SUBLETHAL AND OVIPOSITION- DETERRENT ACTIVITY OF COMBINATION AGAINST MALARIA VECTORS

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Running Title: combination as ecofriendly larvicide

ABSTRACT

The plant extracts reduces the activities of the different developmental stages of the target organisms, therefore, they can be used as a growth inhibitors. The effect of phytochemicals on the inhibition of mosquito growth is also governed with their toxicity by mosquito species, plant species, plant parts, solvents used in extraction and fractions of the same solvent. Such plant extracts have, therefore, attracted the attention of the scientists involved in the vector control programmes. A range of pre emergent effects can occur; such as prolongation of instar and pupae durations, inhibition of larval and pupal molting, morphological abnormalities and mortality especially during molting and melanization processes. *Thus, the growth inhibiting activity of a phytochemical play an important role in the management of the mosquito borne diseases*

KEYWORDS- *Anopheles*, combination, imidacloprid, growth-inhibitor, *Ocimum*

INTRODUCTION

Insects constitute about 65% of living species and about 75% of animal species that's why they are the predominant group of living organisms on the earth. Numerically, there are about 1 million species as recorded until date. Yet the diversity could be far more, with estimate of as high as 30 million. They were evolved more than 350 million years ago and have adapted well to all kinds of vagaries of nature. This evolution has been both beneficial, harmful and indifferent to humans. Pest insects also serve as vectors of plant and animal diseases including those of humans. Millions of people suffer from diseases like malaria, dengue, filarial, chikungunya and plague wherein insects play a major role of transmission of causal organism. The use of chemical insecticides for controlling pests and vector of diseases is undesirable, save and yet effective methods of control are being sought. The use of natural products of plants origin is a new trend that preserves the environment and can be applied effectively. Botanical extracts, termed Insect Growth Regulators (IGRs), can have a pronounced effect on the developmental period, growth, adult emergence, fecundity, fertility and egg hatching resulting in effective control. Thus the growth inhibiting activity of a phytochemical may be essential to its uptake by mosquito control industries. Indeed, the rational application of exceptional phytochemicals may not only lead to new strategies, but may inhibit the development of insect resistance.

Identification of phytochemicals with growth inhibition properties combined with a considerable capacity to reduce adult emergence is the need of a phytogrowth inhibitor in vector management. Another desirable quality would be that a control agent induces IGR effects at dose lesser than the lethal dose so that recruitment is reduced over time.

No proper attention has been paid to study the effect of combination of synthetic insecticide and phytochemical on developmental profile of mosquito. Therefore, the detailed study on the impact of so evolved potent combination on the morphometric and developmental profile of anopheline mosquito was observed.

MATERIALS AND METHODS

TESTED COMPOUND

The tested plant material i.e. leaves of *Ocimum basilicum* were washed to avoid dusts and dirt then lift to dry under shade in the laboratory. Dried part of plant was crushed into small pieces and powdered. Hundred grams of the resulting powdered material of plant was exhaustively extracted with three solvents namely petroleum ether, carbon-tetrachloride and methanol, following the method described by [1].

Next to plant, neonicotinoid insecticide, imidacloprid (97.6% SL) provided by District Malaria Office, Circuit House, Agra (India), was also used for bioassay test against target.

TESTED MOSQUITO

Anopheles stephensi **(Culicidae: Diptera).**

Mosquito larvae at the 3rd-4th instars of a laboratory colony of the mosquito *Anopheles stephensi* were used

TOXICOLOGICAL STUDIES

According to WHO [2] bioassay test was performed with different concentration to assess the larvicidal activity.

JOINT ACTION OF SELECTED WASTE EXTRACTS

The selected most potent plant extract were mixed with selected synthetic insecticide to carry out synergistic activity against anopheline larvae. The combined action of the different mixtures was expressed as the co-toxicity factor and synergistic factor [3].

EFFECT OF SUBLETHAL TREATMENTS OF SELECTED PLANT EXTRACT AND IMIDACLOPRID ON DIFFERENT DEVELOPMENTAL STAGES OF *ANOPHELES STEPHENSI*:

For observing the effect of most potent combination identified on the different developmental stages of the target organism, twenty unhatched eggs of each mosquito species after observing under microscope were selected. These selected unhatched eggs were exposed to different concentrations ranging from 0.0025 to 0.15 ppm of the most potent combination. The percent egg hatched, larval emergence, larval mortality, larval period, pupal emergence, pupal mortality, pupal period and adult emergence were examined and observed the deformalities observed noticed in the developmental stages after every 24 hr. The larvae/pupae were transferred to same fresh concentrations of the combination after each 48 hours, until adult emergence. Experiments were set in triplicates along with control.

Saxena and Sumithra [4] used a simple formula to calculate a Growth Index (GI) based on emergence and the duration of the developmental period as follows:

$$GI = Ae \div Px$$

where Ae is the percentage of adult emergence and Px is the average developmental period.

OBSERVATIONS

LARVICIDAL ACTIVITY OF IMIDACLOPRID

The larvicidal potential of imidacloprid, LC50 values were 0.018 ppm and 0.009 ppm after 24 and 48 hours of treatment. LC90 values were 0.063 ppm and 0.030 ppm after 24 and 48 hours of treatment, respectively [3].

LARVICIDAL ACTIVITY OF *OCIMUM BASILICUM*

The data mentioned in Table 1 reveal that the crude petroleum ether extracts (PEE) of *O. basilicum* were the most effective as compared to their carbon tetrachloride (CEE) and methanol extracts (MEE). LC50 values of the PEE was 8.29 ppm and 4.57 ppm; LC90 values were 87.68 ppm and 47.25 ppm after 24 and 48 h of exposure, accordingly [1].

LARVICIDAL ACTIVITY OF COMBINATIONS

Among different combinations of PEE of *Ocimum basilicum* and Imidacloprid, combinatorial ratio 1:1 show highest toxicity with LC50 values 0.011 ppm and 0.007 ppm; LC90 values were 0.033 ppm and 0.019 ppm after 24 and 48 h of exposure, accordingly in table 2 & 3 [3].

IMPACT ON MORPHOMETRIC AND DEVELOPMENTAL PROFILE

The observations were made on the effect of most potent combination, imidacloprid and PEE of *Ocimum basilicum* on the morphometric and developmental profile of anopheline interpretated in table 4.

The average size of anopheline eggs was 0.495 mm in control along with airfloats at the same time as in treatment they were slightly increased in size 0.527 mm with partly damaged egg shell, dissolved cuticle and shaded airfloats. The hatching was initiated in treated eggs at low concentrations, 0.0025-0.0175 ppm but arrested beyond the concentration 0.025 ppm while in control there is no change (Plate 1 a, b).

The data depicted in table – 4 indicate that the percent hatching was 80.0% at 0.0025 ppm, 65.0% at 0.0075 ppm, 47.0% at 0.0125 ppm, 32% at .0175 and 94% in control. The hatching was completely arrested beyond 0.025 ppm. The larvae were emerged from control eggs be dark brown and cylindrical with average size 3.954 mm in addition to larvae emerged from combination were decreased in size to 3.252 mm show sluggish movement with peculiar coiling. Microscopic examination of dead larvae revealed that larval cuticle had was partly dechitinised in the abdominal region and the alimentary canal, heamolymphatic tissues and fat bodies were found significantly damaged, discarded bristles and disruption of alimentary canal (Plate 2 a, b). The larval mortality was 25.0% at 0.0025 ppm, 30.0% at 0.0075 ppm, 32.0% at 0.0125 ppm, 40% at 0.0175 ppm and 9.0% in control. The larval period was noted as 9 days at 0.0025 and 0.0075 ppm, 10 days at 0.0125 and 0.0175 ppm and 8 days in control.

The percent transformation of larvae into pupae was 75.0% at 0.0025 ppm, 70.0% at 0.0075 ppm, 68.0% at 0.0125 ppm, 60% at 0.0175 ppm and 91.0% in control. The pupae were comma shaped (,), dark brown with average length 3.527 mm during control and 4.129 mm during treatment conditions, slightly increased. Treated pupae had less sclerotization of cuticle, discarded bristles and disturbed trachea. Active histolysis of heamolymphatic tissues were observed but histogenesis was arrested (Plate 3 a, b). The average pupal period was 2 days at 0.0025 and 0.0075 ppm, 3 days at 0.0125 and 0.0175 ppm and 2 days in control conditions. The pupal mortality was 37.0% at 0.0025 ppm, 49.0% at 0.0075 ppm, 52.0% at 0.0125 ppm, 61% at 0.0175 ppm and 12.0% in control. The percent transformation of pupae into adults was recorded as 63.0% at 0.0025 ppm, 51.0% at 0.0075 ppm, 48.0% at 0.0125 ppm, 39% at 0.0175 ppm and 88.0% in control. The percent transformation of eggs into adults was 37.0% at 0.0025 ppm, 23.0% at 0.0075 ppm, 15.0% at 0.0125 ppm, 7% at 0.0175 ppm and 75.0% in control and the average developmental period was noted as 11 days at 0.0025 and 0.0075 ppm, 13 days at 0.0125 and 0.0175 ppm and 10 days in control. The growth index was observed as 3.7, 2.3, 1.15, 0.53 and 7.5 at the concentration 0.0025, 0.0075, 0.0125, 0.0175 and in control, respectively (Fig. 1).

DISCUSSION

The present investigation reveals that potent combination of imidacloprid and PEE of *Ocimum basilicum* induced abnormalities and prolongations during life cycle of exposed mosquito. These observations were supported by number of workers effort in vector control field.

The anopheline and culicine eggs hatching were arrested at higher concentrations of the combination without any change in control. These results supported by findings of [5] who evaluated the ovicidal activities of azadirachtin against *Cx. pipiens* and reported that at 5 and 10 ppm no hatching was recorded. In another study, [6] showed the ovicidal effects of various formulations of azadirachtin against the *Cx. tarsalis* and *Cx. quinquefasciatus* and revealed that 1 ppm of this formulation induced 100% mortality in eggs and interpretated that the ovicidal activity of the extract was influenced by concentration. The percent transformation of eggs into larvae was decreased with the increase in concentration in both anopheline and culicine mosquito.

Induction of morphogenic abnormalities in both mosquito larvae were marked by disorganization of body wall, bristles, alimentary canal, heamolymphatic tissues, fat bodies and tracheal network. These changes were generally attributed to neural or muscular disturbances, disturbed endocrine systems, arrest metabolic activities and disturbance in normal behavior physiology [7, 8, 9, 10, 11 and 12]. [13] recorded morphogenic abnormalities of mosquito larvae treated with plant extract were generally attribute to interference of the active ingredients with the endocrine system. [14] found larval mortality might be due to the chemical constituents present in extract that arrest the metabolic activities of the larvae and increase in turbidity at higher concentration might block the oxygen depletion to the larvae. The average larval period increases with increasing concentration of combination in both the larvae. This may be due to the interference in normal hormonal activity as interpreted by [9]**.**

The percentage of pupal transformation decreases with increase in concentration in both the cases. The negative trend in pupal emergence was corresponding to the positive trend in larval mortality. This observation was in consonance with the investigation with the findings of [10]**.** In another study [11] reported that disruption of growth of the larvae to pupae observed in this study may be the result of disturbances in the digestive process, which led to inadequate supply of nutrition to the larvae.

The pupal mortality generally take place through the structural damage was marked by loss of bristles, trachea, loose arthropodal joints and arrested histogenesis in both the mosquitoes. [11] studied impact of *Clerodendron inerme* on life cycle of *Ae. aegypti* and interpretated that the dead pupae showed less sclerotization of the cuticle compared to untreated ones, and in majority of the pupae, the head capsule remained attached to the pupal head.

The percent emergence of the adults from pupae was influenced on increasing the concentration of combination. The failure of adult emergence has been interpreted by [15] this effect may be due to interference in chitin synthesis. [4] reported that it is due to insufficient availability of chitin during metamorphosis which perhaps caused death since the insect was entangled in the weak integument.

The present morphometric and developmental studies predicts that the effect of combination on the morphogenesis and development of mosquitoes is similar in the mosquitoes exposed to different phytoextracts as reported by [16, 13, 17, 18, 19, 20, 10, 11, 12]. Thus, this combination contributes larvicidal, pupicidal and ovicidal activity against the mosquitoes, as a result in an extension of the duration of development by decreased egg hatchability, increased larval and pupal mortality and decreased adult emergence. The present finding has important implications in the practical control of mosquito population to facilitate suppress vector borne diseases and beneficially applied for future vector control program.

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TABLE.1. TOXICITY OF DIFFERENT LEAVE EXTRACTS OF *OCIMUM BASILICUM* AGAINST ANOPHELINE LARVAE

Solvent extract	Exposure Period (hrs.)	Chi-square	Regression equation	LC_{50}±SE (Fiducial limits) (ppm)	Relative toxicity irrespective of time period	LC_{90}±SE (Fiducial limits) (ppm)	Relative toxicity irrespective of time period
Carbon tetra-chloride	24	3.77	1.89X-1.47	268.61±40.28 (347.56-189.66)	58.78	1282.45±501.34 (2265.07-299.83)	27.14
		2.12	2.34X-	143.85±26.54	31.48	507.80±95.17	10.75

	48		2.39	(195.87-91.84)		(694.33-321.27)	
Methanol	24	26.34	4.06X-9.82	446.61±31.76 (508.86-384.36)	97.73	923.60±140.33 (1198.64-648.56)	19.55
	48	19.69	3.53X-7.67	384.84±30.70 (445.01-324.66)	84.21	887.00±139.01 (1159.46-614.54)	18.77
Petroleum ether	24	3.70	1.25X+2.60	8.29±1.92 (12.05-4.52)	1.81	87.68±34.35 (154.99-20.36)	1.86
	48	6.02	1.26X+2.90	4.57±1.24 (6.99-2.15)	1.00	47.25±16.01 (78.61-15.88)	1.00

TABLE.2. TOXICITY OF DIFFERENT COMBINATIONS OF IMIDACLOPRID WITH PETROLEUM ETHER CRUDE EXTRACT OF *OCIMUM BASILICUM* AGAINST ANOPHELINE LARVAE

Combinations	Exposure Period (hrs.)	Chi-square	Regression equation	LC_{50}±SE (Fiducial limits) (ppm)	Relative toxicity irrespective of time period	LC_{90}±SE (Fiducial limits) ppm	Relative toxicity irrespective of time period
1:1	24	1.162	1.504X+6.401	0.011±0.002 (0.016-0.006)	1.57	0.033±0.027 (0.137-0.028)	1.73
	48	0.424	1.667X+6.838	0.007±0.0019 (0.011-0.004)	1.00	0.019±0.012 (0.071-0.021)	1.00
1:2	24	2.497	1.451X+6.324	0.012±0.0028 (0.017-0.006)	1.714	0.040±0.0343 (0.160-0.025)	2.105
	48	0.764	1.520X+6.605	0.008±0.002 (0.013-0.004)	1.142	0.021±0.0194 (0.099-0.013)	1.105
1:4	24	2.251	2.047X+6.422	0.0201±0.003 (0.026-0.014)	2.857	0.0653±0.022 (0.129-0.041)	3.436
	48	3.574	2.113X+6.618	0.017±0.002 (0.022-0.012)	2.428	0.059±0.016 (0.102-0.036)	3.105

TABLE.3. CATEGORIZATION OF DIFFERENT COMBINATIONS OF IMIDACLOPRID WITH PETROLEUM ETHER CRUDE EXTRACT OF *OCIMUM BASILICUM* AGAINST ANOPHELINE LARVAE

Ratio	Analysis with LC_{50} values				Analysis with LC_{90} values		
	Exposure (Hours)	Co-toxicity coefficient	Combined factor	Nature of action	Co-toxicity coefficient	Combined factor	Nature of action
1:1	24	163.63	1.636	S	190.90	1.90	S
	48	128.57	1.285	S	157.89	1.578	S
1:2	24	150.00	1.50	S	157.50	1.575	S
	48	112.5	1.125	S	150.0	1.50	S
1:4	24	90.00	0.90	A	96.92	0.96	A
	48	52.94	0.529	A	60.00	0.60	A

Table 4. EFFECT OF THE COMBINATION OF IMIDACLOPRID AND PETROLEUM ETHER LEAVES EXTRACT OF *OCIMUM BASILICUM* ON DEVELOPMENT AND GROWTH INDEX OF *ANOPHELES STEPHENSI*

S.No.	Concentration (ppm)	Eggs Treated	% transformation of eggs into larvae	Average larval period (days)	% Larval mortality	% transformation of larvae into pupae	Average pupal period (days)	% pupal mortality	% transformation of pupae Into adults	% transformation of eggs into adults	Average developmental period (days)	Growth Index*
1.	0.0025	20	80.00	9	25.00	75.00	2	37.00	63.00	37.00	11	3.7
2.	0.0075	20	65.00	9	30.00	70.00	2	49.00	51.00	23.00	11	2.3
3.	0.0125	20	47.00	10	32.00	68.00	3	52.00	48.00	15.00	13	1.15
4.	0.0175	20	32.00	10	40.00	60.00	3	61.00	39.00	7.00	13	0.53
5.	0.025	20	-	-	-	-	-	-	-	-	-	-
6.	0.075	20	-	-	-	-	-	-	-	-	-	-

- *Statistical evaluation carried out by using student 't' test ($p<0.001$) compared to the control
- (-) Development arrested

7.	CONTROL	20	94.00	8	9.00	91.00	2	12.00	88.00	75.00	10	7.5

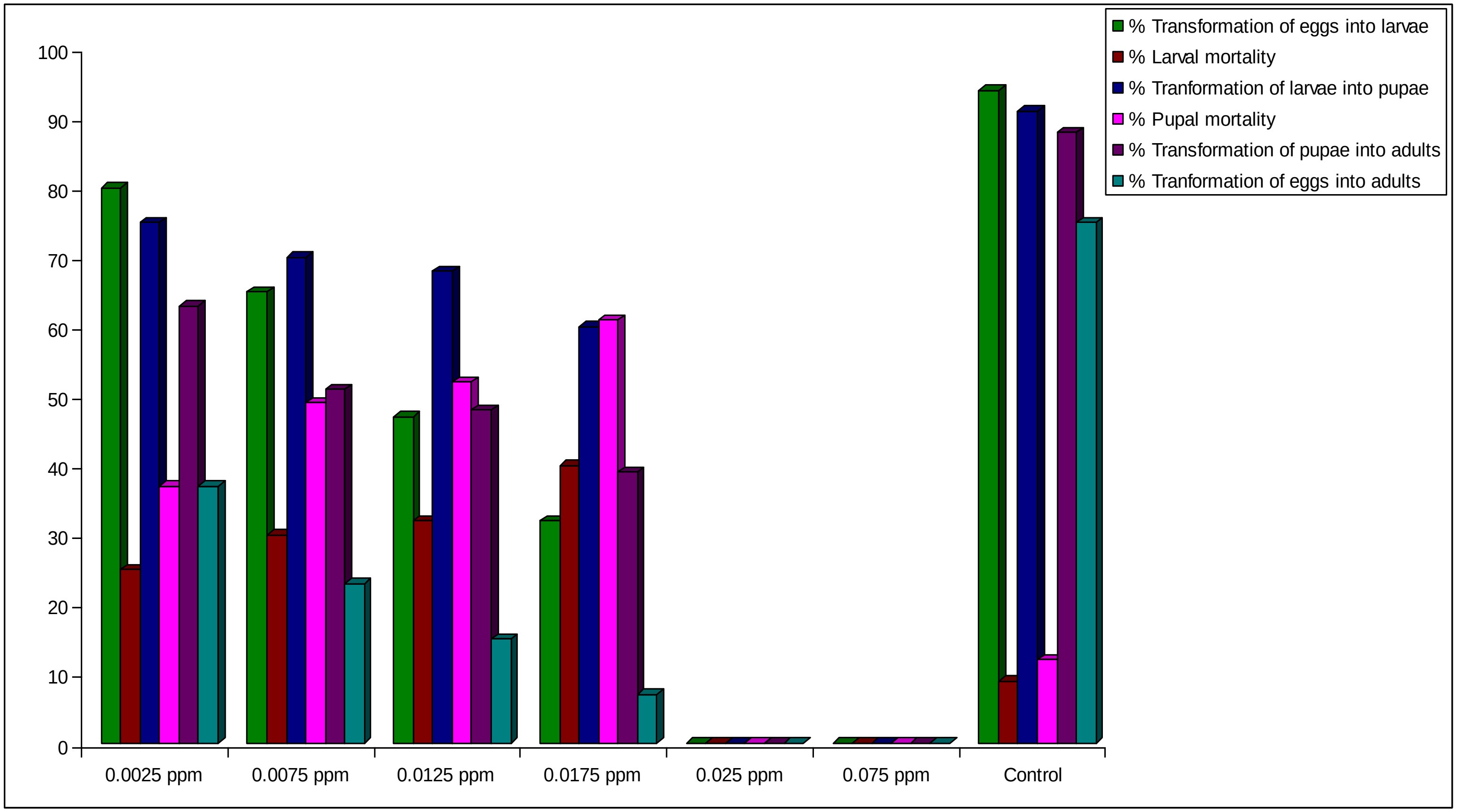


FIGURE. 1. IMPACT OF THE COMBINATION ON THE LIFECYCLE OF ANOPHELINE MOSQUITO.

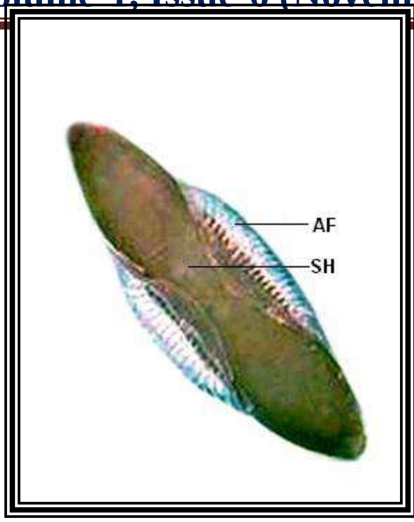


PLATE-1a
AF-Air floats, SH-Shell
***Anopheles* egg (Control)**

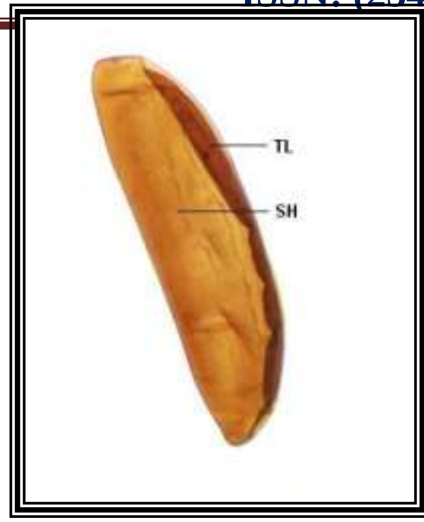


PLATE-1b
SH-Shell
***Anopheles* egg (Treated)**

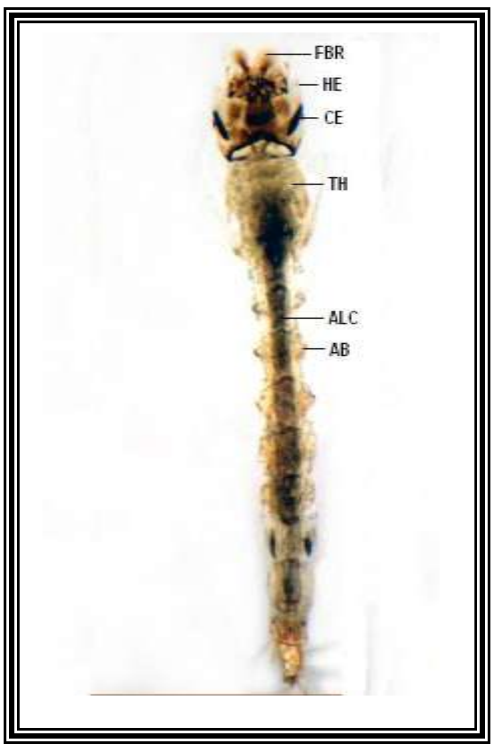


PLATE-2a
CE-Compound eye, TH-Thorex,
ALC-Alimentary canal, AB-Abdomen
***Anopheles* larvae (Control)**

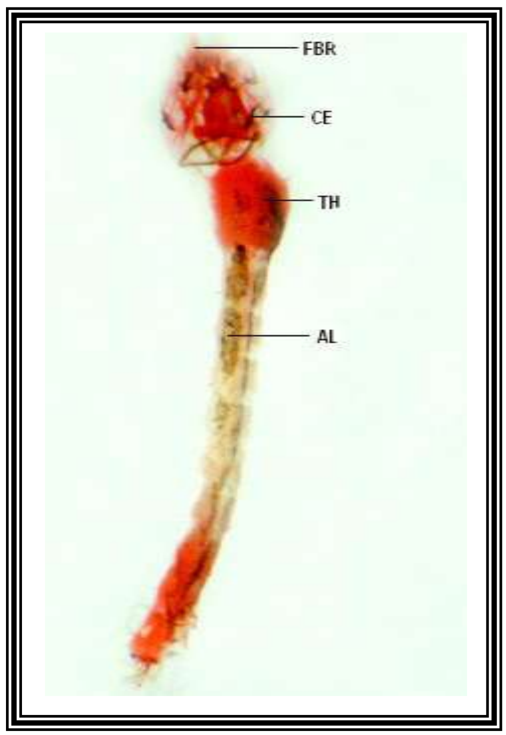
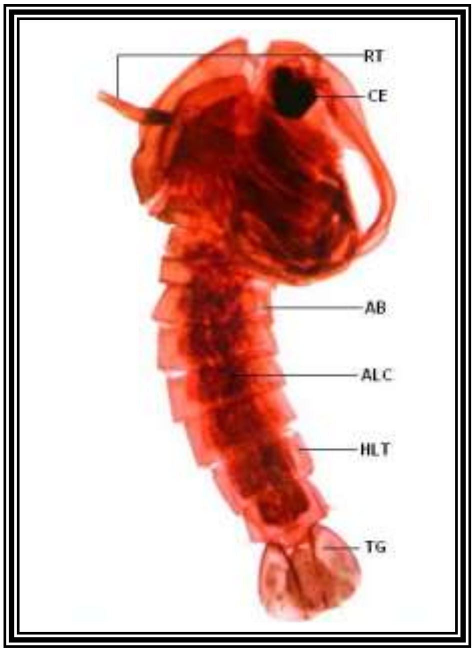


PLATE-2b
CE-Compound eye, TH-Thorex,
ALC-Alimentary canal, AB-Abdomen
***Anopheles* larvae (Treated)**



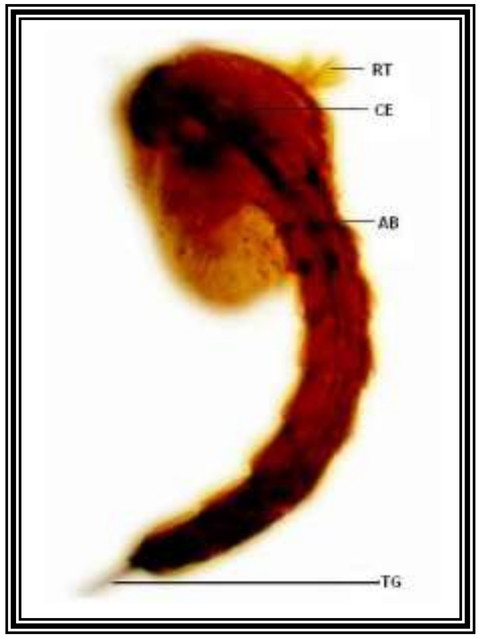


PLATE-3a
CE-Compound eye, AB-Abdomen ,
ALC-Alimentary canal
Anopheles **pupa (Control)**

PLATE-3b
CE-Compound eye, AB-Abdomen
Anopheles **pupa (Treated)**