Larvicidal activity and biochemical effects of cyperstar (synthetic pyrethroid) against elephantiasis vector

*Culex quinquefasciatus*

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Abstract - Laboratory investigation was conducted to evaluate the larvicidal activity of synthetic pyrethroid (Cyperstar) against larvae of *Culex quinquefasciatus* which is the serious vector of filarial worm, *Wuchereria bancrofti* causing lymphatic filariasis in human. Cyperstar was administered for 24h or 96h to the larvae. Exposure of larvae over 24h to sub-lethal doses 40% and 80% of LC50, significantly altered the biochemical parameter levels i.e. Total carbohydrate, total protein, total free amino acids and activities of enzymes-Acetyl cholinesterase, Acid and Alkaline phosphates in whole body tissue of larvae. The alterations in all these biochemical parameters were significant (P<0.05) as well as time and dose dependent. The use of synthetic pyrethroid in restricted and controlled manner can be used in management of population of *Culex quinquefasciatus*.

Index Terms - *Culex quinquefasciatus*, Biochemical effects, *Wuchereria bancrofti* and Synthetic Pyrethroid (Cyperstar).

I. INTRODUCTION

Mosquitoes are responsible for the major public health problem around the globe. Out of 3492 species of mosquitoes more than hundred species are capable of transmitting various diseases in human and other vertebrates[1]. Mosquitoes transmits malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis and chikungunya to humans[2]. Lymphatic filariasis is a mosquito borne disease caused by filarial nematodes (*Wuchereria bancrofti*) whose vector is *Culex quinquefasciatus*. Several kinds of mosquitoes belonging to different genera are vectors for the pathogen of various diseases[3].

Filaria is a vector borne parasitic disease and its global transmission is caused by different species of *Culex mosquitos*[4]. *Culex quinquefasciatus* is an urban vector of lymphatic filariasis which is endemic in 80 countries of the world and recognized as one of six potentially eradicable diseases[5-8]. Synthetic pesticides are mostly used for public health sprays in most parts of the world[9-10]. It’s unlimited, uninterrupted and indiscriminate use as the principal agent, results in development of insecticide resistance in mosquitoes and also poses a threat to life and environment [11-15].

Lymphatic filariasis (LF) is an endemic in 82 countries approximately of the world and recognized as one of six potentially eradicable diseases. LF and Malaria rank amongst the world most prevalent tropical infectious diseases. An estimated 300-500 million people are infected with malaria annually, resulting in 1.5-3 million deaths [16]. Elephantiasis is probably the fastest spreading insect-borne disease of humans in tropical, about 30% (394 million) of the global at risk population is estimated to be in the endemic countries like African region [17]. Elephantiasis is a significant public health and economic problem in many tropical and subtropical regions of the world, including India [18,19,20]. One of the methods to control these diseases is to control the vectors for the interruption of disease transmission. Malaria remains a major health problem in world, accordingly about 20-40% of outpatient clinic visits and 30% of total hospital admissions are due to malaria [21].

II. MATERIALS AND METHODS

Collection and maintenance of mosquitoes-

Fully fed adult females of Culicines were collected from the different residential areas of Gorakhpur. Collections were made from human dwellings with the help of an aspirator supplied by W.H.O. and kept in 30x30x30 cm cages with cotton pads soaked in 10% glucose solution and water containing enamel bowl for egg laying.

Experimental conditions of water determined by the method of APHA/AWWA/WEF [22] were atmospheric temperature 30.2°±1.6°C, water temperature 27.6°±1.1°C, pH 7.3-7.5, dissolved oxygen 7.6-8.1mg/L, free CO₂ 4.1-5.1mg/L, bicarbonate alkalinity 103.5-105.0 mg/L.

Collection of eggs: Individual batches of eggs were obtained by isolation gravid female through inspirator and were introduces into 30x30x30 cm cages. An enamel tray containing filtered pond water was kept inside the cage for egg laying. The eggs were allowed to hatch in the tray in which they were collected. In 2-4 days mosquito eggs were hatched and after hatching the larvae were transferred to another bowl, containing de-chlorinated tap water.

Hatching of larvae: The incubation period last for about two days at 25°C. The eggs float until they hatched, and then young larvae were transferred to breeding pans, having de-chlorinated water.
Feeding of larvae: Newly emerge larvae do not take food during first 24th of their emergence. After 24h, they were transferred to the breeding pan and the food was provided them in form of dry powder. The dry powder consists of yeast powder and dog biscuit in 1:4 ratios. The water of breeding pan was changed every day to avoid contamination through the dry food. The quantity of dry food depends on the number of larvae in the pan and it was increase with the larval growth.

Rearing of adult mosquito: Pupae was picked up from the breeding pan with the help of dropper. After collection they were transferred into an enamel bowl, and placed in the breeding cage. The majority of adults were emerged out in about 2-3 days at 25-27ºC. To maintain the colony the female mosquitoes were left to be fertilized naturally. Mating takes from 4 to 40 seconds and some stay together for over an hour.

Feeding of adult mosquito: The adult females were fed with blood meals twice in a week, to maintain a mosquito colony. Male were fed on 5-10% sugar solution or glucose solution contained in a small glass bottle with a long piece of cotton wool, whose one end was dipped in the solution of the bottle and other was on the top. The males and females readily fed on this solution. The female mosquitoes were also fed on the blood of a rabbit, inside the insectory.

Biochemical Analysis

Total protein: Total protein level was estimated by the method of [23]. Homogenates (10 mg/mL) was prepared in 10% trichloroacetic acid (TCA). Bovine serum albumin was used as standard.

Total free amino acids: Total free amino acids level was estimated by the method of [24]. Homogenates (10mg/mL) were prepared in 95% ethanol. Glycine was used as standard.

Glycogen: Glycogen level was estimated by the method of [25]. Homogenate (10 mg/mL) was prepared in 5% TCA. Glucose was used as standard.

Acetylcholinesterase activity: Acetylcholinesterase activity was measured by the method of [26]. Homogenate (50 mg/ml, w/v) was prepared in 0.1 M-phosphate buffer, PH 8.0 for 5 min in an ice bath. The change in optical density at 412nm, caused by the enzymatic reaction, was monitored for 3 min at 25°C.

Acid and alkaline phosphatase activity: Acid and alkaline phosphatase activity was determined by the method of [27]. Homogenates (2% w/v) were prepared in ice-cold 0.9% NaCl solution and centrifuged at 5000 xg at 0°C. Statistical analysis: Each experiment was replicated at least six times and data has expressed as mean ±SE.

Figure 1 Structure of Synthetic pyrethroids
III. RESULT AND DISCUSSION

(A) Results-

(a) Toxicity Section-
The mortality produced by synthetic pyrethroid (cyperstar) for the periods ranging from 24 to 96hr. The toxicity was time and dose dependent for *Culex quinquefasciatus* larvae. There was a significant negative correlation between LC values and exposure periods. i.e. LC₅₀ values of cyperstar decreased from 1.46 mg/L (24h)>1.32 mg/L (48h)>1.15mg/L (72h)>1.02 mg/L (96h) in case of *Culex quinquefasciatus* larvae (Table 1; Figure 2).

### Table 1: Toxicity (LC values) of different concentrations of synthetic pyrethroid – Cyperstar against *Culex quinquefasciatus* larvae at 24h to 96h exposure period.

<table>
<thead>
<tr>
<th>Exposure Period (hours)</th>
<th>Effective dose (mg/L)</th>
<th>Limits (mg/L)</th>
<th>Slope value</th>
<th>‘t’ ratio</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LCL</td>
<td>UCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>LC₁₀=0.95</td>
<td>0.44</td>
<td>1.12</td>
<td>6.84±0.34</td>
<td>2.84</td>
</tr>
<tr>
<td></td>
<td>LC₅₀=1.46</td>
<td>1.30</td>
<td>1.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₉₀=2.25</td>
<td>1.78</td>
<td>7.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>LC₁₀=0.89</td>
<td>0.48</td>
<td>1.05</td>
<td>7.51±0.31</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td>LC₅₀=1.32</td>
<td>1.17</td>
<td>1.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₉₀=1.96</td>
<td>1.64</td>
<td>3.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>LC₁₀=0.78</td>
<td>0.35</td>
<td>0.95</td>
<td>7.52±0.21</td>
<td>3.17</td>
</tr>
<tr>
<td></td>
<td>LC₅₀=1.15</td>
<td>0.94</td>
<td>1.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₉₀=1.71</td>
<td>1.48</td>
<td>2.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>LC₁₀=0.67</td>
<td>0.18</td>
<td>0.87</td>
<td>7.11±0.89</td>
<td>2.84</td>
</tr>
<tr>
<td></td>
<td>LC₅₀=1.02</td>
<td>0.64</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC₉₀=1.54</td>
<td>1.35</td>
<td>2.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Batches of twenty mosquito larvae were exposed to four different concentrations of the extract.
- Concentrations given are the final concentration (w/v) in the glass beaker containing de-chlorinated tap water. Each set of experiment was replicated six times.
- Mortality was recorded after every 24h.
- Regression coefficient showed that there was significant (P<0.05) negative correlation between exposure time and different LC values.
- LCL: Lower confidence limit; UCL: Upper confidence limit.
- There was no mortality recorded in the control group.

![Figure 2](image-url) - Bar diagram showing cyperstar (synthetic pyrethroid) toxicity on *Culex quinquefasciatus* larvae at different concentrations and at different time intervals

* Values are mentioned in percentage.
* Doses are 40% and 80% of LC₅₀ for period for which animals were exposed.
* Significant (P<0.05) when two way analysis of variance was applied to see biochemical profile was time and dose dependent.
* Significant (P<0.05) when Student ‘t’ test was applied between control and treated group.
(b) Biochemical Section-

Exposure of larvae to 40% and 80% of LC\textsubscript{50} of cyperstar extract for 24h caused significant dose dependent reduction in the protein and glycogen and increased the free amino acid levels in the body tissue of \textit{Culex quinquefasciatus} larvae. Total protein levels were reduced to 64% and 46% of control respectively after treatment with 40% and 80% of LC\textsubscript{50} (24h). The levels of glycogen reduced to 50% and 32% of control in the body tissue respectively after treatment with 40% and 80% of LC\textsubscript{50} (24h). Exposure of 40% and 80% of LC\textsubscript{50} (24h) of cyperstar extract significantly increased the free amino acid level to 127% and 146% of control respectively (Table 2: Figure 3).

Exposure of larvae to 40% and 80% of LC\textsubscript{50} of cyperstar extract for 24h or 96h caused significant dose dependent reduction in the AChE, acid phosphate and alkaline phosphatase activity in the body tissue of \textit{Culex quinquefasciatus} larvae. Acetylcholinesterase (AChE) activity was reduced to 68% and 48% of control respectively after treatment with 40% and 80% of LC\textsubscript{50} (24h) while in case of (96h) of 40% and 80% of LC\textsubscript{50} AChE activity was also reduced to 49% and 30% of control respectively (Table 3; Figure 4). The acid phosphatase activity was reduced to 72% and 59% of control respectively after treatment with 40% and 80% of LC\textsubscript{50} (24h) while in case of (96h) of 40% and 80% of LC\textsubscript{50} acid phosphatase activity was also reduced to 55% and 40% of control respectively (Table 3; Figure 5). Alkaline phosphatase activity was reduced to 66% and 48% of control respectively after treatment with 40% and 80% of LC\textsubscript{50} (24h) while in case of (96h) of 40% and 80% of LC\textsubscript{50} alkaline phosphatase activity was also reduced to 59% and 30% of control respectively (Table 3; Figure 6).

Table 2: Changes in total protein, glycogen and total free amino acid in whole body tissue of \textit{Culex quinquefasciatus} larvae after 24h exposure to sub-lethal doses (40% and 80% of LC\textsubscript{50} of 24h) of synthetic pyrethroid of Cyperstar.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>40% of LC\textsubscript{50} (+, ±£)</th>
<th>80% of LC\textsubscript{50} (+, ±£)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(0.58 mg/L, 24hLC\textsubscript{50})</td>
<td>(1.16 mg/L, 24hLC\textsubscript{50})</td>
</tr>
<tr>
<td>Protein</td>
<td>24h</td>
<td>2.20±0.03 (100)</td>
<td>1.40±0.004 (64)</td>
</tr>
<tr>
<td>Glycogen</td>
<td>24h</td>
<td>1.70±0.004 (100)</td>
<td>0.85±0.006 (50)</td>
</tr>
<tr>
<td>Amino acid</td>
<td>24h</td>
<td>0.52±0.006 (100)</td>
<td>0.66±0.004 (127)</td>
</tr>
</tbody>
</table>

- Values are mean ±SE of six replicates.
- Values in brackets indicate percent biochemical activity with control taken as 100%.
- Doses are 40% and 80% of LC\textsubscript{50} for period for which animals were exposed.
- +, significant (P<0.05) when two way analysis of variance was applied to see whether enzyme inhibition was time and dose.
- £, significant (P<0.05) when Student ‘t’ test was applied between control and treated groups.

Table 3: Changes in acetylcholinesterase activity, acid and alkaline phosphatase activity in whole body tissue of \textit{Culex quinquefasciatus} larvae after 24h or 96h exposure to sub-lethal doses (40% and 80% of LC\textsubscript{50} of 24h) of synthetic pyrethroid of Cyperstar.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AChE activity (μm SH hydrolyzed/min/mg protein)</th>
<th>Acid phosphatase</th>
<th>μm-nitrophenol formed/30 min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE</td>
<td>24h</td>
<td>96h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.085±0.004 (100)</td>
<td>0.082±0.004 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.058±0.003 (68)</td>
<td>0.040±0.006 (49)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.041±0.004 (48)</td>
<td>0.025±0.005 (30)</td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>24h</td>
<td>96h</td>
<td></td>
</tr>
<tr>
<td>phosphatase</td>
<td>0.180±0.003 (100)</td>
<td>0.200±0.003 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.130±0.003 (72)</td>
<td>0.110±0.003 (55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.107±0.003 (59)</td>
<td>0.080±0.003 (40)</td>
<td></td>
</tr>
<tr>
<td>μm-nitrophenol formed/30 min/mg protein</td>
<td>24h</td>
<td>96h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.440±0.003</td>
<td>0.290±0.003</td>
<td>0.210±0.003</td>
</tr>
<tr>
<td></td>
<td>0.390±0.003</td>
<td>0.290±0.003</td>
<td>0.210±0.003</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>96h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(66)</td>
<td>(48)</td>
</tr>
<tr>
<td></td>
<td>0.460±0.003</td>
<td>0.220±0.004</td>
<td>0.140±0.003</td>
</tr>
</tbody>
</table>

- Values are mean ±SE of six replicates.
- Values in brackets indicate percent biochemical activity with control taken as 100%.
- Doses are 40% and 80% of LC₅₀ for period for which animals were exposed.
- +, significant (P<0.05) when two way analysis of variance was applied to see whether enzyme inhibition was time and dose.
- £, significant (P<0.05) when Student ‘t’ test was applied between control and treated groups.

**Figure 3** Bar diagram showing effect of Cyperstar (synthetic pyrethroid) on biochemical parameters of mosquito larvae at different concentrations.

**Figure 4** Bar diagram showing effect of Cyperstar (synthetic pyrethroid) on % activity of AchE activity at different concentrations at different time intervals.

* Values are mentioned in percentage.
* Doses are 40% and 80% of LC₅₀ for period for which animals were exposed.
* Significant (P<0.05) when two way analysis of variance was applied to see biochemical profile was time and dose dependent.
* Significant (P<0.05) when Student ‘t’ test was applied between control and treated groups.
Figure 5 Bar diagram showing effect of Cyperstar (synthetic pyrethroid) on % activity of Acid phosphatase activity at different concentrations at different time intervals.

Figure 6 Effect of Cyperstar (synthetic pyrethroid) on % activity of Alkaline phosphatase activity at different concentrations at different time intervals.

* Values are mentioned in percentage.
* Doses are 40% and 80% of LC$_{50}$ for period for which animals were exposed.
* Significant (P<0.05) when two way analysis of variance was applied to see biochemical profile was time and dose dependent.
* Significant (P<0.05) when Student ‘t’ test was applied between control and treated groups.

(B) Discussion-

In the present study cyperstar (synthetic pyrethroid) showed larvicidal activity of Culex quinquefasciatus mosquitoes. Exposure to sub-lethal doses of cyperstar against larvae of Culex quinquefasciatus significantly altered the level of total protein, total free amino acid, glycogen and enzyme activity of acetylcholinesterase, acid and alkaline phosphatase activity.

The protein acts as the next alternative source of energy to meet the increase energy demand. The reason of protein depletion in treated mosquito larvae of Culex quinquefasciatus may be due to their degradation and the possible utilization for metabolic activities. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains [28,29]. The decreased protein content resulted in destruction or necrosis of cells and consequent impairment in protein synthesis machinery [30]. The total free amino acids content showed a significant increase in whole body tissue of mosquito larvae exposed to cyperstar. The rise in total free amino acids level in the whole body tissue depicted high proteolytic activity. The accumulation of free amino acids can also be attributed to lesser use of amino acids [31] and their involvement in the equilibrium of an acid base balance [32]. Another reason for the rise of free amino acid level might be due to transamination and amination to keto acids. Stress conditions induce elevation in the transamination pathway [33]. In stress condition, carbohydrate content reduced to meet energy demand. The low glycogen content in body tissues of Culex larvae indicates its fast utilization for energy generation caused by cyperstar. Finally, glycogenolysis seems to be the result of increased secretion of catecholamine by the larvae in excess amount due to stress of plant extracts treatment [30] which depletes glycogen reserves [34]. Anaerobic and aerobic segments are two important components of carbohydrate metabolism. During anaerobic segment, breakdown of glucose or glycogen through glycolysis occurs while the next one consists oxidation of pyruvate to acetyl co-A to be
utilized through citric acid cycle [35]. Effect of synthetic pyrethroid on enzymes activity is one of the most important biochemical parameters, which affect physiology of body. When an organ is diseased due to toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured. Acetylcholinesterase, or acetyl-hydrolase, is a serine protease that hydrolysies the neurotransmitter acetylcholine. AChE found mainly at neuromuscular junctions and brain synapse, where its activity serves to terminate synaptic transmission. It belongs to carboxyl esterase family of enzymes. Enzyme alkaline phosphatase plays an important role in animal metabolism. Vorbrodt [36] has reported that this enzyme helps in the transport of metabolites across the membrane. The enzyme has been shown to be intimately associated with protein synthesis and is thus involved in the synthesis of certain enzymes [37].

IV. CONCLUSION

In conclusion, the larvicidal activity of the cyperstar is highly toxic to larvae of Culex quinquefasciatus mosquito. It significantly suppressed the population of the mosquito larvae by structural and functional action on insect. Sub-lethal doses of cyperstar significantly altered the level of protein, amino acids, glycogen, enzyme activity like acetylcholinesterase, acid and alkaline phosphatase activity of Culex quinquefasciatus larvae. Therefore the cyperstar (synthetic pyrethroids) may be of great value for the management of the vector say Culex quinquefasciatus larvae in aquatic stage in standard protocol and restricted manner.

REFERENCES