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Efficacy and the disruptive effects of amalgamation on development and morphogenesis of the *Culex quinquefasciatus* (Culicidae: Diptera)

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Abstract

Phytolarvicides represent a prosperous store for the discovery of novel pesticides that are effective, cheap and environmentally safe. The main targeted insect pests are mosquito vectors of most serious human and animal diseases in the world, such as the mosquito *Culex quinquefasciatus.* The frequent cases of filariasis incidences are leading cause of illness and death in urban areas and *Cx. quinquefasciatus* is a primary vector for its transmission in India**.** Bioassays showed that larvae are highly susceptible to the selected plant extract, *Ocimum basilicum* and synthetic insecticide, imidacloprid as well as their combinations. Histopathological examination showed that 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, synthetic insecticides and the active ingredients in plant extracts have important effects on physiological processes of the mosquito, therefore, could implicate its life cycle and vectorial capacity for disease transmission and thus, present new source for the development of insecticides against mosquitoes and other vectors.

Keywords: Culex quinquefasciatus, Ocimum basilicum, imidacloprid, mortality, growth, development, morphogenesis

1. Introduction

Mosquito versus men have never ending fight, wherein mosquito always get upper hand due to underestimation of this tiny creature over centuries, as man could land on Mars but failing to overcome to this. These blood-feeding mosquitoes transmitted deadly vector-borne diseases, such as malaria, filariasis, dengue hemorrhagic fever and Japanese encephalitis, they are increasing in prevalence worldwide, particularly in tropical and subtropical regions. *Culex quinquefasciatus* is a vector of lymphatic filariasis, which affects 120 million people worldwide, and approximately 400 million people are at risk of contracting filariasis, resulting in an annual economic loss of 1.5 billion dollars [1]. Lymphatic filariasis is a serious public health problem in India, constituting one third of the infected population in the world [2]. Control of mosquito is essential as they constitute an intolerable biting nuisance. Biotechnologists and entomologists agree that mosquito control efficiency should be with selectivity for a specific target organism. New control methodologies aim at reducing mosquito breeding sites and biting activity by using a combination of chemical–biological control methods soothing several advocated biocontrol methods to reduce the population of mosquito and to reduce the man–vector contact [3]**.** Recently, there has been a major concern for the promotion of botanicals as environment friendly pesticides, microbial sprays, and insect growth regulators amidst other control measures such as beneficial insects and all necessitate an integration of supervised control [3-8]. With the progress of phytolarvicides research, many laboratories around the world have investigated around the world have investigated their anti-juvenile activity.

Insect growth regulators (IGRs) have received a great deal of attention as so-called "Third-generation insecticides [9]. These compounds including insect juvenile hormone mimics and other compounds controlling the insect development have mode of action disports from other insecticides and low toxicity against non-target organisms. The use of IGRs is increasing of controlling various insects of agricultural, horticultural, stored product and public health pests. There are number of research paper deals with the toxicological, developmental and morphogenic effects of synthetic insecticides and phytoextracts. But 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 culicine mosquito was observed in present investigation.

2. Materials and Methods

2.1 The Experimental Material

2.1.1 Synthetic Insecticide

The imidacloprid (97.6% SL) provided by District Malaria Office, Circuit House, Agra (India), were used as experimental synthetic insecticide for assessing the impact of combination on life cycle of culicine mosquito after studying its bioassay test against *Culex quinquefasciatus* mosquito larvae.

2.1.2 Phytoextract

Leave extracts of the medicinal plant, *Ocimum basilicum* (Plate.1) were tested for their bioefficacy against culicine larvae, thereafter, petroleum ether extract has been found more potent than other extracts tested [10].

Therefore, petroleum ether extract of the *O. basilicum* was selected as experimental phytoextract for trace its impact in combination with imidacloprid on culicine mosquito lifecycle.

2.1.3 Combination

The bioefficacy of different combinations of synthetic insecticide, Imidacloprid and crude petroleum ether leave extract of *Ocimum basilicum* against culicine larvae is shown that the combinatorial ratio 1:1 has been highly toxic as compared to other ratios.

2.1.4 Target Organism
For observing the effect of most potent combination (i.e. 1:1) on the different developmental stages of the target organism, twenty unhatched eggs of culicine mosquito species after observing under microscope were selected (Plate. 2).

2.2 Methodology
The selected unhatched eggs were exposed to different concentrations ranging from 0.0025 to 0.075 ppm of the 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 occurred during 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 [11]. used a simple formula to calculate a Growth Index (GI) based on emergence and the duration of the developmental period as follows:
GI = Ae ÷ Px
Where Ae is the percentage of adult emergence and Px is the average developmental period.

3. Results
The effect of synthetic insecticide, imidacloprid; phytoextract, *Ocimum basilicum;* combination ratio 1:1 and developmental & morphogenetic indices were screened against target culicine mosquito and the obtained results can be assorted as follows.

3.1 Lethal effects
3.1.1 Synthetic insecticide, Imidacloprid
The susceptibility of insecticides were represented as LC_{50} values 0.022±0.004 with 0.031 and 0.014 ppm upper and lower fiducial limits and 0.014±0.004 ppm with 0.022 and 0.006 ppm upper and lower fiducial limits and LC_{90} values were 0.117±0.040 with 0.19 and 0.03 ppm upper and lower fiducial limits and 0.105±0.041 ppm with 0.187 and 0.023 ppm upper and lower fiducial limits after 24 and 48 hours of exposure, respectively (Table.1).

3.1.2 Phytoextract, Petroleum ether extract (PEE) of *Ocimum basilicum*
The PEE of leaves of *Ocimum basilicum* were observed more effective with LC_{50} values of 10.06±2.69 ppm with 15.28 and 4.71 ppm upper and lower fiducial limits, 6.06±1.80 ppm with 9.60 and 2.53 ppm upper and lower fiducial limits after 24 and 48 hrs. Of exposure, respectively. Subsequently, LC_{90} values were 129.32±53.48 ppm with 234.15 and 24.49 ppm upper and lower fiducial limits after 24 hrs. and 65.68±21.37 with 107.57 and 23.78 ppm upper and lower fiducial limits after 48 hrs. of exposure against culicine larvae, respectively (Table.1).

3.1.3 Combination (ratio 1:1) of imidacloprid with Petroleum ether extract (PEE) of *Ocimum basilicum*
The combinatorial bioassay of the ratio 1:1 have LC_{50} 0.014±0.002 ppm with 0.020 and 0.008 ppm being upper and lower fiducial limits and LC_{90} 0.067±0.034 ppm with 0.164 and 0.030 ppm upper and lower fiducial limits after 24 hours and LC_{50} was 0.010±0.002 ppm and LC_{90} was 0.058±0.017 along with 0.014 and 0.005 ppm upper and lower fiducial limits for former and 0.091 and 0.024 ppm for later after 48 hours of exposure period, respectively (Table.1).

3.2. Effects on development and morphogenesis
The observations were made on the effect of combination, imidacloprid and *Ocimum basilicum* on the morphometric and developmental profile of target organism interpretated in table 2 and Fig.1. During developmental metamorphosis, time taken for total egg, larval and pupal developmental time (in days), per cent larval and pupal mortality and adult emergence inhibition were recorded.
Culicine eggs were brownish and cigar shaped in raft with average size in control and treated was 0.398 mm and 0.453 mm, respectively, slightly increased (Plate.3 a,b). Treated eggs were slightly damaged shell and dissolved cuticle. The hatching was initiated in treated eggs at low concentrations but arrested beyond the concentration 0.05 ppm while in control there was no change.
The developmental data of culicine mosquito are mentioned in table –2. The data indicate that the percent hatching was 75.0% at 0.005 ppm, 68.0% at 0.007 ppm, 59.0% at 0.009 ppm, 30.0% at 0.025 ppm, and 80% in control. The hatching was completely arrested beyond 0.075 ppm. The larvae of culicine were dark in color and having respiratory siphon in control with average size 4.124 mm. On the exposure to so evolved combination, the culicine larvae shows shrinkage and the average body size reduced to 3.986 mm. Treated larvae show some abnormality in their movement, they were found to be less active or may be inactive. The body wall was sclerotized, lacking melanization, damaged alimentary canal and tracheae, degerated compound eyes, disturbed bristles and destroyed heamolymphatic tissues (Plate 4 a, b). The larval mortality was 30.0% at 0.005 ppm, 65.0% at 0.007 ppm, 82.0% at 0.009 ppm, 100.0% at 0.025 ppm and 17.0% in control. The larval period was noted as 8 days at 0.005, 9 days at 0.007 and 0.009 ppm, and 8 days in control conditions. In 0.025 larval mortality was 100% after 4 days, therefore, no larval period was mentioned.
The percent transformation of larvae into pupae, 70.0% at 0.005 ppm, 35.0% at 0.007 ppm, 18.0% at 0.009 ppm and 83.0% in control. The average pupal period was 2 days at 0.005 and 0.007 ppm, 3 days at 0.009 ppm and 2 days in control conditions.The pupae of culicine were comma shaped (,) and dark brown with an average length of 3.789 mm and 4.542 mm in control and treated, respectively. The pupae molted from treated larvae were inactive and slightly increased in size. The body wall was slightly damaged along with loose arthopodal joints and histolysis was initiated but histogenesis was restricted (Plate. 5a,b). The pupal mortality was 18.0% at 0.005 ppm, 20.0% at 0.007 ppm, 30.0% at 0.009 ppm and 19.0% in control. The percent transformation of pupae into adults was recorded as 82.0% at 0.005 ppm, 80.0% at 0.007 ppm, 70.0% at 0.009 ppm and 81.0% in control. The percent transformation of eggs into adults was 43.0% at 0.005 ppm, 19.0% at 0.007 ppm, 7.0% at 0.009 ppm and 53.0% in control and the average developmental period was noted as 10 days at 0.005 ppm, 11 days at 0.007 ppm, 12 days at 0.009 ppm and 11 days in control. The growth index was observed as 4.3, 1.9, 0.56 and 4.82 at the concentration 0.005, 0.007, 0.009 ppm and in control, respectively.
Further, this combination reduces the overall performance of mosquito vector by arresting egg hatchability, egg-larval-pupal period prolongation and decrease adult emergence. This extract has, therefore, been more suitable to environment because the concentration needed for mosquito larval mortality has no

negative impact on certain non-targets; they need highest concentration to show any side effect.

4. Discussion

The effect of selected combinatorial ratio on the growth and morphogenesis of the mosquitoes are remarkably greater than those reported for other plant extracts in the literature.

The culicine eggs hatching were arrested at higher concentrations of the combination without any change in control. These results supported by findings of Soliman and Tewfick [12]. 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, Su and Mulla [13]. 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. Muthukrishnan *et al*. [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 Supavaran *et al*. [15].

For example 50% inhibition of the emergence of the adult mosquitoes was observed by the use of *C. inophyllum*, *S. suratense*, *S. indica* and *Rhinocanthus nasutus* leaf extracts [16]. The Meliaceae plant family is used as growth regulator against many insect pests [17, 18, 19, 20, 21, 22]**.** The growth regulatory effect is the most important physiological effect of *M. azedarach* on insects. It is because of this property that family Meliaceae has emerged as a potent source of insecticides. Exposure of *A. stephensi* larvae to sub-lethal doses of neem leaves extract in the laboratory prolonged larval development, reduced pupal weight and ovipostion [13]. In the field, delayed phenology of surviving larvae and reduced pupal weight are common occurrence after treatment with neem [23]**.** Furthermore, Dhar *et al*. [24] revealed that exposure to neem extract suppressed rather than inhibited oviposition in mosquitoes.

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 [25, 26, 27, 28, 29, 30, 31] recorded morphogenic abnormalities of mosquito larvae treated with plant extract were generally attribute to interference of the active ingredients with the endocrine system.

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 Sharma [29]**.** In another study Patil *et al*. [30] 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. Patil *et al*. [30]. 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 Saxena and Yadav [32]**.** this effect may be due to interference in chitin synthesis. Saxena and Sumithra [12]. 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 Attri and Prasad (1980), Zebitz (1986), Bhagwan *et al*. (1995), Koul and Shanker (1995), Dhar *et al*. (1996), Jayaprakasha *et al*. (1997), Shaalen *et al*. (2005), Patil *et al*. (2006) and Kuppusamy and Murugan (2008) [33, 24, 34, 35, 25, 36, 37, 30, 31].

Elimam *et al*. [38]. Experimental results reveal that the crude extract of *R. communis* possesses remarkable larvicidal, adult emergence inhibition and oviposition deterrent properties against *Anopheles arabiensis* and *Culex quinquefasciatus* and can be used as biological control means. Gunasekaran *et al*. [39, 40]. Results indicated that because of its emergence inhibition activity, NeemAzal T/S 1.2 per cent EC could be a promising candidate for the use in integrated vector management (*Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*) programme and replace chemical insecticides.

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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