquefasciatus* Say, 1823 is responsible for Filariasis in India as it acts as a vector for *Wuchereria bancrofti* [6].
Since 1950's the use of highly toxic chemicals in the form of pesticide to control these mosquitoes are being practiced in India. As a result these synthetic pesticides have helped these vectors to gain physiological resistance, instead of killing them, and also their application causes poisonous residual effects on different beneficial and other living organisms and other environmental components. So, the control of these mosquitoes using toxic chemicals as insecticides has failed in part. Recently, many different plants were found to be of great insecticidal properties. *Lantana camara* L., *Catharanthus roseus* (L.) G.Don and *Ricinus communis* L. are common locally available medicinal plants and are with different insecticidal properties.
Thus, a comparative study on larvicidal potentials of *Lantana camara*, *Catharanthus roseus* and *Ricinus communis* leaf extracts against the larvae of *Culex quinquefasciatus* using different solvents were carried out during the present study.

Materials and methods

1. Model Organism: *Culex quinquefasciatus* Say, 1823. was chosen as model organism.

2. Plants used: The leaves of *Lantana camara*, *Catharanthus roseus* and *Ricinus communis* were collected locally.

3. Preparation of Extract

The leaves were collected and then air dried at room temperature for about 1 week. Then the leaves were finely grounded to powder using a mixer-grinder.

After grinding the powdered leaves were transferred to a conical flask. Then a suitable solvent (70% Ethanol) was used to homogenize the solution.

The amount of ethanol added was double the amounts of powdered leaves. Then shaken vigorously so that a proper mixture is formed. The extracts were left for 2-3 days in between shaken vigorously. Then after 2-3 days the top layer extract was isolated and kept in a petri dish and the supernatant was discarded. The mixture collected in petri dish was dried in incubator for 2-3 days. Then after 2-3 days the dried extract was obtained.

4. Mosquito collection and rearing

The mosquito larvae was obtained locally and then kept in an aquarium using optimum conditions.

5. Larvicidal bioassay

Different concentrations of the crude extracts was prepared by dissolving 0.1 grams of crude extract in suitable milliliters of distilled water. The concentrations prepared were 250 ppm, 500ppm, 750ppm and 1000 ppm. The concentrations were transferred to suitable cups. For each concentration 3 cups were prepared (labeled R1, R2 and R3). About 10 larvae were transferred to each cup using dropper. Then a control was prepared using 3 cups containing distilled water. Mosquito was added in that cup as well. Then the death rate of the larvae was calculated for 24hrs, 48hrs and 72hrs. Using the mortality assay probity analysis was done.

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

The mosquito larval death rate or mortality was calculated in percentage using the following formula:

6. Statistical analysis

Statistical analysis was performed using MS Excel 2003 and anti-log calculator to find out the Lethal concentration 50 (LC50), regression equations, mean larval mortality, etc.

Probit analysis was done following Finney, 1952 [7].

5. Results

During the present experiment 3 most common and locally available medicinal plant species leaf extracts were applied on the 4th instars larvae of *C. quinquefasciatus* at different time intervals. Mosquito larvae also shows different morphological changes after exposure with different concentrations of three medicinal plant at different time**.** The results were presented in the following tables (Table. 1; Fig. 1-7).

Table 1: Percentage Mortality of *C. quinquefasciatus* larvae after different intervals of application.

Treatments (ppm)	Pre-treatment Populations	Mortality Rate		
		24HRS	48HRS	72HRS
1.	**Lantana**			
200 ppm	10	45%	66%	82%
300 ppm	10	56%	78%	95%
500 ppm	10	77%	90%	98%
Control	10	0	0	0
2.	**Nayantara**			
200 ppm	10	36%	50%	75%
300 ppm	10	45%	69%	82%
500 ppm	10	52%	81%	96%
Control	10	0	0	0
3.	**Castor**			
200 ppm	10	28%	39%	50%
300 ppm	10	36%	51%	71%
500 ppm	10	53%	72%	90%
Control	10	0	0	0

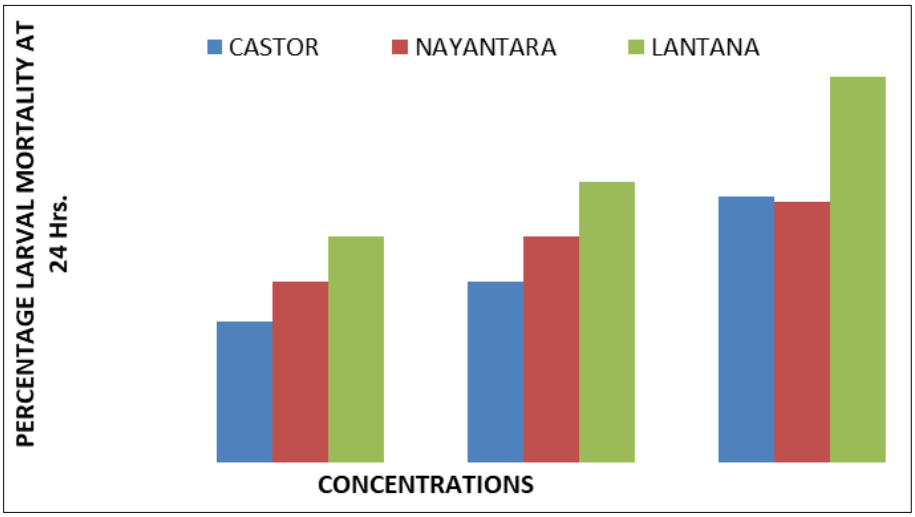

Fig 1: Percentage mortality of *C. quinquefasciatus* larvae at 24 hrs. Intervals of post-application of three medicinal plant extracts with different concentrations.

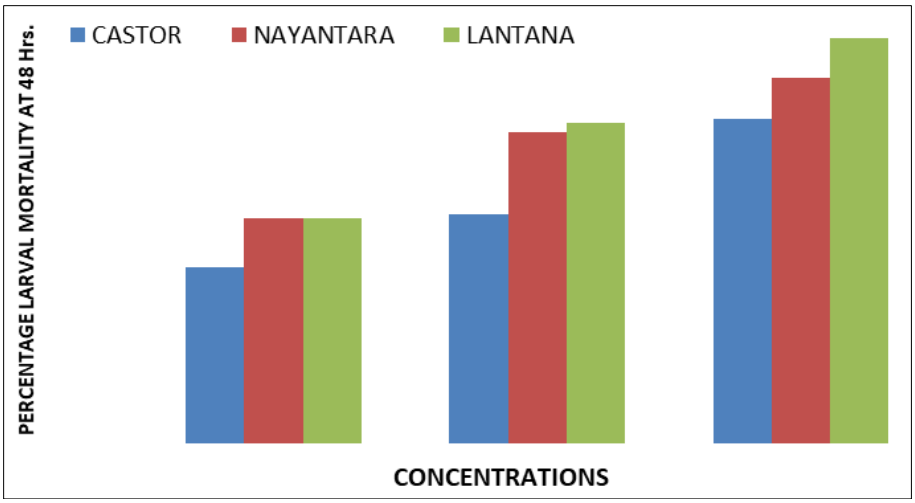

Fig 2: Percentage mortality of *C. quinquefasciatus* larvae at 48 hrs. intervals of post-application of three medicinal plant extracts with different concentrations.

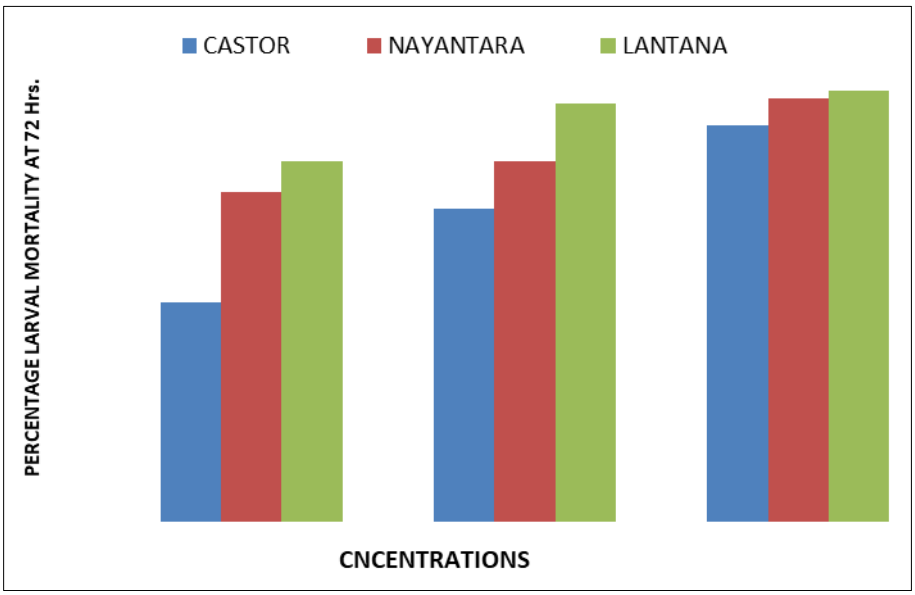

Fig 3: Percentage mortality of *C. quinquefasciatus* larvae at 72 hrs. intervals of post-application of three medicinal plant extracts with different concentrations.

The % of mortality of different intervals over different medicinal plants given below

- **24 hrs after treatment**
  After 24 hrs. mortality of mosquitoes (*C. quinquefasciatus*) ranged 45% for Lantana, 36% for Nayantara and 28% for Castor at 200 ppm concentration. At 300 ppm concentration 56% for Lantana, 45% for Nayantara and 36% for Castor. In other cases the mortality was 77% for Lantana, 52% for Nayantara and 53% for Castor in 500 ppm respectively (Table. 1; Fig. 1-3).
- 48 hrs after treatment- The Lantana and Nayantara show the most higher mortality rate of mosquito larva than the other spices. After 48 hrs mortality of mosquitoes ranged 66% for Lantana, 50% for Nayantara and 39% for Castor at 200 ppm concentration. At 300 ppm concentration 78% for Lantana, 81% for Nayantara and 51% for Castor respectively. At 500 ppm concentration the larval mortality rate recorded was 90% for Lantana, 72% for Nayantara and 28% for Castor Table. 1; Fig. 1,2&3).
- **72 hrs after treatment-**
  After treatment after 72 hrs it shows 82% at 200 ppm concentration 95% at 300 ppm concentration 98% at 500 ppm concentration for Lantana. For Nayantara 75% at 200 ppm concentration 82% at 300 ppm concentration 96% for 300 ppm concentration. For Castor the larval mortality rate was 50% at 200 ppm concentration 71% at 300 ppm concentration 90% at 500 ppm concentration (Table.1; Fig. 1,2&3).
- No larval deaths were recorded in case of control medium

Study on Probit Analysis and linear Regression:

The present experiment observed that the forth instars *C. quinquefasciatus* larvae shows significantly higher mortality rates when exposed to higher concentrations of each crude extracts viz., 200 ppm, 300 ppm and 500 ppm at 24, 48, and 72 hrs of exposure (Table: 1 - 3). Results of regression analysis revealed that there is a positively correlation of mortality rate (Y) with the concentration of exposure (X) with regression coefficient (R) closer to 1 (Table 2-5; Figure: 5-13). The log- probit analysis recorded that the LC50 values gradually decreased with the exposure period with the lowest value at 72 hrs of exposure (Table 2-5; Figure: 5-13). The 3 medicinal plant extracts showed different mortality frequencies, viz., *Lantana camara* > *Catharanthus roseus* > *Ricinus communis* (Table-1; Figure: 1-3).

Table-2: The larvicidal effect of Ethanol extract of *Lantana camara* leaves on *C. quinquefasciatus* larvae after different time intervals.

Treatments (ppm)	Log C	Mortality Rate 24HRS	Probit	Mortality Rate 48HRS	Probit	Mortality Rate 72HRS	Probit
200 ppm	2.30	45%	4.87	66%	5.41	82%	5.92
300 ppm	2.47	56%	5.15	78%	5.77	95%	6.64
500 ppm	2.69	77%	5.74	90%	6.28	98%	7.05

Table 3: The larvicidal effect of Ethanol extract of *Catharanthus roseus* leaves on *C. quinquefasciatus* larvae after different time intervals.

Treatments (ppm)	Log C	Mortality Rate 24HRS	Probit	Mortality Rate 48HRS	Probit	Mortality Rate 72HRS	Probit
200 ppm	2.30	36%	4.64	50%	5.00	75%	5.67
300 ppm	2.47	45%	4.87	69%	5.50	82%	5.92
500 ppm	2.69	52%	5.05	81%	5.88	96%	6.75

Table 4: The larvicidal effect of Ethanol extract of *Ricinus communis* leaves on *C. quinquefasciatus* larvae after different time intervals.

Treatments (ppm)	Log C	Mortality Rate 24 hrs	Probit	Mortality Rate 48HRS	Probit	Mortality Rate 72 hrs	Probit
200 ppm	2.30	28%	4.42	39%	4.72	50%	5.00
300 ppm	2.47	36%	4.64	51%	5.03	71%	5.55
500 ppm	2.69	53%	5.08	72%	5.58	90%	6.28

Table 5: Log probit analysis and regression analysis of larvicidal activity of *Lantana camara*, *Catharanthus roseus*, *Ricinus communis* on fourth instar larvae with a confidence limit of 95%.

Larvae	Samples	Parts used	Time	Regression equations	r^2 value	LC_{50} (ppm)
Culex spp.	*Lantana camara* L.	Leaves	24hrs	y = 2.252x - 0.347	0.983	234.43
			48hrs	y = 2.235x + 0.262	0.999	131.82
			72hrs	y = 2.847x - 0.545	0.947	89.12
	Catharanthus roseus (L.) G.Don	Leaves	24hr	y = 1.040x + 2.266	0.979	426.57
			48hrs	y = 2.231x - 0.087	0.976	186.20
			72hrs	y = 2.817x - 0.892	0.949	100.00
	Ricinus communis L.	Leaves	24hrs	y = 1.707x + 0.468	0.986	398.10
			48hrs	y = 2.219x - 0.408	0.992	295.12
			72hrs	y = 3.283x - 2.555	0.999	199.52

Statistical analysis observed that the LC50 value was 234.43 ppm, 131.82 ppm and 89.12 ppm at 24hrs, 48hrs. and 72hrs. time intervals respectively in case of *Lantana camara*; 426.57 ppm, 186.20 ppm and 89.12 ppm at 24hrs, 48hrs. and72hrs. time intervals respectively in case of *Catharanthus roseus*; 398.10 ppm, 295.12 ppm and 199.52ppm at 24hrs, 48hrs. and72hrs. time intervals respectively in case of *Ricinus communis* (Table 5).

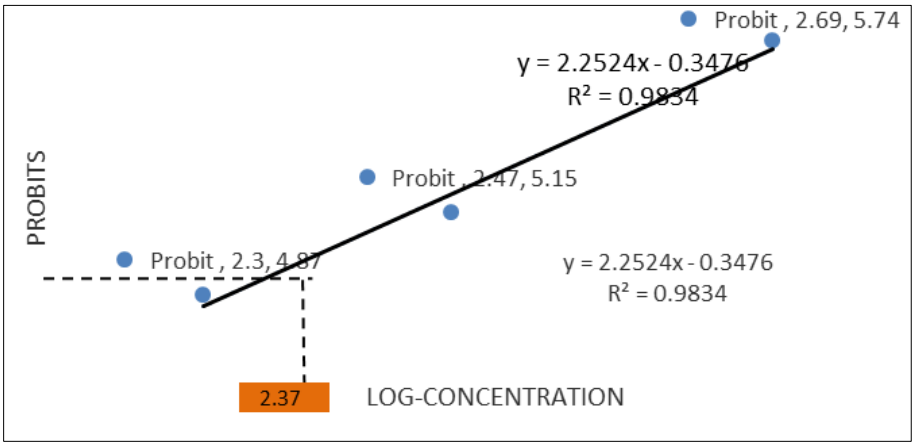

Fig 5: Log- Probit curve of action of ethanol extract of Castor leaves on mosquito larvae (24hrs).

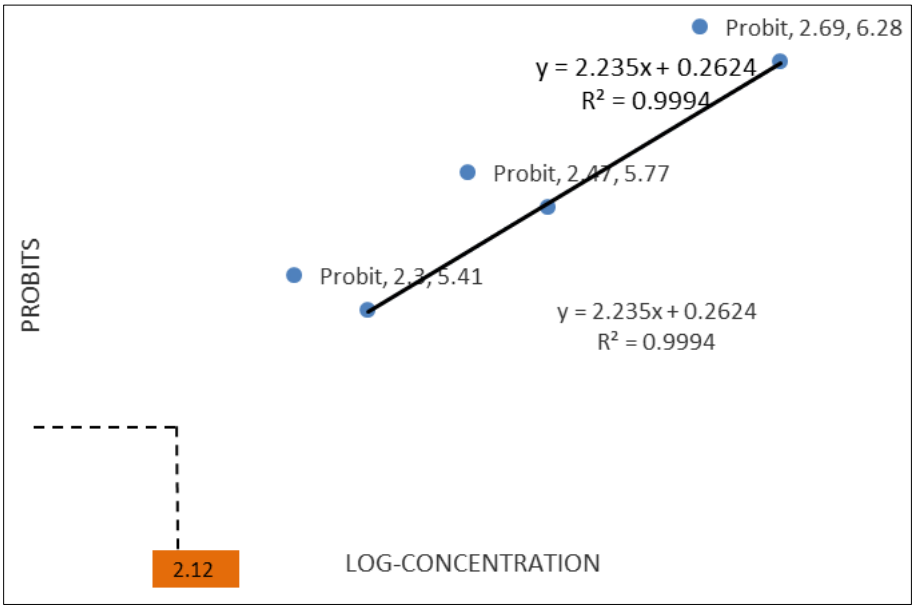

Fig 6: Log- Probit curve of action of ethanol extract of Castor leaves on mosquito larvae (48hrs).

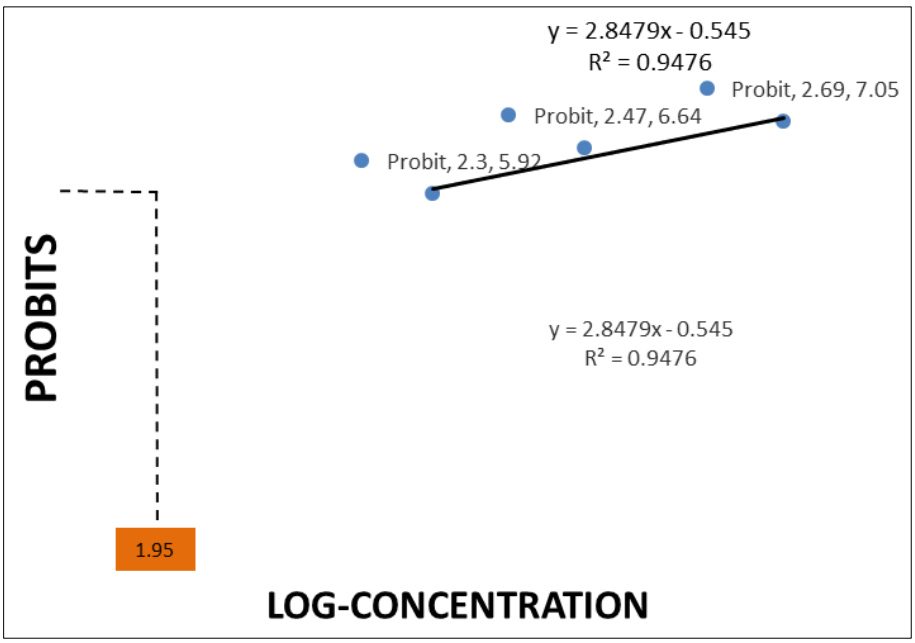

Fig 7: Log- Probit curve of action of ethanol extract of Castor leaves on mosquito larvae (72hrs).

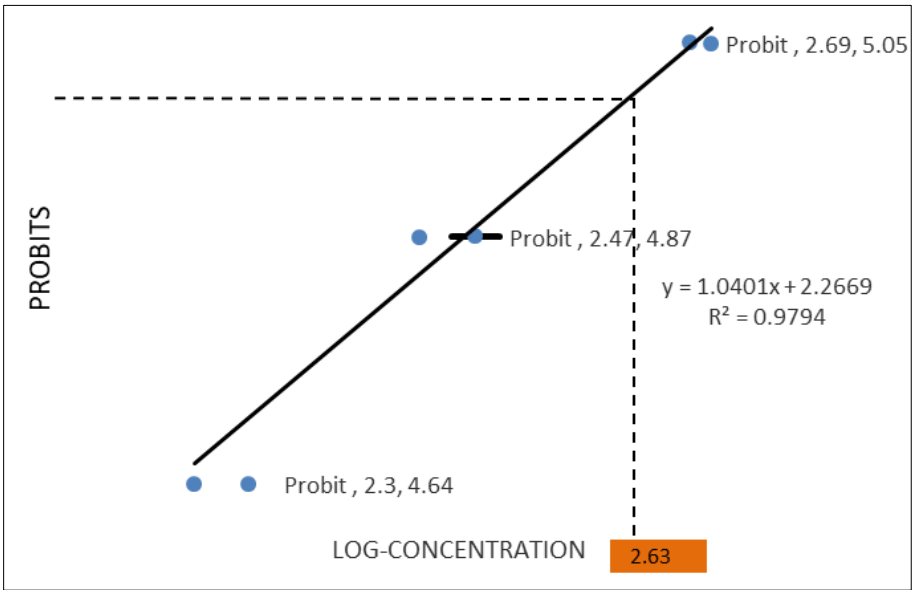

Fig 8: Log- Probit curve of action of ethanol extract of Nayantara leaves on mosquito larvae (24hrs).

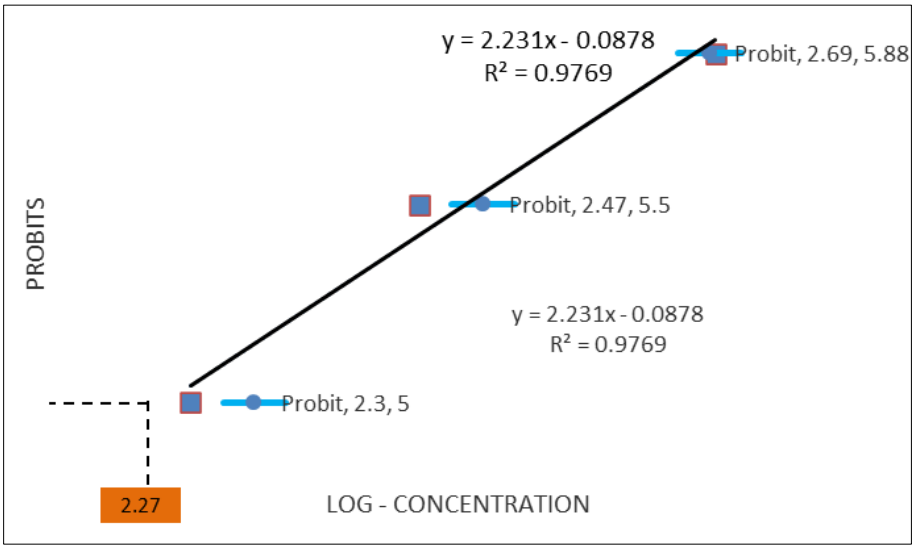

Fig 9: Log- Probit curve of action of ethanol extract of Nayantara leaves on mosquito larvae (48hrs).

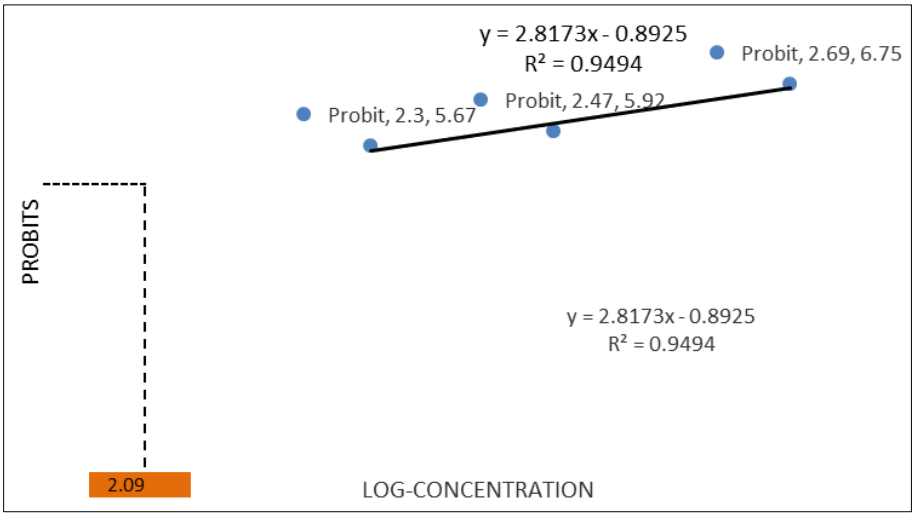

Fig 10: Log- Probit curve of action of ethanol extract of Nayantara leaves on mosquito larvae (72hrs).

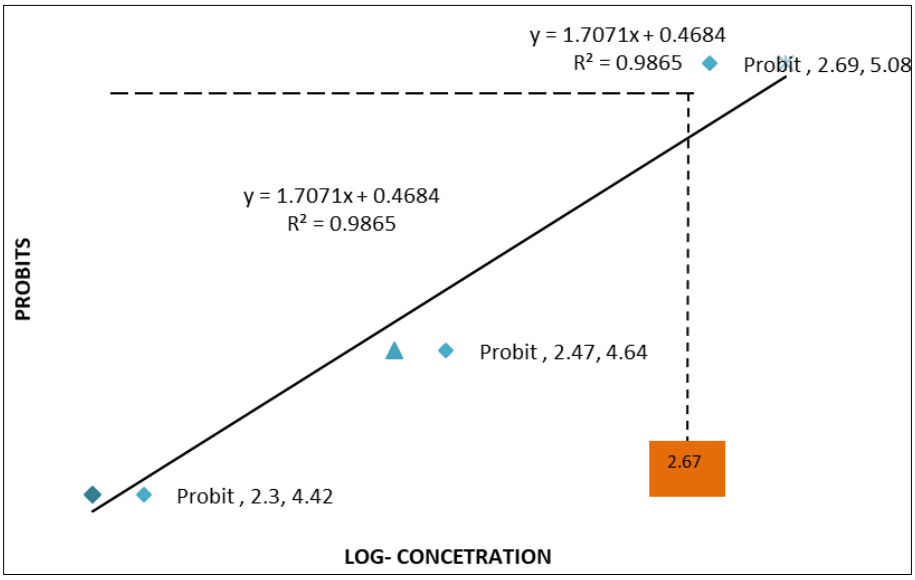

Fig 11: Log- Probit curve of action of ethanol extract of Castor leaves on mosquito larvae (24hrs).

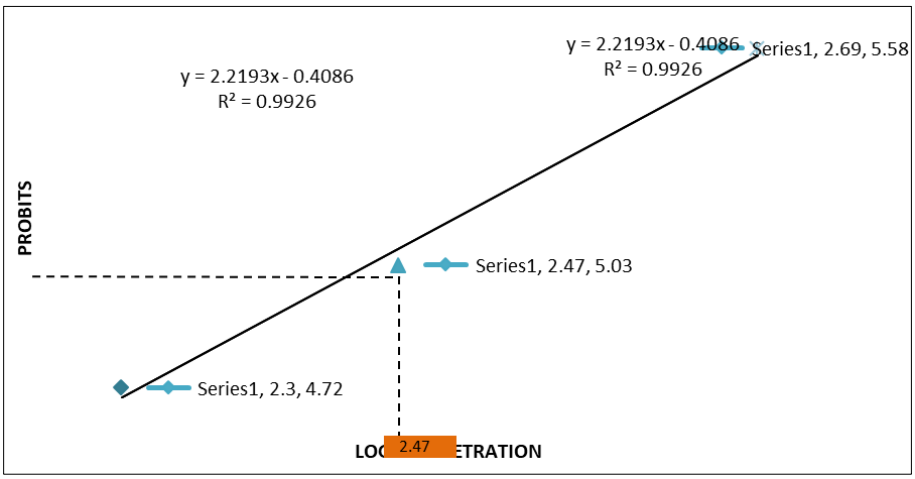

Fig 12: Log- Probit curve of action of ethanol extract of Castor leaves on mosquito larvae (48hrs).

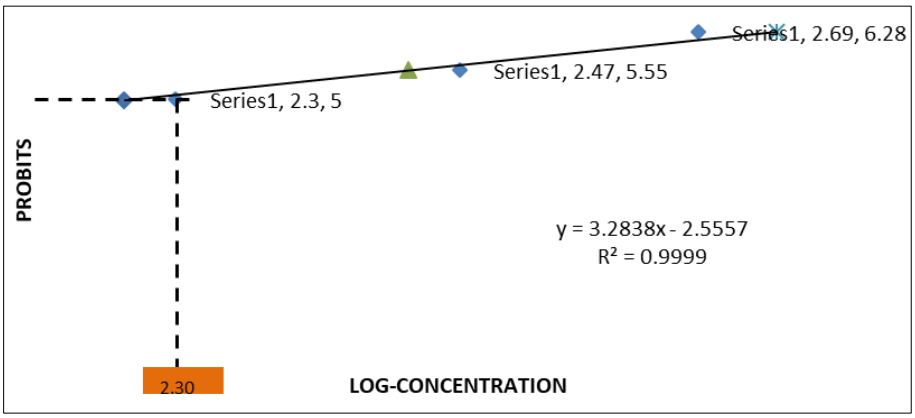

Fig 13: Log- Probit curve of action of ethanol extract of Castor leaves on mosquito larvae (72hrs).

Discussion

The present study recorded that in the ethanol extract, the LC_{50} value was 234.43 ppm, 131.82 ppm and 89.12 ppm at 24hrs, 48hrs. and 72hrs. time intervals respectively in case of *Lantana camara*; 426.57 ppm, 186.20 ppm and 89.12 ppm at 24hrs, 48hrs. and 72hrs. time intervals respectively in case of *Catharanthus roseus*; 398.10 ppm, 295.12 ppm and 199.52 ppm at 24hrs, 48hrs. and 72hrs. time intervals respectively in case of *Ricinus communis*. The present study also observed that the ethanol leaf extracts of *Lantana camara* > *Catharanthus roseus* > *Ricinus communis*.

Vairavan, Thangapandiyan and Alif Alisha, 2018 [8], observed that the LC_{50} values of ethanol leaf extract of *Catharanthus roseus* against *Culex quinquefasciatus* were found to be 471.84 ppm, 473.16 ppm, 397.99 ppm, 354.55 ppm, 301.81 ppm, 229.26 ppm, 161.99 ppm, 90.38 ppm at different

concentrations for the time intervals of 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr and 120 hr respectively. According to Alghamdi and Basher, 2020 [9], ethanol leaf extracts of *Lantana camara* the LC_{50} was 21.37 ppm for *C. quinquefasciatus* larvae for 24 hrs. time intervals. Osman, Taha and Sidahmed, 2011 [10], ethanol leaf extracts of *Lantana camara* and *Ricinus communis* the LC_{50} was 80ppm and 27.5ppm respectively for *C. quinquefasciatus* larvae at 24 hrs. time intervals.

Conclusion

The result of the present study recorded that the ethanol leaf extracts of *Lantana camara*, *Catharanthus roseus* and *Ricinus communis* have high larvicidal properties against the 4th instars of *C. quinquefasciatus*. The larvicidal efficacy of these medicinal plant leaf extracts should be applied and evaluated under the field conditions. In addition to this, investigation on the action of these 3 medicinal plants on other non-target organisms are also needed. These medicinal plants are very commonly available and easy to grow. The commercial production of new plant-based insecticide from these medicinal plants could provide an alternative to the expensive and toxic chemical insecticides.

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