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VD Mendhulkar
Department of Botany, Institute of Science, 15 Madame Cama Road, Mumbai - 32.
& National Malaria Institute, Champal, Panji, Goa, India

LA Yeragi
Research Fellow, Department of Botany, National Malaria Institute, Champal, Panji, Goa, India

Hemanth Kumar
Senior scientist, National Malaria Institute, Champal, Panji, Goa, India

Correspondence
VD Mendhulkar
Department of Botany, Institute of Science, 15 Madame Cama Road, Mumbai - 32.
& National Malaria Institute, Champal, Panji, Goa, India

Bioassay of vector Larvaes with Latex of blind eye Mangrove plant *Excoecaria agallocha* Linn

VD Mendhulkar, LA Yeragi and Hemanth Kumar

Abstract
The present work deals with the studies of latex of mangrove plant *Excoecaria agallocha* to evaluate the larvicidal efficacy against three mosquito species i.e. *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* mosquito and one housefly species *Musca domestica*. The latex of the *Excoecaria agallocha* was collected from the Mithbav creek and larvicidal tests were performed in National Malaria Institute, Champal, Panji, and Goa. The tests were performed by WHO standard method-15. Total of nine aqueous concentrations in the range of 1.17 to 300 ppm of the plant latex were tested against the third stage larvae of *Anopheles stephensi* and *Culex quinquefasciatus.* In trial experiment *Aedes aegypti* indicated resistance to the doses of latex solution two higher doses were introduced and the range of 4.69 to 1200 ppm were used for the treatment *Aedes aegypti* larvae. Mortality rate was observed and recorded after 24 hrs. The third instar larvae of *Musca domestica* was treated with the higher latex concentrations in the ranging from 2000 to 20000 ppm along with control. For *Culex quinquefasciatus* and *Anopheles stephensi* the highest mortality observed was 100 % in 300 ppm latex concentration. In case of *Aedes aegypti* 1200 ppm latex concentration showed 100% mortality. For *Musca domestica* larvae, the highest 100 % mortality was recorded in 18000 ppm and above concentration. Our analysis revealed that the latex of the plant potent enough to induce 100% mortality in all studied mosquito larvae and house fly. The latex of *Excoecaria agallocha* possesses promising larvicidal activities against the studied mosquito species of malaria, dengue and filarial. Further photochemical analysis of the latex with respect to its active components will add a high value to these findings.

Keywords: *Excoecaria agallocha, Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus, Musca domestica*, larvicidal, latex.

1. Introduction
Excoecaria agallocha is a medicinally important mangrove plant belongs to the family Euphorbiaceae. The latex of the plant has antimicrobial and larvicidal properties against the salt marsh mosquito *Culex sitiens* [1]. The leaves are crushed and dropped in the water as a result; fishes are stupefied and float to the surface. An amorphous saponin isolated from the fresh twigs and bark of *Excoecaria agallocha* is recognized as the active principal constituents with piscicidal properties[2]. Hippomanin A and B and crystalline tannins are the toxic principles of the extracts[2]. Timber used as firewood, converted charcoal and in carpentry. Sap and wood are used as purgative often used as fish poison. It is interesting to note that *Excoecaria* paste is used as a cure for leprosy in the Asia-Pacific region [2]. This plant has been traditionally used to treat sores and stings from marine creatures and ulcers, as a purgative and an emetic, and the smoke from the bark to treat leprosy [3]. Even dried and powdered leaves contain the poison which can kill fish very quickly. Owing to its complex chemistry, the plant has many medicinal uses**.** The latex of this plant is acrid milk sap which is a strong irritant and injurious to the human eyes, hence the plant is known as "blinding tree". The present work was undertaken to investigate the effect of latex for the larvicidal properties against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* mosquito and *Musa domestica* housefly species. Mosquitoes are responsible for transmission of many diseases and parasites to human, birds, dogs, horses. India has a favorable climate for *Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus* and *Musa domestica* which are transmitter of malaria, dengue, filarial and Typhoid respectively in urban and rural areas of India. Extensive application of chemical insecticides for many years in this area caused irreparable damages to the environment as a consequence, these insect becomes resistant to the chemical insecticides or larvicides. Phytochemicals offer great promise for the control of mosquitoes. To this context, the present work has been carried out to investigate the application of latex content of this plant against some mosquito larvae.

2. Material and methods

2.1 Administration of treatment for mosquito larvicidal activity.

The larvicidal test on larvae of *Anopheles stephensi* and *Culex quinquefasciatus* and *Aedes aegypti* was performed using latex of the *Excoecaria agallocha* at National Malaria Institute, Champal, Panjim, Goa. All tests were performed using WHO standard method-15 [4]. Latex is water soluble therefore concentrations were prepared in water. The studied concentrations of latex were determined on the basis of conducting the pilot experiments that provided a clue for the mortality of larvae minimum upto 5%. This criteria followed for all the other experimental samples. Total of nine concentrations i.e. 1.17, 2.34, 4.69, 9.38, 18.75, 37.50, 75, 150 and 300 ppm of the plant latex along with control were tried against the third stage larvae of *Anopheles stephensi* and *Culex quinquefasciatus. Aedes aegypti was* noted to be resistant for these doses therefore higher doses were used for this organism i.e. (4.69, 9.38, 18.75, 37.50, 75, 150 300, 600 and 1200 ppm). For every studied concentration, four batches (each of 25 larvae) were tested with a batch of control in plastic container with 100ml latex solution. Tested larvae were maintained under the insectary conditions (Temperature 27-29 ℃, relative humidity 70-75% and 12: 12 light: dark period). The rate of mortality was observed and recorded after 24 hrs.

2.2 Administration of treatment for Housefly larvicidal activity.

This experiment was performed in Department of Botany, Institute of Science Mumbai. The third instar larvae of *Musca domestica* was used for the present study. The concentration used for the treatment of larvae were 2000, 4000, 6000, 8000, 10000, 12000, 14000, 16000, 18000 and 20000 ppm along with control. Twenty five number of late 3rd instar larvae of *Musca domestica* identified by shortening and thickening of size and shape, darker in color and presence of three spiracles at the posterior end of the body were selected for each set of treatment. Ten numbers of glass petriplates of 10cm diameter were taken and labeled for different concentrations in addition to one for check and one for control. Each glass petriplates was filled with blotting paper and then 2 ml of each concentration poured in each petriplates. Twenty five numbers of late 3rd instar larvae were transferred in each petriplates. Each experiment was conducted in triplicates along with the control group. Tested larvae were maintained under the insectary conditions (Temperature 27-29°C, relative humidity 70-75% and 12: 12 light: dark period without food). The susceptibility test was performed as prescribed by WHO standard procedure (1975) in petriplates [4]. Mortality rate was observed and recorded after 24 hrs. LC50 was calculated using Karber's method. The LC50 value was determined following the formula as - 50 - 100 Mean death X Concentration difference LC = LC – No. of organisms per group.

3. Result and Discussion

In present study larvae of three mosquito species *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* were given treatment of varying doses of latex of *Excoecaria agallocha. Anopheles stephensi* is a vector of malaria*, Culex quinquefasciatus* is a vector of filarial and *Aedes aegypti* is vector of dengue and chikungunya. Total of nine concentrations i.e. 1.17, 2.34, 4.69, 9.38, 18.75, 37.5, 75, 150 and 300 ppm of the plant latex were tried against the third stage larvae of *Anopheles stephensi* and *Culex quinquefasciatus* and two higher concentrations i.e. 600 and 1200 ppm were used due higher resistance of *Aedes aegypti* larvae.

The highest mortality was 100% for *Anopheles stephensi* recorded in 300 ppm followed by 94 % in 150ppm. The latex concentration of 1.17 ppm indicated lowest mortality 5% (Table- 1, Fig -1). Latex treatment with 9.38ppm was below 50% of mortality for *Anopheles stephensi*. Published report on *Anopheles stephensi* revealed that the larvae of this mosquito are sensitive to various mangrove plant extract [5].

For *Culex quinquefasciatus,* the highest mortality observed was 100 % in 300 and 600 ppm latex concentration. Whereas the middle dosage indicated percent mortality in the range of 66 to 93% i.e. up to 4.69ppm concentration. The lowest mortality rate 14% was evident for 1.17 ppm concentration.

The *Aedes aegypti* mosquito species were observed to be more compare to other two mosquito species. The highest mortality was 100 % for *Aedes aegypti* in 1200ppm followed by 82 % 600ppm and 63 % in 300 ppm of latex solution (Table- 1, Fig -1). It is reported that the methanol and hexane leaf extract of *Excoecaria agallocha* shows 100% mortality against the larvae of *Aedes aegypti* and *Culex quinquefasciatus* [6]. Similarly positive mosquito larvicidal activity of seaweeds extracts observed against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* [7].

Musa domestica larvae are found to be comparatively more resistance in earlier studies hence higher doses was tried for the treatment. The concentrations were used from 2000 to 20,000 ppm with difference of 2000 ppm between the two concentrations. The results obtain are discussed for the third stage larvae of *Musca domestica.* For *Musca domestica* larvae, the highest 100 % mortality was recorded in 18000 ppm and above concentration, followed by 98 % mortality in 16000 ppm, 91 % mortality in 14000 ppm, 75 % mortality in 12000 ppm, 64% mortality in 10,000ppm (Table- 1, Fig -2). The antilarval activity of *Aloe vera* leaf and crude fruit extract of *Capsicum annuum* was also experimented on *Musca domestica* [8, 9]. Similarly the effect of *Eucalyptus globules* leaf extract has been studied for the insecticidal activity [10]. Similar larvicidal effect of aqueous leaf extract of Tobacco (*Nicotiana tabacum*) studied on the Third Instar larvae of *Musca domestica* [11].

Table 1: Mortality Percentage in mosquito larvae treated with different concentrations of latex of *Excoecaria agallocha*

Sr. No	Latex Concentration in ppm	Mortality mosquito larvae		
		Anopheles stephensi	***Culex quinquefasciatus***	***Aedes aegypti***
1	1200	NA	NA	100 ± 0.00
2	600	NA	NA	82 ± 1.290
3	300	100 ± 0.00	100 ± 0.00	63 ± 2.872

4	150	94 ± 1.290	93 ± 0.5	29 ± 2.5
5	75	73 ± 1.707	84 ± 0.816	23 ± 0.5
6	37.5	64 ± 0.957	78 ± 0.816	16 ± 0.816
7	18.75	53 ± 0.957	71 ± 1.258	14 ± 0.577
8	9.38	44 ± 2.160	68 ± 0.816	10 ± 0.577
9	4.69	31 ± 2.629	66 ± 0.577	08 ± 0.00
10	2.34	20 ± 1.414	33 ± 1.290	NA
11	1.17	05 ± 0.816	14 ± 1.290	NA
12	Control	00.00	00.00	00.00

* NA- Not Applicable

Table 2: Percentage Mortality in *Musca domestica* larvae

Sr. No	Latex concentration in ppm	Percentage of Mortality
1	20,000	100 ± 00
2	18,000	100 ± 00
3	16,000	98 ± 0.557
4	14,000	91 ± 0.957
5	12,000	75 ± 1.258
6	10,000	64 ± 0.816
7	8000	46 ± 1.290
8	6000	35 ± 0.957
9	4000	27 ± 0.5
10	2000	10 ± 0.557
11	Control	00 ± 00

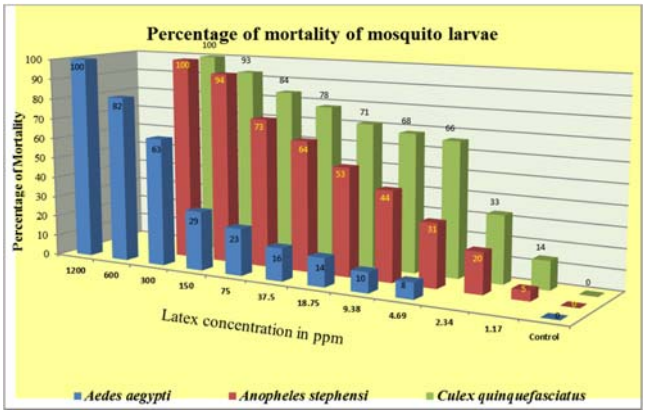

Fig 1: Mortality Percentage in Mosquito larvae

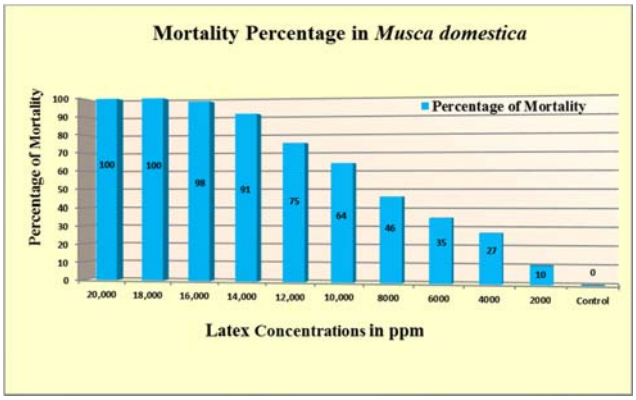

Fig 2: Percentage mortality in *Musca domestica* larvae treated by the latex of *Excoecaria agallocha*

4. Conclusion

Extensive application of chemical insecticides for many years in this area caused irreparable damages to the environment as a consequence, these insect becomes resistant to the chemical insecticides or larvicides. Phytochemicals offer great promise for the control of mosquitoes. From the present studies it is concluded that aqueous latex extracts of *Excoecaria agallocha* Linn. exhibit mosquito larvicidal activities against the studied mosquito species of malaria, filarial and dengue. 300ppm concentration was found to be most effective against *Anopheles stephensi* and *Culex quinquefasciatus*. The *Aedes aegypti* larvae responded at higher concentrations and 1200 ppm concentration was effective to induce 100% mortality. The resistance capacity of studied mosquito larvae can be sequence in the ascending order as *Anopheles stephensi* ≤ *Culex quinquefasciatus* ≤ *Aedes aegypti* for the latex treatment. Further photochemical analysis of the latex with respect to its active components will add a high value to these findings. In case of *Culex quinquefasciatus* larvae, the mortality count was 100% in 300 ppm. It can be remarkably noted that the plant exude i.e. latex of *Excoecaria agallocha* possesses promising larvicidal activities.

The *Musa domestica* larvae found to be more resistant to the latex of *Excoecaria agallocha* and responded at much higher concentrations compared to other three mosquito larvae. From the results obtained indicated that *Excoecaria agallocha* possesses high larvicidal activities against *Anopheles stephensi* ≤ *Culex quinquefasciatus* ≤ *Aedes aegypti* ≤ *Musca domestica* species. 300, 300, 1200 and 18,000 ppm concentration was found to be most effective against studied mosquito and housefly larvae. Thus, biological methods can be used to achieve high acceptance to induce enhanced mortality rate because of high level biocompatibility mode of interaction.

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6. Reference

1. Reddy TKK, Rajasekhar A, Jayasunderamma B, Ramamurti R. Studies on marine bioactive substances from the Bay of Bengal: Bioactive substances from the latex of the mangrove plant *Excoecaria agallocha* L: Antimicrobial activity and degradation. In: Thompson, M. F., Sarojini, R. and Nagabhushanam, R. (eds) Bioactive compounds from marine organisms with emphasis on the Indian Ocean: An Indo-United States Symposium, 1991, 75-78.

2. Bandarnayake WM. Traditional and Medicinal uses of mangroves, Mangroves and Salt Marshes; 1998;

2(3):133-148.
3. Ghani A. Medicinal Plants of Bangladesh. 2nd edition. The Asiatic Society of Bangladesh, 2003, 228-229.
4. World Health Organization Division of Malaria and other Parasitic Diseases. Manual on practical entomology in malaria. Part II: Methods and techniques. World Health Organization Offset, Geneva Publication 1975, 13.
5. Thangam TS, Kathiresan K. Toxic effect of mangrove plant extracts on mosquito larvae *Anopheles stephensi* L. Current Science (Bangalore) 1988; 57(16):914-915.
6. Pradeepa P, Subalakshmi K, Saranya A, Dinesh P, Raj VA, Ramanathan T. Milky Mangrove *Excoecaria agallocha* L. Plant as a source for potential mosquito larvicides. Journal of Applied Pharmaceutical Science. 2015; 5(3):102-105.
7. Ali MYS, Ravikumar S, Beula JM. Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus.* Asian Pacific Journal of Tropical Disease 2013; 3(3):196-201.
8. Jesikha M. *Capsicum annuum* Larvicidal Activity on Larval Stages of *Musca domestica,* International Interdisciplinary Research Journal. 2012 a; 2(4):9-11.
9. Jesikha M. Evaluation of Larvicidal Efficacy of *Aloe vera* Extract against *Musca domestica,* IOSR Journal of Environmental Science, Toxicology and Food Technology. 2012 b; 2(2):01-03.
10. Halim AAS, Morsy TA. The insecticidal activity of *Eucalyptus globulus* oil on the development of *Musca domestica* third stage larvae. Journal of Egypt Society of Parasitology; 2005; 35:631-6.
11. Ogbalu OK, Bobmanuel RB, Membere O. Larvicidal Effect of Aqueous Leaf Extract of Tobacco (*Nicotiana tabacum*) On the Third Instar Larvae of *Musca domestica.* IOSR Journal of Agriculture and Veterinary Science. 2014; 7(12):35-40.