TOXICITY OF INSECTICIDES TO *TOXORHYNCHITES SPLENDENS* AND THREE VECTOR MOSQUITOS AND THEIR SUBLETHAL EFFECT ON BIOCONTROL POTENTIAL OF THE PREDATOR

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Abstract. Toxicity of six larvicides *ie* fenthion, temephos, malathion, deltamethrin, alphamethrin (Fendona), OMS 3031 and five adulticides *ie* malathion, fenitrothion, propoxur, deltamethrin, permethrin to *Aedes aegypti, Culex quiquefasciatus, Anopheles stephensi* and the predator, *Toxorhynchites splendens* was studied for evaluating safety margin. Concentrations of alphamethrin that killed 50% larvae of *T. splendens* were 53 and 12 times more than that which killed *Cx. quinquefasciatus* and *Ae. aegypti*. In case of deltamethrin, concentrations required to kill 50% larvae of *T. splendens* were 14 and 5 times more than that required against other two species. Other larvicides tested were equally toxic to both *T. splendens* and vector mosquitos. There was no significant difference in the toxicity of larvicides to *T. splendens* and *An. stephensi*. Deltamethrin was 25-132 times less toxic to adults of *T. splendens* in comparison to vector mosquitos. For other adulticides the range was 1-10. Immature developmental time of *T. splendens* was not affected by any of the insecticides tested. However, predation rate was lowered when larvae of *Ae. aegypti* previously exposed to fenthion and temephos were offered. Whereas, alphamethrin and OMS 3031 did not affect the feeding rate of the predator. There was a significant reduction in the pupal weight and pupation as a result of the predator feeding on the insecticide treated prey. There was a significant negative relationship between rate of pupation and dosage. The present study indicates that synthetic pyrethroids owing to their higher safety margin can be used in an integrated vector management program.

INTRODUCTION

Integrated vector management (IVM) strategies require optimum blending of various methods including insecticides to get the desired control of the pest. When the pest is abundant, there is a need to bring immediate reduction in its number. Normally, that can be achieved by one round of insecticide application. Subsequently, the pest population can be maintained at low level by releasing natural regulators such as predators and that will reduce the need for repeated application of insecticides (Focks *et al*, 1978). However, to achieve this successfully, one should select such insecticides that do not upset the dynamics of predator-prey interaction. Dynamics of predator-prey interaction may be disturbed either by directly killing or dampening biotic potential of prey and the predator. When an insecticide kills equally both predator and prey, prey population will resurge before establishment of the predator population. Therefore, informations on comparative toxicity of insecticides to both predator and prey and sub-lethal effects of insecticides on biotic potential of the predator are prerequisite for the integration of insecticide and a predator in IVM.

Little information is available on the sublethal effect of insecticides on members of the genus *Toxorhynchites* (Van Halteren, 1971; Wiedl, 1977; Focks *et al*, 1979, 1986; Rawlings and Ragoonansingh, 1990). Hence, studies were undertaken to get information on the insecticide susceptibility of vector mosquitos and the predator *Toxorhynchites splendens*. *Culex quinquefasciatus* and *Anopheles stephensi* do not share the breeding habitat with *T. splendens* and IVM strategy involving *T. splendens* is largely targeted against *Aedes aegypti*. However, susceptibility status of *Cx. quinquefasciatus* and *An. stephensi* was studied in comparison with *T. splendens* as the use of adulticides against these species may affect *T. splendens* and that may jeopardize the control of *Ae. aegypti*. Sublethal effects of insecticides on biocontrol potential of *T. splendens* were also studied and the results are presented here.

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MATERIALS AND METHODS

For determining the toxicity of larvicides to *T. splendens, Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus,* standared procedures were followed (WHO, 1963, 1975, 1981, 1982).

Early fourth instar larvae were exposed to various concentration of the insecticides. Four test replicates for each concentration and four control beakers with tap water were set up. Mortality was recorded after 24 hours. Since *T. splendens* is known to be cannibalistic, individual larvae were isolated in 500 ml beaker prior to treatment. LC_{50} values were determined by probit analysis (WHO, 1975). Control mortality if any, was corrected by using Abbott's formula (WHO, 1963). In case of Insect Growth Regulator (IGR), third instar larvae were continuously exposed to the insecticide. Mortality in immatures and inhibition in adult emergence were recorded until the entire lot of test immatures was exhausted.

For determining the toxicity of adulticides, 5 females of *T. splendens* and 20 blood fed females of other species that were of the same age were exposed to deterministic dose using adult test kits. Mortality was determined after 24 hours for various exposure periods. LT_{50} values were calculated using probit analysis. Each set of experiment was replicated four times.

Tests were conducted for determining the sublethal effects of insecticides on various aspects of predator biology such as immature development, rate of predation, rate of pupation and pupal weight. Insecticides used were fenthion (OP), temephos (OP), alphamethrin (Fendona: a synthetic pyrethroid) and OMS 3031 (IGR). Laboratory strain of *Ae. aegypti* was used as prey. Concentrations were chosen from the susceptibility of early fourth instar of *Ae. aegypti* to these insecticides. Concentrations at which there would be 10%, 25% and 50% mortality in the prey species were chosen for test. Each test was replicated ten times (n = 10). Controls were maintained for each set of experiment. Late first instar larvae of *T. splendens* were released individually in 500 ml beaker with water. Twenty alive early fourth instar larvae of *Ae. aegypti* that had been treated for 24 hours with respective insecticide at different concentrations were placed in each test beaker containing a single *T. splendens* larva. At the end of 24 hours exposure period, the number of *Ae. aegypti* larvae left uneaten in each beaker was counted, removed and twenty fresh insecticide-treated larvae were placed. This was continued until the predator pupated. Water in the test beakers was changed every day. The number of prey eaten each day and time required for *T. splendens* to complete immature development was recorded. Rate of predation was estimated by dividing the total number of prey eaten per perdator by immature duration. Individual pupa was transferred or removed to a No. 2 Whatman filter paper inorder to remove water molecules on the body surface and weighed with a Dhona chemical balance to a precision of 0.1 mg. Mean immature developmental time, pupal weight and percentage pupation were calculated from the number of replicates in which *T. splendens* completed the immature development and reached the pupal stage. The data were classified in two ways by insecticide type and dosage. The results were analyzed through Model-I two-way ANOVA. Means were compared by setting 95% confidence limits. The functional relationship between dosage and percentage pupation was studied by regression analysis. Dosage and percentage pupation were ln transformed before being regressed on dosage following the procedure of Sokal and Rohlf (1981).

RESULTS

Comparative toxicity of larvicides to *T. splendens* and vector mosquitos

The comparative toxicity of larvicides to the predator *T. splendens* and three vector mosquitos is presented in Table 1. The relative toxicity of insecticides on larvae of *T. splendens* was determined by comparison to the susceptibility of vector mosquitos. Concentrations of alphamethrin that killed 50% (LC_{50}) larvae of *T. splendens* were 53 and 12 times more than that of *Cx. quinquefasciatus* and *Ae. aegypti*. In case of deltamethrin, concentrations required to kill 50% larvae of *T. splendens* were 14 and 5 times more than that required against other two species. Other larvicides tested were equally toxic to both *T. splendens* and vector mosquitos. There was no significant difference in the toxicity of larvicides to *T. splendens* and *An. stephensi.*

Table 1

Susceptibility of the predator *T. splendens* and three mosquito vectors to larvicides.

Compound	Species	LC_{50} (mg/l)	*T.splendens* Relative toxicity LC_{50}
Fenthion	*T. splendens*	0.01176	-
	Ae. aegypti	0.01851	0.64
	An. stephensi	0.01737	0.68
	Cx. quinque-fasciatus	0.00569	2.07
Temephos	*T. splendens*	0.00035	-
	Ae. aegypti	0.00104	0.34
	An. stephensi	0.00162	0.22
	Cx. quinque-fasciatus	0.00014	2.50
Malathion	*T. splendens*	0.22208	-
	Ae. aegypti	0.23219	0.96
	An. stephensi	0.63378	0.35
	Cx. quinque-fasciatus	0.06211	3.58
Deltamethrin	*T. splendens*	0.00117	-
	Ae. aegypti	0.00024	4.88
	An. stephensi	0.00246	0.48
	Cx. quinque-fasciatus	0.00008	14.27
Alphamethrin	*T. splendens*	0.00264	-
	Ae. aegypti	0.00023	11.48
	An. stephensi	0.00135	1.96
	Cx. quinque-fasciatus	0.00005	52.80
		EI_{50}	
OMS 3031	*T. splendens*	0.00021	-
	Ae. aegypti	0.00011	1.91
	An. stephensi	0.00022	0.95
	Cx. quinque-fasciatus	0.00009	2.33

EI_{50} = Emergence inhibition (50)

$$\text{Relative toxicity} = \frac{LC_{50} \text{ of } T. \text{ splendens}}{LC_{50} \text{ of vector mosquitos}}$$

Comparative toxicity of adulticides to *T. splendens* and vector mosquitos

The LT_{50} values and relative toxicity (Table 2) showed that deltamethrin was 25-132 times less toxic to adults of *T. splendens* in comparison to vector mosquitos. Deltamethrin, malathion and permethrin were 32, 6 and 4 times less toxic to *T. splendens* in comparison to *Ae. aegypti*. However, there was a little difference in the susceptibility of these two species to fenitrothion and propoxur. Differences in the susceptibility of *T. splendens* and *An. stephensi* to deltamethrin and propoxur were conspicuous. Deltamethrin was 25 times less toxic on *T. splendens* than on *An. stephensi*. Propoxur was 9 times less toxic to *T. splendens* than to *An. stephensi*. However, there was a little difference in their susceptibility to rest of the compounds tested. Regarding relative toxicity of compounds on *T. splendens* and *Cx. quinquefasciatus*, only malathion and deltamethrin were less toxic to *T. splendens*. Finally, the order of toxicity of the compounds on *T. splendens* was fenitrothion > permethrin > propoxur > malathion > deltamethrin.

Table 2

Susceptibility of the predator *T. splendens* and three mosquito vectors to adulticides.

Compound	Species	LT_{50} (min)	*T.splendens* Relative toxicity LT_{50} (min)
Malathion	*T. splendens*	66.95	-
	Ae. aegypti	11.17	6.0
	An. stephensi	34.71	1.93
	Cx. quinque-fasciatus	6.42	10.43
Fenitrothion	*T. splendens*	101.74	-
	Ae. aegypti	84.83	1.2
	An. stephensi	31.28	3.25
	Cx. quinque-fasciatus	36.14	2.82
Propoxur	*T. splendens*	155.18	-
	Ae. aegypti	116.1	1.34
	An. stephensi	17.12	9.06
	Cx. quinque-fasciatus	150.0	1.03
Deltamethrin	*T. splendens*	172.47	-
	Ae. aegypti	1.31	131.55
	An. stephensi	6.9	24.98
	Cx. quinque-fasciatus	6.43	26.82
Permethrin	*T. splendens*	69.72	-
	Ae. aegypti	16.01	4.36
	An. stephensi	17.54	3.98
	Cx. quinque-fasciatus	30.5	2.85

$$\text{Relative toxicity} = \frac{LT_{50} \text{ of } T. \text{ splendens}}{LT_{50} \text{ of vector mosquitos}}$$

Effect of sublethal concentration of insecticides on predator biology

Variation in the mean developmental time of immatures of *T. splendens* fed on insecticide exposed larvae of *Ae. aegypti* was analyzed by two-way ANOVA. The results showed that there was no significant ($F = 0.83$, $p > 0.05$) difference in the immature developmental time of the predator. Neither the type of insecticide nor the dosage affected the predator's developmental time ($F = 0.65$, 0.91; $p > 0.05$) (Fig 1).

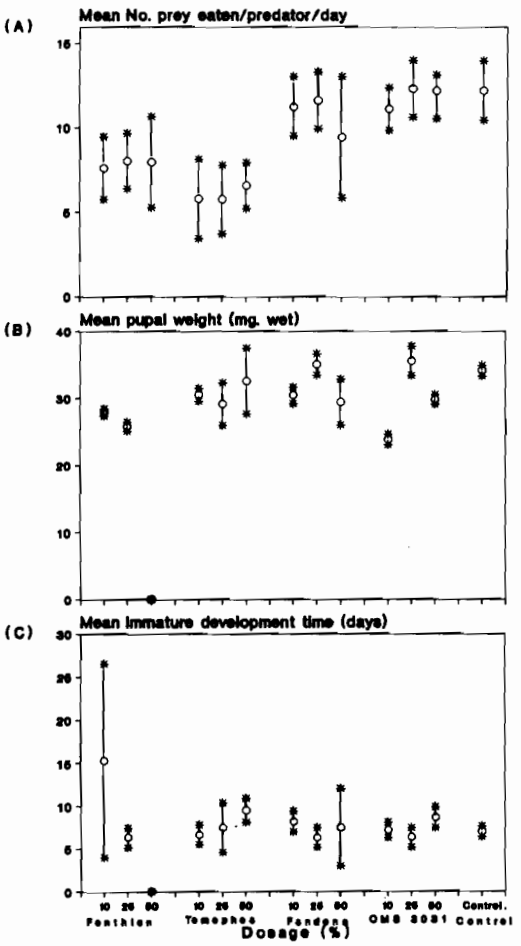

Fig 1–Effect of feeding *T. splendens* with insecticide exposed larvae of *Ae. aegypti* on (A) feeding rate (No. prey eaten/predator/day), (B) pupal weight (wet) and (C) immature duration. Vertical lines are 95% confidence limits for the means.

There was a significant ($F = 18.72$, $p < 0.001$) reduction in the prey consumption when predator was offered prey previously exposed to fenthion and temephos at dosages causing 10% mortality in the prey. Setting of 95% confidence limits for the means showed no further decrease in prey consumption at higher dosages (Fig 1). For fenthion and temephos, the average number of prey eaten/predator/day were 7.86 and 6.04 respectively. However, alphamethrin and OMS 3031 did not affect the feeding rate of the predator. Computation of the feeding rate (No. of prey eaten/predator/day) from the total number of prey eaten during the entire developmental time of the predator was possible as neither insecticides nor the dosage affected the developmental time of the predator.

The effect of insecticides on the predators' pupal weight was studied by analysis of variance. There was a significant reduction in the pupal weight ($F = 90.38$, $p < 0.001$) as a result of the predator feeding on the insecticide treated prey. The presence of significant interaction between insecticide and dosage ($F = 82.47$, $p < 0.001$) implies that reduction in pupal weight was not dosage dependent (Fig 1). Results of regression of percentage pupation on insecticide dosage indicated that percentage pupation decreased with increase in dosage (t-range = -4.99 to -12.57; $df = 2$; $p < 0.05$). Predator could not survive to pupal stage when offered prey treated with fenthion at higher dosage. When the predator was offered prey treated with temephos, alphamethrin and OMS 3031 at higher dose, the percentage pupation were 44.44, 22.22 and 33.33 respectively (Figs 2 and 3).

DISCUSSION

The integrated control approach requires integration of various control methods including insecticide application to manage the pest population without disrupting the natural regulatory mechanism. Therefore, there is a need for studying the effect of insecticides on the biology of the predator (Schoof, 1962; Steffan, 1975). The results of the present study show that pupation of *T. splendens* is significantly affected by the insecticides. Hence, it appears that there is a cumulative effect of eating prey previously exposed at sublethal doses. Data on time to pupation besides percentage pupation is required when trying to maintain a stable predator

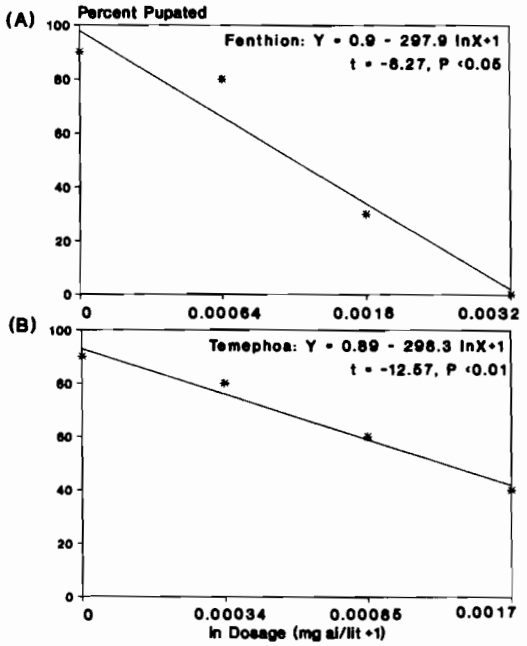

Fig 2–Functional relationship of percentage pupation to dosages of (A) fenthion and (B) temephos. Percentage pupation was regressed on ln transformed dosage.

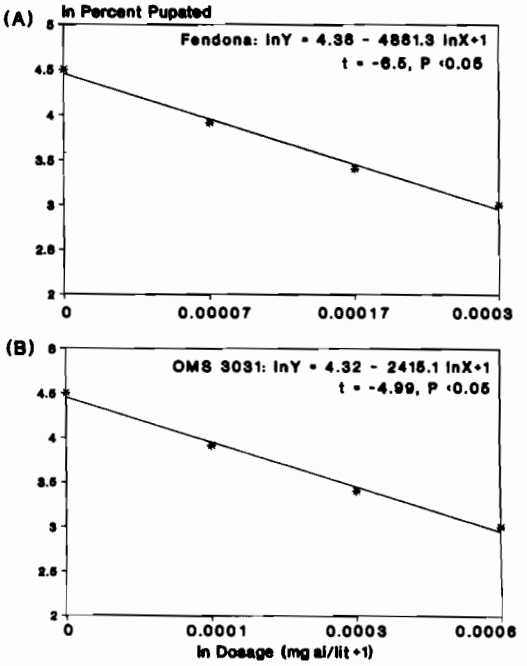

Fig 3–Functional relationship of percentage pupation to dosages of (A) alphamethrin (Fendona) and (B) OMS 3031. Percentage pupation was ln transformed before being regressed on ln dosage.

population. The present study did not show any significant effect of insecticides on the pupation time. Studies of Wiedl (1977) showed that the daily consumption rate of *T. brevoipalpis* was adversely affected and accompanied by an increase in the time of pupation. The present study indicates that there was significant difference in the number of prey consumed between fenthion and temephos treated and control subjects. These differences were not apparent in the case of alphamethrin and OMS 3031. Insecticides also affected the pupal weight. *Cx. quinquefasciatus* does not share the breeding habitat with *T. splendens*. Therefore, eventhough larvae of *T. splendens* and *Cx. quinquefasciatus* were equally susceptible to fenthion, malathion, temephos and OMS 3031, use of these larvicides against *Cx. quinquefasciatus* will not disrupt the dynamics of *T. splendens* and *Ae. aegypti* interaction in the natural condition.

In conclusion, it can be stated that synthetic pyrethroids such as alphamethrin and deltamethrin owing to their higher safety margin can be used in integrated vector management program. However, direct exposure to OC, OP and carbamates and the predation on insecticide treated prey reduces the biotic potential of the predator. In a control effort based upon regularly repeated inundative release of the predator, the contribution that recycled predator makes is limited. So, insecticide application and predator release schedules should be worked out in such a way that insecticide pressure on the predator should be kept minimum. At the same time abundant prey population could be reduced to the level for the predator to exert further pressure upon it. This would be more applicable when there is danger of epidemic outbreak of dengue fever and the immediate necessity for the control of the vector *Ae. aegypti* to a low threshold level to arrest the disease transmission. In this way, insecticide usage can be minimized.

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