3.0 TOLERANCE LIMIT OF NONTARGET ORGANISM
DIPLONYCHUS RUSTICUS EXPOSED TO SELECTED
PESTICIDES

3.1 INTRODUCTION
3.1.1 Pesticides
3.1.2 I - Generation pesticides
3.1.3 II - Generation pesticides
3.1.4 III - Generation pesticides
3.1.5 IV - Generation pesticides
3.1.6 V - Generation pesticides
3.1.7 Integrated Control operations (ICO)
3.1.8 Aim of the present study

3.2 MATERIALS AND METHODS
3.2.1 Materials
3.2.1.1 Water bug - Diplonychus rusticus
3.2.1.2 Insecticides
3.2.1.3 Maintenance
3.2.2 Methods
3.2.2.1 Selection of pesticide concentrations
3.2.2.2 Acute toxicity test

3.3 RESULTS
3.3.1 Tolerance limit of male D.rusticus
3.3.2 Tolerance limit of female D.rusticus

3.4 DISCUSSION
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PESTICIDES

3.1 INTRODUCTION

An important factor of pesticides is to cause the susceptibility in the
target pest species. Insecticide resistance management is of central importance
to an increasing number of vector control programmes (Bonning and
Hemingway, 1991). The behaviour of mosquito larvae is influenced by the level
at which the pesticide undergoes degradation in freshwater environment and
is directly dependent on the sustenance of toxic effect of the pesticide used.

The resistance of organophosphate compound in Culex quinquefasciatus
is inherited as monofactorial partial dominant character (Georghiou et al,
1980). Adult mosquitoes are controlled well by residual spraying of the
pesticides (Taylor et al, 1981). High resistance to DDT in Culex
quinquefasciatus is already shown (Brown and Tadano, 1965; Guneidy et al,
(1988). Deltamethrin is a synthetic pyrethroid known for its outstanding
insecticidal activities. Larvae of indian strains of vector mosquitoes are highly
susceptible to Deltamethrin (Thomas and Pillai, 1986).

3.1.1 Pesticides

To face the universal challenge for increased production of food material
for the sustenance of ever - escalating population, pesticidal use along with
other agrochemicals has been an indispensable modern strategy. But the
prolonged usage of these chemical agents results in the development of
insecticide resistance.
Insecticide resistance has been in the driver's seat in the succession of pesticide management from one decade to the next. Its major effect has been to replace the persistent organochlorines with less persistent organophosphates and carbamates, which on environmental grounds is a movement in the right direction.

However, where the original insecticide was not replaced, but simply applied in higher concentration and more frequently in the hope of somehow obtaining control, the effect was simply to intensify the resistance by eliminating all the natural enemies of the pest (Mallett, 1989).

3.1.2. I-Generation Pesticide

Since the advent of DDT, reliance has been placed almost exclusively on chemical pesticides for vector control programmes. Although it was originally hoped that reliance on the broad spectrum organochlorine insecticides, such as DDT, HCH and Dieldin, could lead to the control or even eradication of many vector borne-diseases, this has not been the case.

The appearance of second order problems of steadily increasing severity, such as vector resistance, enviromental contamination and increasing costs, has seriously jeopardized successful vector control operations in large areas, which are inhabited by millions of people. High resistance to DDT in *Culex quinquefasciatus* was shown by Guneidy et al (1988). Cross resistance between DDT and pyrethroids was shown by Amin and Hemingway (1989). Larvae of *Aedes aegypti* gain resistance to DDT (Rathor and Wood, 1981).
3.1.3 II-Generation Pesticide

Organophosphorous insecticides as malathion and fentrothion are being used as substitutes in residual house spraying against DDT-resistant and Dieldrin-resistant *Anopheles* species. Malathion is one of the insecticides of choice as a powder and for ultra-low-volume (ULV) spraying, and fenthion, chlorpyrifos and Abate are most effective larvicides. All these compounds are readily biodegradable and cause only limited environmental contamination. However, vector resistance to organophosphorus insecticides is becoming widespread and is now found in 27 species.

*Anopheles flavinostris*, malaria vector in Philippines, developed resistance to Dieldrin in 1959 (WHO, 1996). Fenthion (Baytex) and Temephos (Abate) are used in urban malaria control programme (Vittal *et al*, 1984).

3.1.4 III-Generation Pesticide

Carbamate insecticide propoxus has been used in residual house spraying against resistant *Anopheles* and shows promise for the control of triatomine bugs. However, resistance to propoxus has already appeared in *An. albimanus* and its high cost is a discouragement to wider use. Carbaryl is effective as a powder. Several workers on the WHO have emphasized the importance of collection of data in the development of physiological resistance in malaria vector. Concern is noticed that if malathion is to serve as a last report weapon, its judicial use is necessary (Vittal *et al*, 1982).
3.1.5 IV-Generation Pesticide

This includes the synthetic pyrethroids, such as resmethrin, bioresmethrin and prothrin. They are relatively safe to man and higher animal candidates for integrated vector control (WHO, 1984). Penetration of permethrin was faster in the resistance than the susceptible strains. Higher penetration rate in the resistance strain was attributed to their continued activity throughout the exposure period (Priester and Georghiou, 1979).

3.1.6 V-Generation Pesticide

This includes the insect growth regulators, such as methoprene and OMS 1804, offer more specific toxicity to target species. However, since they act by inhibition of growth and maturation of cuticle development, applications of these compounds must be timed precisely to expose the vector at a critical developmental period (WHO, 1984).

3.1.7 Integrated Control Operations (IPO)

Biological control of mosquitoes involving the use of several predators and parasites has gathered greater momentum in the recent years mainly because certain vectors have been proved difficult to be controlled only by chemical means. The potential use of predatory fishes, water bugs, parasites, fungi, bacteria and other protozoans enhance the effective control of vector borne diseases of human beings (Chauhan, 1996 and Vas Dev, 1998). Several species of predatory fishes like Gambusia, Poecilia and Catla and majority of the aquatic insects of the sub-order Heteroptera have shown their capacity to be used as potential biocontrol agents against the mosquito population.
3.1.8 Aim of the Present Study

In the context of resistance mechanism gained by mosquito larvae against chemical pesticides, it was felt worthwhile to estimate the tolerance capacity of the water bugs that are not only reported as bioagents but as nontarget organism to the pesticides used in mosquito control. Hence, in the present study, an attempt has been made to determine the tolerance limit of such a water bug *D. rusticus* to the larvicide Abate (Organophosphorus) and adulticide Solfac (Synthetic pyrethroid).

3.2 MATERIALS AND METHODS

3.2.1 Materials

3.2.1.1 Water Bug - *Diplonychus rusticus*

The belostomatid bugs *D. rusticus* were collected from the Chetpet pond, Chennai, India. The margin of the pond is rich in vegetation. The level of water is in accordance with the season and the water body is noted to be rich in floating vegetation such as *Eichhornea* and *Hydrilla*. These vegetation were dragged on and spread over the ground to pick up the bugs that cling on to the roots of the vegetation.

The water column with poor vegetation did not hold higher population of the belostomatid bug. Sometimes the bugs were directly collected from the water body with larger kitchen strainer. Early hours of a day were preferred since the bugs are distributed in abundance in the edges and on water surface during these hours.
3.2.1.2 Insecticides

Abate and Solfac were used as the insecticides in order to determine the tolerance limit of the belostomatid bugs. Abate is an organophosphate that represents temephos 50% EC used to control mosquitoes. Solfac is a synthetic pyrethroid compound called as cyfluthrin and it is a fifth generation insecticide.

3.2.1.3 Maintenance

The collected bugs from the study site were brought to the laboratory with aquatic vegetation to avoid high rate of mortality. These bugs were maintained in an aquarium at room temperature along with ample vegetation like Hydrilla and Eichhornia to make it a favourable habitat. The bugs were fed with Culex and Chironomus larvae periodically. Water in the container was periodically changed and the dead ones were removed to avoid the fouling of the medium. Male and female bugs were separated for the experiment. The sex determination was done based on the presence of tufts of hairs on the external and internal margins of the respiratory siphons in males.

3.2.2 Methods

3.2.2.1 Selection of pesticide concentration

Selected pesticides namely Abate and Solfac were introduced into beakers containing water with the bug. Male and female bugs were exposed to specific concentration of pesticides. These concentrations are lethal
concentrations to 50% mortality of the bug. The log concentration of the pesticide Abate was 1.28, 1.15, 1.10 and 1.02 μg/l and 1.36, 1.30, 1.22 and 1.14 μg/l for Solfac in male. In female it was 1.30, 1.21, 1.16 and 0.15 μg/l in Abate 1.42, 1.26, 1.22 and 1.19 μg/l in Solfac for the period of 24, 48, 72 and 96 hrs respectively.

3.2.2.2 Acute toxicity test

To study the toxicity of pesticide, the static bioassay method (APHA, 1980) was followed. The test individuals were exposed to selected and serially diluted pesticides. For each acute toxicity test, 20 male bugs and 20 female bugs were separately exposed to each concentration of the pesticide using pesticide free water as control. Experimental beakers were not aerated and animals that were pre-starved for 1 day were not fed even during the treatments.

All the bugs in control survived during the test-period. Mortality was recorded continuously for 24, 48, 72 and 96 hrs using the method of Sprague (1973). Percent mortality was calculated and the values were transformed into the probit scale. Probit analysis was carried out as per Finney (1971). Regression line of probits against logarithmic transformation of calculations were made. Slope(s) function and confidential limit (Upper confidence and lower confidence limits) of the regression line with chisquare test (UNEP/FAO/IAEA 1987) were calculated as follows:
\[ S = \frac{\frac{\text{LC}_{90}}{\text{LC}_{50}} + \frac{\text{LC}_{50}}{\text{LC}_{20}}}{2} \]

\[ F = \text{Antilog} \left( \frac{2.77 \log S}{\sqrt{N}} \right) = S \cdot 2.77\sqrt{N} \]

Where \( N \) is the number of animals tested whose expected effects are between 20% and 90% mortality. Upper confidence limit (UCL) = \( \text{LC}_{50} \times f \), lower confidence limit (LCL) = \( \text{LC}_{50} / f \).

Based on acute toxicity test, four lethal concentrations were derived for 24, 48, 72 and 96 hrs durations, which have been used as the experimental concentration of the pesticide toxicants in the subsequent experiments.

### 3.3 RESULTS

#### 3.3.1. Tolerance limit of male \textit{D.rusticus}

Tolerance limit of male \textit{D.rusticus} was estimated after it was exposed to the selected pesticides of varied concentrations to specific periods. The mortality of the bugs of various pesticidal concentrations was plotted in the graph and the \( \text{LC}_{50}, \text{LC}_{20}, \text{LC}_{90} \) values were determined. In male \textit{D.rusticus}, the \( \text{LC}_{50} \) concentrations were 1.28 \( \mu \text{g/l} \), 1.15 \( \mu \text{g/l} \), 1.10 \( \mu \text{g/l} \) and 1.02 \( \mu \text{g/l} \) and at 24, 48, 72 and 96 hrs respectively against Abate. In Solfac treated male \textit{D.rusticus}, the log concentrations of 50% mortality were 1.36 \( \mu \text{g/l} \), 1.30 \( \mu \text{g/l} \), 1.22 \( \mu \text{g/l} \) and 1.14 \( \mu \text{g/l} \) at 24, 48, 72 and 96 hrs time intervals. On comparison, the tolerance
Table: 3.1(a) Tolerance limit of male *Diplonychus rusticus* to the larvicide Abate

<table>
<thead>
<tr>
<th>Exposure period (hrs)</th>
<th>Regression equation</th>
<th>Chi square at 0.05% level</th>
<th>Slope</th>
<th>Lethal limit</th>
<th>Confidence level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>Table</td>
<td>LC 20</td>
<td>LC 50</td>
</tr>
<tr>
<td>24</td>
<td>Y=1.22468+4.72229X</td>
<td>0.566</td>
<td>15.5</td>
<td>1.70</td>
<td>1.10</td>
</tr>
<tr>
<td>48</td>
<td>Y=1.18234+5.13341X</td>
<td>1.964</td>
<td>12.6</td>
<td>1.60</td>
<td>1.001</td>
</tr>
<tr>
<td>72</td>
<td>Y=1.16233+5.21363X</td>
<td>0.627</td>
<td>15.5</td>
<td>1.91</td>
<td>0.89</td>
</tr>
<tr>
<td>96</td>
<td>Y=1.11762+5.47146X</td>
<td>0.443</td>
<td>15.5</td>
<td>1.67</td>
<td>0.85</td>
</tr>
</tbody>
</table>

All values indicate log concentration µg/l and are statistically significant.
### Table: 3.1(b) Tolerance limit of male *Diplonychus rusticus* to the adulticide Solfac

<table>
<thead>
<tr>
<th>Exposure period (hrs)</th>
<th>Regression equation</th>
<th>Chi square at 0.05% level</th>
<th>Slope</th>
<th>Lethal limit</th>
<th>Confidence level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>Table</td>
<td>LC 20</td>
<td>LC 50</td>
</tr>
<tr>
<td>24</td>
<td>Y=1.26404+4.60693X</td>
<td>1.364</td>
<td>15.5</td>
<td>2.00</td>
<td>1.14</td>
</tr>
<tr>
<td>48</td>
<td>Y=1.25987+4.77735X</td>
<td>1.860</td>
<td>12.6</td>
<td>1.77</td>
<td>1.12</td>
</tr>
<tr>
<td>72</td>
<td>Y=1.23925+5.04488X</td>
<td>0.822</td>
<td>15.5</td>
<td>1.71</td>
<td>1.05</td>
</tr>
<tr>
<td>96</td>
<td>Y=1.19923+5.34545X</td>
<td>0.65</td>
<td>15.5</td>
<td>1.50</td>
<td>1.009</td>
</tr>
</tbody>
</table>

All values indicate log concentration µg/l and are statistically significant.
Fig. 3.1 Regression analysis of Abate and Solfac concentrations for 24, 48, 72 and 96 hrs for the male Diplonychus rusticus.
Table: 3.2(a) Tolerance limit of female *Diplonychus rusticus* to the larvicide Abate

<table>
<thead>
<tr>
<th>Exposure period (hrs)</th>
<th>Regression equation</th>
<th>Chi square at 0.05% level</th>
<th>Slope</th>
<th>Lethal limit</th>
<th>Confidence level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>Table</td>
<td>LC 20</td>
<td>LC 50</td>
</tr>
<tr>
<td>24</td>
<td>Y=1.21416+4.66031X</td>
<td>0.190</td>
<td>15.5</td>
<td>2.02</td>
<td>1.07</td>
</tr>
<tr>
<td>48</td>
<td>Y=1.20759+4.9398x</td>
<td>0.608</td>
<td>12.6</td>
<td>1.67</td>
<td>1.05</td>
</tr>
<tr>
<td>72</td>
<td>Y=1.17998+5.08368X</td>
<td>0.412</td>
<td>15.5</td>
<td>1.63</td>
<td>1.003</td>
</tr>
<tr>
<td>96</td>
<td>Y=1.18169+5.12146x</td>
<td>1.081</td>
<td>15.5</td>
<td>1.73</td>
<td>0.97</td>
</tr>
</tbody>
</table>

All values indicate log concentration μg/l and are statistically *α*-significant
Table: 3.2 (b) Tolerance limit of female *Diplonychus rusticus* to the adulticide Solfac

<table>
<thead>
<tr>
<th>Exposure period (hrs)</th>
<th>Regression equation</th>
<th>Chi square at 0.05% level</th>
<th>Slope</th>
<th>Lethal limit</th>
<th>Confidence level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>Table</td>
<td>LC 20</td>
<td>LC 50</td>
</tr>
<tr>
<td>24</td>
<td>Y=1.25042+4.58229X</td>
<td>0.280</td>
<td>15.5</td>
<td>3.02</td>
<td>1.07</td>
</tr>
<tr>
<td>48</td>
<td>Y=1.24910+4.91200X</td>
<td>1.875</td>
<td>12.6</td>
<td>1.76</td>
<td>1.08</td>
</tr>
<tr>
<td>72</td>
<td>Y=1.23935+5.07621X</td>
<td>2.391</td>
<td>15.5</td>
<td>1.65</td>
<td>1.06</td>
</tr>
<tr>
<td>96</td>
<td>Y=1.22525+5.17027X</td>
<td>0.551</td>
<td>15.5</td>
<td>1.61</td>
<td>1.03</td>
</tr>
</tbody>
</table>

All values indicate log concentration μg/l and are statistically non-significant.
Fig: 3.2 Regression analysis of Abate and Solfac concentrations for 24, 48, 72 and 96 hrs for the female Diplonychus rusticus.
limit of the male bugs to the pesticidial concentration of Solfac was higher than Abate. Results obtained with the tolerance of male *D.rusticus* to the pesticides Abate and Solfac were summarised (Table 3.1 a,b) and graphically represented (Fig. 3.1).

### 3.3.2 Tolerance limit of female *D.rusticus*

Female *D.rusticus* were exposed to the pesticides Abate and Solfac at the time interval of 24, 48, 72 and 96 hrs. The lethal concentration of the pesticides responsible for the 50% morality of the bugs were determined. Results obtained were tabulated (Table 3.2 a,b) and were graphically represented (Fig. 3.2). The log values of the lethal concentration for the pesticide Abate was 1.30 µg/l, 1.21 µg/l, 1.16 µg/l and 1.15 µg/l at 24, 48, 72 and 96 hrs. From which the LC$_{20}$ and LC$_{90}$ values were calculated. With regard to the pesticide Solfac, the log concentration of the 50% lethal concentrations were 1.42 µg/l, 1.26 µg/l, 1.22 µg/l and 1.19 µg/l at 24, 48, 72 and 96 hrs. On comparison, female *D.rusticus* were more tolerant to the pesticides Abate and Solfac than male.

### 3.4 DISCUSSION

The greatest challange in the control of mosquito menace is the ever growing resistance of mosquito population to the widely used larvicides and adulticides. Substantial advances are made in improving the larvicidal activity of synthetic pyrethroid against wide range mosquitoes (Mulla *et al*, 1980). However the extent of mosquito menace has not gone down drastically. Bisset
et al (1990) have reported the development of the cross resistant spectra in the strains of mosquito to organophosphorus compounds such as malathion and fenthion. More than 90% of the current annual malaria incidence is in Africa, where the major vector is *Anopheles gambiae* that has pyrethroid resistance (Martineztorres et al, 1998).

The carbamate resistance in *Anopheles albinanus* is conferred by an altered acetylcholineesterase (AChE) based resistant mechanism (Patricia et al, 1998). Resistance to permethrin induced a decrease in mortality not only for pyrethroid compounds but also for deltamethrin in the most resistant population (Chandre, 1998).

The major mechanism of organophosphorus insecticide resistance in *Culex* mosquitoes involves the elevation of one or more esterases. The general mechanism underlying their resistance is the amplification of the structural genes (Hemingway et al, 1998). Increased resistance to malathion but not to the other organophosphate or carbamate insecticides resulted from deltamethrin selection. The high resistance to deltamethrin after just four generations of lavicidal selection indicates that this pyrethroid is not a good candidate for long term suppression of *Culex quinquefasciatus* in Cuba (Bisset et al, 1996).
The insecticides applied in an aquatic ecosystem apart from killing the
target organisms not only pollute the environment but also cause adverse
effects to the nontarget beneficial organisms ultimately disturbing the
ecological balance. As the pyrethroids are designed to control arthropod pests,
they are generally very toxic to aquatic insects and crustaceans (Bradbury and
Coats, 1989).

In the present investigation, LC$_{50}$ values of organophosphorus (Abate)
insecticide was slightly higher than the synthetic pyrethroid (Solfac). The LC$_{50}$
values of certain pyrethroids viz., deltamethrin, cypermethrin, fenvalerate and
permethrin for a variety of mosquito and midge larvae and pupae (target
organisms) range between 0.02 - 13 mg/l. The toxicity values for nontarget
insect species are generally in the same range. The may fly nymphs are very
sensitive to pyrethroids whereas aquatic bugs and beetles are relatively more
tolerant (Webber et al, 1989).

The pyrethroids are highly toxic to freshwater (Cladoceran, amphipods,
isopods, crayfish) and marine (Shrimps and lobsters) crustaceans. LC$_{50}$ values
of permethrin and fenvalerate for amphipod Gammarus pseudolimnaeus are
much less than LC$_{50}$ values for aquatic insects viz., mayfly, stonefly, caddisfly
and Artherix (Anderson, 1995).
The range of pesticide concentration for the 50% mortality of the target organism remains the same for the 50% mortality of the nontarget aquatic insect *D. rusticus* also. Generally insects are more tolerant to insecticidal toxicity. However, some are equally or even more sensitive than fish and other vertebrates (Sphehar *et al*., 1978).

It is pertinent to quote the work of Darwazeh *et al* (1978) that Decamethrin at 0.0025 lb/A produces complete control of *Aedes* larvae, Rajvanshi *et al* (1982) have also reported that two new synthetic pyrethroids namely Decamethrin and Fenvalerate had higher efficacy when tested against mosquito larvae. The degradation of insecticides is an important phenomenon in the context of persistence of pesticidal residue. Organophosphorus pesticides are less biodegradable than the synthetic pyrethroids. Mulla *et al* (1978) have stressed the role of photodecomposition and hydrolytic degradation of synthetic pyrethroid as the factors probably responsible for their inactivity in the field.

On comparison of a synthetic pyrethroid with that of an insect growth regulator, the latter was noted to be highly toxic. The resistance to organophosphorus compounds has been developed in many insects. So far organophosphorus insecticide resistance has been observed in 27 species of mosquitoes (Georghiou *et al*., 1980).

The proposed bioagent in control of mosquito larvae *D. rusticus* is capable of withstanding salinity and temperature stress (Venkatesan, 1986). Male *D. rusticus* is of great adaptive value in the insecticidal system because the male bugs are reported to be dominant in the population (Venkatesan, 1978),
since belostomatid bugs exhibit distinct egg carrying mechanism (Smith, 1974). The survival of male bug promotes the encumberence and keeps the egg population viable in growth.

In the present investigation the insecticides are found to kill the belostomatid bug. However, their tolerance to insecticides was noted to be at the higher dosage. The mortality rate of both the sexes were not similar when exposed to selected pesticides (Abate and Solfac). The females were more tolerant to the pesticides than males.

Mulla et al (1980) have reported that highly active pyrethroids were toxic to larvae and adults of diving beetle. The present study further reveals the occurrence of sex dependant tolerance of the belostomatid bug \textit{D.rusticus}. Though their lethal dosage of insecticides was at a higher rate of their tolerance to pesticides status of organic content of the body, enzymological and haemotological responses towards pesticide pollution will provide an indepth knowledge about the predatory potential of the bug to consider them for effective integrated pest management.