

# VECTOR CONTROL



## 4.1. Laboratory evaluation of a flowable formulation of the metabolite of *Pseudomonas fluorescens* against the immatures of the mosquito vectors *Anopheles stephensi*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

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Duration : One year

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Funding Agency : Intramural project

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### Objective

- To test the VCRC-PFFC formulation under laboratory conditions against larvae and pupae of different species of vector mosquitoes to determine the  $LC_{50}$  and  $LC_{90}$  dosages.

A microbial metabolite of *Pseudomonas fluorescens* Migula was identified, formulated and tested at Vector Control Research Centre, Pondicherry and found to be active both against larvae and pupae of mosquito vectors. As part of multi-centric evaluation, the laboratory evaluation results of *Aedes aegypti* and *Culex quinquefasciatus* were presented in the previous year Annual Report. In continuation, the laboratory evaluation results of *Anopheles stephensi*, the vector of malaria, *Ae. albopictus* the vector of dengue, and *Cx. tritaeniorhynchus* the vector of Japanese encephalitis are presented below.

### Laboratory bioassay

For bioassays, the standard method of WHO for testing of larval susceptibility was conducted. All concentrations and control were replicated 3 times and tests were repeated atleast 3 times. The mortalities were averaged and the data were analyzed with a computer statistical package to calculate  $LC_{50}$  and  $LC_{90}$  values with corresponding 95% confidence intervals.

## Results

A comparison of the median lethal concentration ( $LC_{50}$ ) values of bioassay results showed that the formulation was having a high degree of activity against both the larvae and pupae of *An. stephensi* and *Cx. tritaeniorhynchus*. The larvae of *Cx. tritaeniorhynchus* were more sensitive to the formulation than the larvae of *An. stephensi* (0.019 vs., 0.060). The dosage requirement for pupal mortality of *Ae. albopictus* is about 15 times less than that required for larval mortality (0.042 vs. 0.654  $\mu\text{g/ml}$ ).

$LC_{50}$  values of the larvicidal efficacy of the formulation against *An. stephensi*, *Ae. albopictus* and *Cx. tritaeniorhynchus* were respectively 0.060, 0.654 and 0.019  $\mu\text{g/ml}$  for larvae and 0.032, 0.042 and 0.009  $\mu\text{g/ml}$  for pupae (Table 15 & Fig. 15). The pupae of all the species tested were susceptible to low concentrations of the formulation and compared to the larvae.

## Conclusion

This formulation is unique in two respects: first, the formulation has larvicidal as well as pupicidal activity whereas other biocides have only larvicidal property against all the vector species: second, the formulation is found to be highly active against *Aedes* vectors when compared to other biocides which are less active. Further field experiments are in progress to evaluate the field efficacy of the formulation against immatures of vector mosquitoes.

**Table 15. Toxicity of the formulation of protein metabolite of *Pseudomonas fluorescens* to immatures of vector mosquitoes**

Species	Immature stage	LC50 $\mu\text{g/ml}$	Fiducial Limits with 95% Confidence Intervals		LC90 $\mu\text{g/ml}$	Fiducial Limits with 95% Confidence Intervals	
			Upper	Low er		Upper	Low er
<i>An. Stephensi</i>	Larvae	0.0600	0.0700	0.0500	0.2160	0.2760	0.1680
	Pupae	0.0320	0.0360	0.0290	0.0700	0.0820	0.0600
<i>Ae. Albopictus</i>	Larvae	0.6540	0.9260	0.4620	1.5270	2.7470	0.8490
	Pupae	0.0420	0.0570	0.0310	0.0910	0.1490	0.0560
<i>Cx. tritaeniorhynchus</i>	Larvae	0.0190	0.0250	0.0150	0.0700	0.0110	0.0040
	Pupae	0.0090	0.0100	0.0080	0.0180	0.0240	0.0140

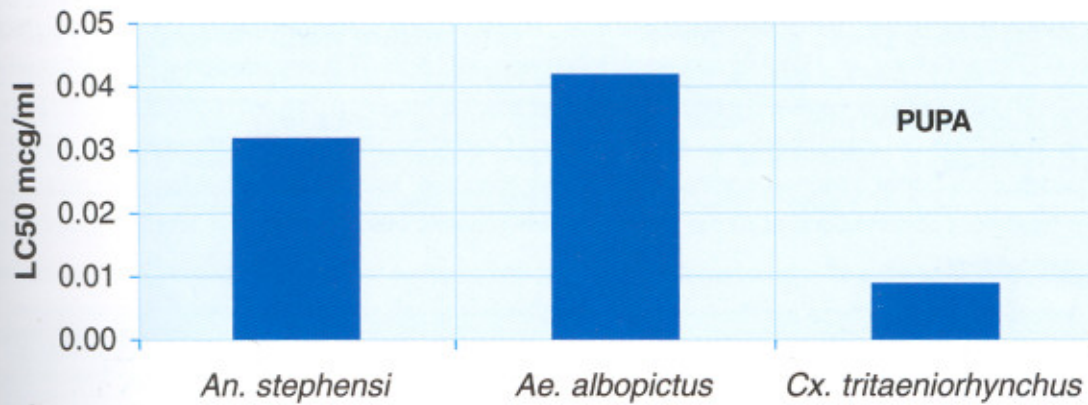
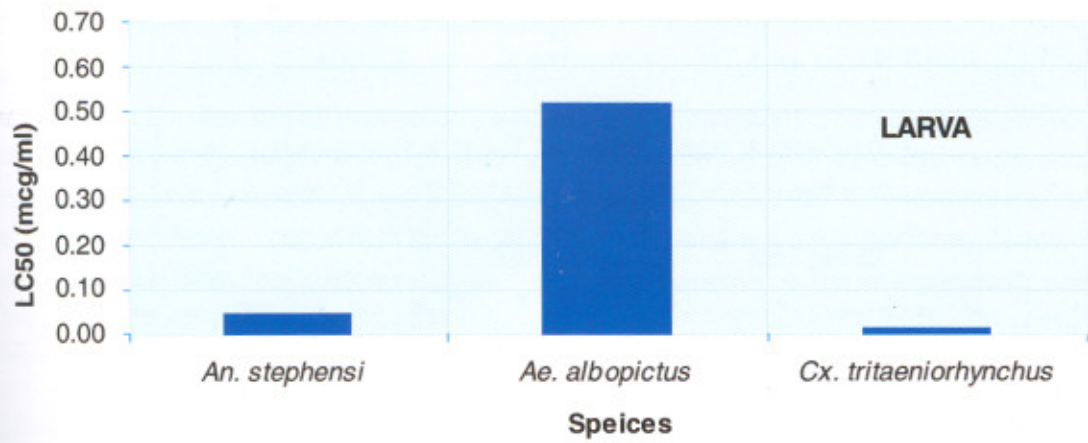


Fig. 15. Susceptibility of immatures of vector mosquitoes to *P. fluorescens*

## 4.2. Report on CRME/DRDE project on Oviposition attracticide trials at Chennai, Tamil Nadu and Thiruvananthapuram, Kerala

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Duration : 6 months  
Funding Agency : DRDE, Gwalior

### (I) Trial at Chennai (October 2005 to June 2006)

A trial was started in Chennai on 15<sup>th</sup> October 2005 by selecting two index areas with high adult, larval and egg densities of *Aedes aegypti*. Before starting the trial entomological, topographical and socio-economic parameters were recorded in index areas, which were verified by DRDE scientists also. Three (A, B & C) concentrations with check were tried in 2 localities, viz; (1) Binny Colony, and (2) Arumbakkam. In experiment I & II, 'A' concentration with 50 replicates (40+10), and 'B' and 'C' with 10 replicates each were placed inside/outside the houses along with control replicates on 15<sup>th</sup> & 16<sup>th</sup> October 2005. In addition, 3<sup>rd</sup> experiment was set up at Binny Colony on 18.10.05 with conventional (CRME) black-tin ovitrap and paddle/filter paper attached against only 'A' concentration, along with control for each replicate. The first round of Post-treatment observations of all the three experiments was taken on 21<sup>st</sup>, 24<sup>th</sup> and 25<sup>th</sup> October in both the index areas respectively.

Subsequently, there were unprecedented heavy rains recorded from 28<sup>th</sup> October 2005 onwards for nearly 30hrs continuously, such rainfall having been recorded earlier nearly 100 years ago only. Chennai remained under deluge and our experimental areas were inundated for more than 3 weeks. This had completely disrupted the whole experiment for about a month and half. Houses were damaged and people had lost their properties. We too had lost more than 50% of ovitraps in both the areas. In view of this nearly after 42 days we could restart the experiment on 7<sup>th</sup> December 2005 at Binny Colony and Arumbakkam on 22<sup>nd</sup> December 2005. Therefore experiment was extended up to June 2006 to have sufficient replicates. Here the vector was *Ae. aegypti*. Altogether, 26 weekly observations were made in both the localities between 14<sup>th</sup> December 2005 and 21<sup>st</sup> June 2006.

In another experimental site viz., Arumbakkam, 23 observations were made for testing all 3 concentrations (A, B & C). The MED was lower (ranging from 0.0 to 9.71) in this area when compared to that in Binny Colony. The experiment was terminated by 30<sup>th</sup> June 2006.

*(II) Trial at Thiruvananthapuram (October 2005 – March 2006)*

The experiment was set up in urban areas of Thiruvananthapuram in a similar pattern as in the case of Chennai. Two localities viz., (1) Kumarapuram, and (2) Kannamoola were selected and traps were set up using same concentrations (A, B & C) of attracticide with the control replicates on 21<sup>st</sup> and 22<sup>nd</sup> October respectively. Ovitrap were mainly placed in peridomestic areas in view of prevalence of *Aedes albopictus* in that area. Altogether 42 observations were made w.e.f. 27<sup>th</sup> October 2005 to 29<sup>th</sup> March 2006 in both the localities. A sample of egg batch (randomly selected) from each observation was reared in laboratory to know the prevalence of *Aedes* species. All the emerged adults were examined and found to be *Ae. albopictus*. The experiment in Thiruvananthapuram went on continuously without any interruption and completed by 30<sup>th</sup> March 2006.

*Summary of analysis*

The initial observations during October / December 2005 against *Aedes aegypti* and *Ae. albopictus* showed that 'A' concentration had better efficacy when compared to 'B' & 'C'. However, after obtaining sufficient number of replicates the results were emerged different. The final analysis showed that mean egg density (MED) in all the treated concentration (A, B & C) were comparable with Check ( $P>0.05$ ). The comparison among the treated showed in Chennai that 'B' concentration having higher among mean value (average MED 2.29) than 'A' (average MED 1.10) & 'C' (average MED 1.38) in one locality (Binny Colony) whereas in another locality (Arumbakkam) 'C' was better attractant (average MED 3.08) when compared to 'B' (average MED 1.21) and 'A' (average MED 1.19). In experiment III, relatively more egg laying was recorded in conventional ovitraps. The details of analysis were illustrated in Tables 16 & 17.

**Table 16. Analysis of mean egg density in oveitraps with "A, B & C" concentrations at Chennai against *Ae. aegypti***

Study Area	Parameters	Experiment - I						Experiment - II	
		Concentrations Used							
		A		B		C		A	
	Treated	Check	Treated	Check	Treated	Check	Treated	Check	
Binny Colony	No. tested	25	25	25	25	25	25	25	25
	Mean eggdensity	1.070	3.944	2.286	3.565	1.380	1.143	2.227	3.237
	Standard deviation	2.201	6.371	3.938	4.183	5.616	4.106	2.266	2.988
	Standard error of mean	0.440	1.274	0.788	0.837	1.123	0.821	0.453	0.598
	t	2.131		1.113		0.170		1.347	
	df	48		48		48		48	
	p	<0.05		>0.05		>0.05		>0.05	
Arumbakkam	No. tested	22	22	22	22	22	22	22	22
	Mean eggdensity	1.991	1.649	1.220	2.494	3.080	2.100	2.303	3.575
	Standard deviation	3.161	1.900	1.363	3.003	5.248	3.141	2.149	5.858
	Standard error of mean	0.674	0.405	0.291	0.640	1.119	0.670	0.458	1.249
	t	0.435		1.812		0.751		0.956	
	df	42		42		42		42	
	p	>0.05		>0.05		>0.05		>0.05	

**Table 17. Analysis of mean egg density using 'A' Concentration in conventional ovitraps with paddle & filter paper at Chennai**

Study Area	Parameters	Experiment - III			
		With Paddle		With Filter paper	
		Treated	Check	Treated	Check
Binny Colony	No. tested	25	25	25	25
	Mean egg density	3.004	8.526	10.174	12.598
	Standard deviation	3.996	11.461	9.593	16.464
	Standard error of mean	0.799	2.292	1.919	3.293
	t	2.275		0.636	
	df	48		48	
	p	<0.05		>0.05	

Similar trend was observed in Kerala (Thiruvananthapuram) also for *Ae. albopictus* when no significant difference in average MED was recorded between treated and control replicates. The average MED was comparable between treated and checks. However, among treated ones the 'B' was more efficacious (average MED 8.96) in one locality (Kumarapuram) whereas in another locality (Kannamoola) 'A' (average MED 4.61) was found more effective than 'B' (average MED 1.96) & 'C' (average MED 1.89). The details are shown in Table 18. No larval / pupal mortality was recorded in all the 3 concentrations applied in the field.

**Table 18. Analysis of mean egg density in ovitraps with A, B & C concentrations at Trivandrum against *Ae. albopictus***

Locality	Parameters	Concentration					
		A		B		C	
		Treated	Check	Treated	Check	Treated	Check
Kumarapuram	No. tested	21	21	21	21	21	21
	Mean egg density	6.298	6.775	8.956	8.884	5.217	6.400
	Standard deviation	3.932	4.815	7.640	9.376	5.517	6.438
	Standard error of mean	0.858	1.051	1.667	2.046	1.397	1.405
	t	0.352		0.027		0.152	
	df	40		40		40	
	p	>0.05		>0.05		>0.05	
Kannamoola	No. tested	21	21	21	21	21	21
	Mean egg density	4.605	3.831	1.959	2.369	1.894	1.655
	Standard deviation	3.288	2.387	2.199	2.733	2.300	2.946
	Standard error of mean	0.718	0.521	0.480	0.596	0.502	0.643
	t	0.873		0.536		0.293	
	df	40		40		40	
	p	>0.05		>0.05		>0.05	