Research Article

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HERBAL CONTROL OF MOSQUITO LARVAE

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Abstract: Mosquitoes are the vectors for the dreadful diseases of mankind. For control of larval stages of mosquito, herbal plant extracts/ botanical insecticides are being tried. In the present study aqueous extract of some traditional medicinal herbal plants i.e. Neem (*Azadirechta indica*), Tobacco (*Nicotiana tabacum*), Turmeric (*Curcuma longa*), Tulasi (*Ocimum santum*), and Ginger (*Zingiber officinale*) were tested for their Larvicidal activity. The successful attempt is made to kill the larvae, the premature stage of mosquitoes by using safe and socio-economical herbal plant extract mixtures. Ginger+Tobacco, Neem+Tobbaco and Ginger Neem, Turmeric, Tobacco and Tulasi showed highest larvicidal activity. The results obtained show that this plant material exhibited larvicidal activity and could be considered as potent natural larvicidal agent without any toxic effects.

Keywords: Cold Hot Extraction Method, Larvicidal Activity, Plant Extracts, Significant Larvicide.

INTRODUCTION

Integrated pest management is being practiced for controlling vectors like mosquitoes which are capable of transmitting potential pathogens to human beings, and they are responsible for several infectious diseases like malaria, filariasis, Japanese encephalitis, yellow fever, dengue, and chikungunya. Vector control is facing a threat due to the emergence of resistance to synthetic insecticides.

Mosquitoes are the vectors for the dreadful diseases of mankind. Of all the insects that transmit diseases, mosquitoes represent the greatest menace. WHO has declared the mosquito "public enemy number one" because mosquitoes are responsible for the transmission of various dreadful diseases causing pathogens[1]. Malaria deaths totaled 1.124 million i.e. 3.080 people per day. Around 90% of these deaths occur in Africa, mostly in young children. It is a heavy death toll, especially amongst children and pregnant women. Treatment and control have become more difficult with the spread of drug-resistant strains of parasites and insecticide-resistant strains of mosquito vectors[2]. Millions of people die in India by mosquito vector borne diseases such as malaria, dengue, chikungunya, filariasis etc. every year[1].

The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides. Larviciding is a successful way of reducing mosquito. Larviciding largely depends on the use of synthetic chemical insecticides organophosphates (*e.g.* temephos, fenthion, malathion), insect growth regulators (*e.g.* diflubenzuron, methoprene), etc. Although effectiveness of their repeated use has disrupts the natural biological control systems and sometimes resulting in the widespread development of resistance. These problems have warranted the need for developing alternative strategies using ecofriendly products. *Bacillus thuringiensis* H-14 is a bacterial larvicide, available in a slow release briquette that dissolves over about 30 days. They are intended for the treatment of small breeding sites and may not be effective in slightly polluted water. It is safe to use in drinking water. Temephos, B.t. H-14 and larvivorous fish can all be used in wells to prevent the breeding of anopheline mosquitoes[3].

Early mosquito control methods included source reduction, mosquito-eating fish, and chemicals. These methodologies continue today, in a much more sophisticated manner[4] Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment. Much effort has, therefore, been focused on plant extracts or phytochemicals as potential sources of mosquito control agents[5]. Neem components show multiple effects against different insects such as mosquitoes. Azadirachtin (3.2% w/w)) was tested for its effects against fourth instars larvae of *Culex pipiens* (Diptera: Culicidae) under laboratory conditions[5]. An insecticide containing azadirachtin, a tree (*Azadirachta indica*)

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extract, was tested against Culex pipiens mosquito larvae and pupae in east of the Republic of Algeria under laboratory conditions[6].

Insecticides of botanical origin have been reported as useful for control of mosquitoes. *Azadirachta indica* (Meliaceae) and its derived products have shown a variety of insecticidal properties[7]. The traditional medicinal plants were utilized for curing various ailments, their insecticidal property also tested for insect pest and vectors like mosquito and its life stages. Cytotoxicity and larvicidal properties of the leaf extracts of 3 Annonaceous plants, *Annona muricata* L., *A. senegalensis* Pers and *A. squamosa* L. were tested against brine shrimp larva and the late 3rd instar of *Culex quinquefascintus* Say. The larval mortality was observed 24 h post-exposure[8]. Larvicidal property of Karela, *M. charantia* against three mosquito species *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae)[9]. The essential oil extracted from the indigenous plant *Ipomoea cairica* Linn., commonly known as railway creeper, was found to be most highly toxic to the larvae of *Culex tritaeniorhychus*, *Ades aegypti, Anopheles stephensi, Culex quinquefasciatus* mosquitoes[10]. A qualitative study of ethanolic extracts of *A. squamosa* were screened for mosquito larvicidal activity against larvae of *Culex quinquefasciatus* Say and *An. gambiae* s.s.[11]. The larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf and bark of *Ficus racemosa* (Moraceae) was assayed for their toxicity against the early fourth-instar larvae of *Culex quinquefasciatus* (Diptera: Culicidae)[12]. Badly done mosquito control is costly and provides little or no protection. It is a public health tool better left to people trained to evaluate the presence of mosquitoes, operate the equipment for pesticide application and to judge the right time, place and method of control to get the best results[13]. Mosquitoes are the major disease vectors as well as nuisance insects responsible for malaria. Most of the NGO programmes still depend on the techniques that eliminate adult mosquitoes. Therefore, natural herbal larvicide is the key strategy highlights the action against mosquito larvae and their breeding sites.

Many studies have drawn attention to the effects of plant extracts on adult eclosion[15, 18]. The benefit of elongation is that mosquito larvae numbers are reduced due to the longer period needed for a new generation to complete the mosquito life cycle[14]. Six medicinal plants extracts, *C. porrectum*, *P. pulcher*, *A. occidentale*, *M. siamensis*, *A. graveolens* and *A. muricata* evinced high larvicidal activity against the fourth instar larvae of *Ae. aegypti*. Furthermore, *M. siamensis*, *A. graveolens*, and *A. muricata* were all found to have a chronic effect on the fourth instar larvae of *Ae. aegypti* after permanent exposure, leading to a reduction in the number of resultant eggs and subsequent larvae of the mosquitoes[17].

Present study focuses the new control ecofriendly methodologies for eradication of malaria. The successful attempt is made to kill the larvae, the premature stage of mosquitoes by using safe and socio-economical herbal plant extract mixtures. In the present paper we report the larvicidal activity of plant extracts of Neem (*Azadirechta indica*), Tobacco (*Nicotiana tabacum*), Turmeric (*Curcuma longa*), Tulasi (*Ocimum santum*), and Ginger *(Zingiber officinale)*. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control.

MATERIALS AND METHODS

The extracts of Leaves of Neem (*Azadirechta indica*), Tobacco (*Nicotiana tabacum*), Turmeric (*Curcuma longa*), Tulasi (*Ocimum santum*), and Ginger *(Zingiber officinale)*. lemon grass *Cymbopogon citriodora. Custard apple Annona squamosa, Eucalyptus, Pudina-Mint Mentha arvensis, Sabja Ocimum basilicum, Chrysanthemum indicum, Camphor Cinnamomum camphora, Jaiphal Nutmeg Myristica fragrans, Zendu-Marigold Tagetus Erecta*, and *Alloe vera* was prepared in distilled water. 100gm leaves of each of these plants were crushed in water with the help of mixer grinder separately. Then each extract was tasted separately on mosquito larvae which were collected from nearby fresh water ponds. 10 larvae were used in each replica, death was recorded every after thirty minutes. After this preliminary study on 15 medicinal plants selected five plants, Neem (*Azadirechta indica*), Tobacco (*Nicotiana tabacum*), Turmeric (*Curcuma longa*), Tulasi (*Ocimum santum*), and Ginger *(Zingiber officinale)*. Results of these plant extracts only reported here.

To test herbal mixture extracts of two herbal plants fresh leaves of Neem, Turmeric, Tobacco, Ginger and Tulsi were obtained directly from the plants. Plant extracts were prepared by cold to hot extraction methods. The plant extracts mixture was prepared as 1:1, 2:3, and 3:2 proportions (Table.1).

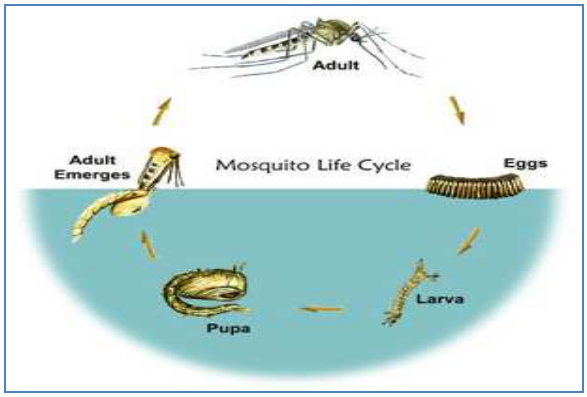

Fig.1. Mosquito life cycle

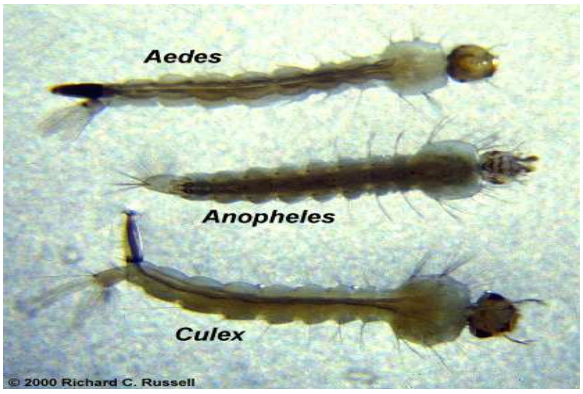

Fig.2. IVth larval instars of *Aedes, Anopheles* and *Culex spp*

The proportion of plant extract mixture was selected after optimization of their ratio by gradual method. Evaluation carried out for their safe larvicidal activity. The third and fourth instar larvae of Aedes aegypti mosquito were collected from ponds and stagnant water pools from Rahuri Muncipal (Ahmednagar district) area And Wagholi (Pune). The study was conducted on third and fourth instar mosquito larvae of *A. aegypti*, the extracts mixture of two herbal plants were tried in addition to single plant extract. The data obtained tabulated and statically analyzed.

RESULTS AND DISCUSSION

Study report by Suwannee et al 2006 112 species of medicinal plant collected from the southern part of Thailand for their mosquitocidal activity, 14 species (12.5%) showed toxicity against the third and the fourth instar larvae of *Ae. aegypti* while eight out of these 14 species (7.1%) demonstrated toxicity to the selected non-target organism. Six of the fourteen species (5.4%) showed excellent larvicidal properties against the fourth instar larvae of *Ae. Aegypti.* In the present study larvicidal activity was performed by counting larval death at the time interval of 5 min (Table 1). From statistical analysis it was found that all plant extracts were having effective larvicidal activity.

Table1. Larvicidal activity of plant extracts mixtures.

S.No	Extracts of Plants	Larval Death (Out of 10)																	
		3:2						1:1						2:3					
	Time (Min)	5	10	15	20	25	30	5	10	15	20	25	30	5	10	15	20	25	30
1	Tobacco+Ginger	2	5	6	8	10	-	3	6	8	10	-	-	1	3	6	8	10	-
2	Tobacco+Tulsi	2	4	6	9	10	-	2	5	7	10	-	-	1	3	6	8	9	10
3	Tobacco+Turmeric	2	4	5	7	9	10	2	4	7	9	10	-	1	3	4	7	9	10
4	Neem+Tobacco	2	4	8	10	-	-	5	8	10	-	-	-	2	4	5	7	10	-
5	Neem+Tulsi	3	5	7	10	-	-	4	7	10	-	-	-	2	3	5	7	10	-
6	Tulsi+Ginger	1	2	5	7	9	10	1	3	5	7	9	10	1	3	5	7	9	10
7	Tulsi+Turmeric	1	2	3	5	7	10	1	3	5	6	8	10	1	3	5	6	8	10
8	Turmeric+Neem	1	3	5	8	9	10	1	4	5	6	10	-	1	3	4	6	8	10
9	Turmeric+Ginger	1	2	4	6	8	10	1	3	5	7	9	10	1	3	5	7	8	10
10	All Plant extract mixtures	-	-	-	-	-	-	5	7	10	-	-	-	-	-	-	-	-	-
11	Malathion (0.0379 µg/ml)	-	-	-	-	-	-	3	5	8	10	-	-	-	-	-	-	-	-

Analysis of variance i.e. t-test and z-test interprets, the plant extract mixture 1,4,10 showing highest t-value and least z-value were found significant and promising larvicides (Table 2 and 3). Difference between the mean of each pair is less than value of critical difference 8.72 reveals that all plant extract mixture are significant larvicides. The study by Suwannee et al 2006 report six medicinal plant extracts, *C. porrectum*, *P. pulcher*, *A. occidentale*, *M. siamensis*, *A. graveolens*, and *A. muricata* evinced high larvicidal activity against the fourth instar larvae of *Ae. aegypti.* Furthermore, *M. siamensis*, *A. graveolens*, and *A. muricata* were all found to have a chronic effect on the fourth instar larvae of *Ae. aegypti* after permanent exposure, leading to a reduction in the number of resultant eggs and subsequent larvae of the mosquitoes. Mode of action of three medicinal plant extracts, *M. siamensis*, *A. graveolens* and *A. muricata* as it impacted on the life cycle of *Ae. aegypti* was studied [18]. Some of the larvae did not die within the 48 hour period, but instead they died at the pupal or at the adult stage, due to the chronic effects of chemical compounds attributable to the medicinal plant extract [16]. In this study when plants extracts of single plant used showed delayed mortality of fourth larval instars.

Table2. Statistical data for larvicidal activity of plant extract mixture (1:1).

Sr.No	Plan Extract Mixtures	SD	RSD	CV	SEM	Confidence limit		Regression Equation	R^2	t-Value	z-value
						Lower	Upper				
1	Tobacco+Ginger	2.98	0.44	44.23	1.12	5.62	11.16	y=0.46x+1.00	0.98	3.91	1.09
2	Tobacco+Tulsi	3.36	0.56	56.10	1.32	4.67	10.07	y=0.52x-0.05	0.99	3.08	1.19
3	Tobacco+Turmeric	3.36	0.52	52.52	1.28	5.22	11.30	y=0.42x+0.10	0.98	3.80	1.07
4	Neem+Tobacco	2.51	0.32	32.82	1.02	6.63	12.09	y=0.50x+2.66	0.99	4.30	0.93
5	Neem+Tulsi	3.00	0.42	42.85	1.13	5.86	10.74	y=0.70x-0.33	0.98	3.29	1.00
6	Tulsi+Ginger	3.48	0.67	59.79	1.38	4.44	11.01	y=0.35x-0.86	0.99	3.73	1.19
7	Tulsi+Turmeric	3.27	0.59	59.47	1.31	4.18	10.45	y=0.34x-0.60	0.99	3.75	1.37
8	Turmeric+Neem	3.27	0.62	62.90	1.95	3.24	11.41	y=0.44x-1.40	0.99	3.17	1.33
9	Turmeric+Ginger	3.48	0.59	59.79	1.44	4.38	11.23	y=0.35x-0.86	0.99	3.73	1.19
10	All plant extract mixture	2.51	0.34	34.31	1.45	1.34	13.32	y=0.70x-0.66	0.99	4.12	1.05
11	Malathion (0.0379 µg/ml)	3.10	0.47	47.83	1.55	0.00	13.00	y=0.48x+0.50	0.99	4.18	1.12

Table3. Statistical data showing significance of larvicidal activity of plant extract mixture (1:1).

Sr.No	Plant extract mixture	5 min	10min	15 min	20 min	25 min	30 min	Mean
1	Tobaco+Ginger	3.00	6.00	8.00	10.00	0.00	0.00	4.50
2	Tobacco+Tulsi	2.00	5.00	7.00	10.00	0.00	0.00	4.00
3	Tobacco+Turmeric	2.00	4.00	7.00	9.00	10.00	0.00	5.33
4	Neem+Tobacco	5.00	8.00	10.00	0.00	0.00	0.00	3.83
5	Neem+Tulsi	4.00	7.00	10.00	0.00	0.00	0.00	3.50
6	Tulsi+Ginger	1.00	3.67	5.00	7.00	9.00	10.00	5.94
7	Tulsi+Turmeric	1.00	3.00	5.00	6.00	8.00	10.00	5.50
8	Turmeric+Neem	1.00	3.67	5.00	6.00	10.00	0.00	4.28
9	Turmeric+Ginger	1.00	3.00	5.00	7.00	9.00	10.00	5.83
10	All Plant Extract Mix.	5.00	7.00	10.00	0.00	0.00	0.00	3.67
11	Malathion(0.037µg/ml)	3.00	5.00	8.00	10.00	0.00	0.00	4.33
Mean		2.55	5.03	7.27	5.91	4.18	2.73	4.61

Particulars	Plant extract mixture	Time
S.E (±)	3.11	2.30
CD at 5%	8.72	6.44
CD at 1%	11.52	8.51

Fig.3: Death of larva in plant extract mixtures.

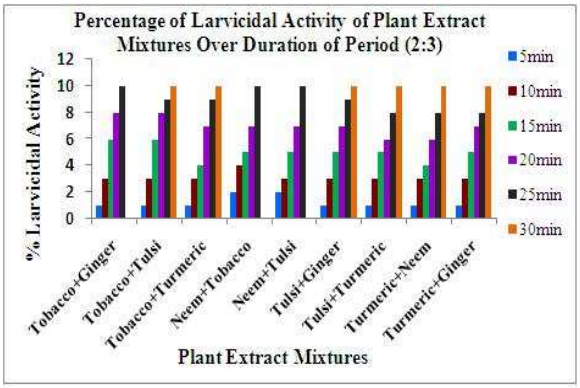

Fig.5: Larvicidal activity of plant extract mixtures(2:3)

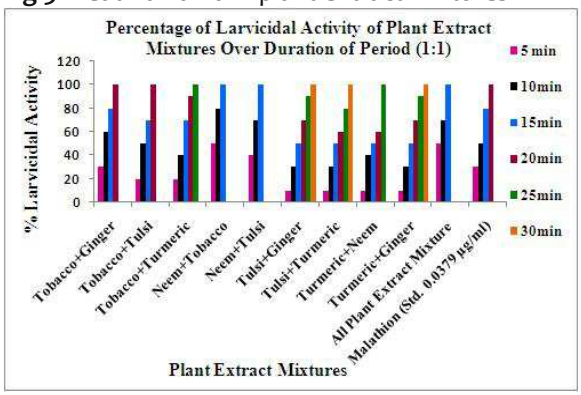

Fig.4: Larvicidal activities of plant extract mixtures (1:1)

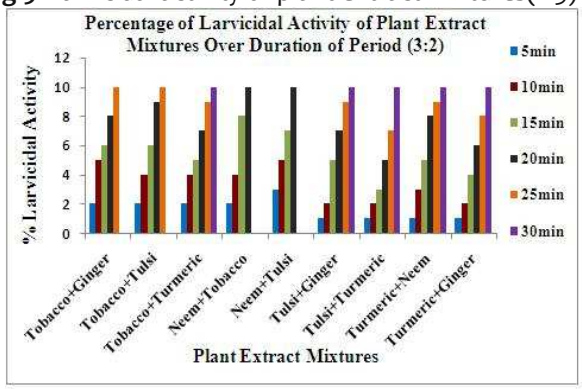

Fig.6: Larvicidal activity of plant extract mixtures(3:2).

CONCLUSION

From statistical analysis, plant extract mixtures no. 1, 4 & 10 showing highest t-value and least z-value was found to be highly effective and promising larvicide. All the results were compared with standard malathion (0.0379 µg/ml) & was found that plant extract mixtures has higher margin of safety than existing larvicide. Difference between the mean of each pair is less than value of critical difference 8.72 reveals that all plant extract mixture are significant larvicides.

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