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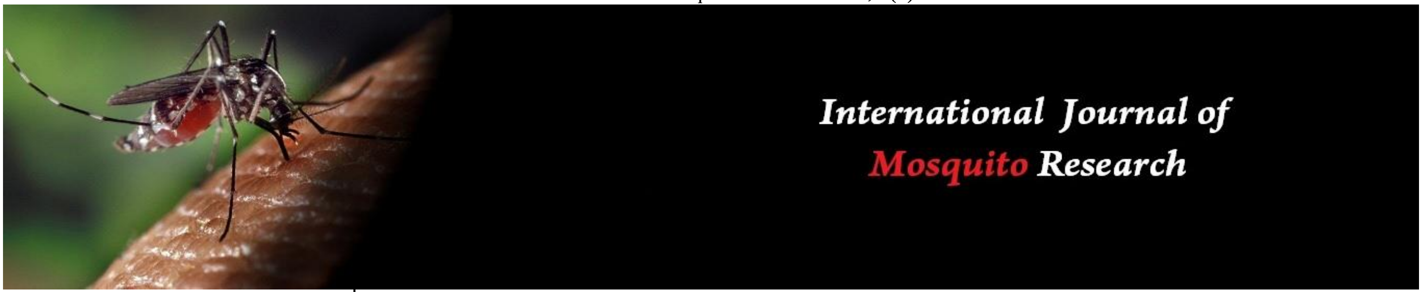

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Comparative studies of three potent bioagent against mosquito larvae

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Abstract
Mosquitoes are menace to human for centuries with their capability to spread dreaded diseases like malaria, dengue, filariasis etc. the control or managing them is the first priority of the humans ever since the cause of such diseases has been revealed. The usual ways to control include the spraying of insecticides or fumigation. Utilization of predacious insect larvae as well as the mosquito larvae are eco-friendly, sustainable and cost effective methods for the purposes. In the present study three insect larvae viz, Odonata (*Sympatrum* sp.), Hemiptera (*Diplonychus* sp.) and Diptera (*Lutzia tigripes*) were reared in the laboratory to check their efficacy on controlling the mosquito larvae efficiently. The larvae feeding them were the *Culex* species larvae. To find out the insect consuming larvae significantly more than others Turkey's HSD for 3 means and 2 means are calculated and is found to be 61.19 and 48.79 respectively. Comparing the differences between the means, all means are found to be significantly different. The insect consuming larvae significantly than others is Odonata. In the same way, to find out the instar of the larvae at which insects consume them in significant amount Turkey's HSD is calculated for 4, 3 and 2 means and they are 79.77, 70.66 and 56.34 respectively. Comparing the differences in the means with these values, it is found that insects consume larvae at 4th instar significantly more than the larvae at other stages. Out of the three larvae for managing or even controlling the mosquito larvae, *Lutzia* larvae might be the most efficient candidate for releasing in wild to control for they can survive and well adapted to the any habitats whether polluted or non-polluted but the Odonata and Hemiptera prefer somewhat non polluted aquatic habitats having moderate amount of dissolved oxygen. The main focus of the study is to select a bioagent that has no habitat boundary or constrain and nature of aquatic habitat. Hence the efficient candidate for controlling the mosquitoes of a particular habitat will be to utilize natures' best weapon against the mosquitoes that is *Lutzia tigripes.*

Keywords: Predacious insects, Odonata, Hemiptera, *Lutzia tigripes*, mosquito larvicides, management

1. Introduction

Mosquitoes are menace to human for centuries with their capability to spread dreaded diseases like malaria, dengue, filariasis etc. The control or managing them is the first priority of the humans ever since the cause of such diseases has been revealed. Efforts of managing in worldwide yet jet-linked world remains on the edge of resurgence and out- breaks of old and new mosquito-borne disease epidemics [1, 2]. Biological control is most suitable in this context as few attempt for introduction of biological agents like larvivorus fishes i.e. gambusia and guppy have been successful [3], bacteria [4, 5], fungi [6, 7] and predatory mosquitoes of subgenera *Mucidus* [8], *Culex fuscanus, Toxorhynchites* spp. [9, 10] to control *Aedes aegypti and Culex quinquefasciatus* larvae [11]. Utilizing biological organisms to control mosquito larvae is not only eco-friendly, but constitutes a means by which more effective and sustainable control can be achieved.

Among the predacious insects promising as biological control are Hemipteran, Odonata and Diptera (*Lutzia tigripes*) predators which are cosmopolitans and locally available. The backswimmers (Family: Notonectidae) are the most common bugs preying upon mosquito larvae, important factor in reducing immature mosquito population and considered promising in mosquito control. The role of hemipteran predators in controlling mosquito larvae has been recognized since 1939 in New Zealand, when stock troughs with *Anisops assimilis* were found to be free of mosquitoes whereas puddles in depressions surrounding the troughs contained mosquitoes [12].

Bay [13] found that almost 100% of mosquito emergence was prevented in field-situated, screened, 100 gallon fiberglass tubs with one square meter of water surface and *Notonecta unifasciata* compared to more than 12000 adult mosquitoes emerged from the control tubs. For instance, emergent vegetation in ponds and other water bodies provide partial protection for mosquito immatures. This effect was experimentally investigated and confirmed by Shaalan [14] and Shaalan *et al.* [15] whereas predation potential of *Anisops* and *Diplonychus* bugs was significantly reduced by the presence of vegetation. Although the costs of colonization and mass production, coupled with the logistics of distribution, handling and timing of release at the appropriate breeding site, impede the use of notonectids in mosquito control [16], results of a recent study for mass rearing and egg release of the predatory backswimmer *Buenoa scimitar* for the biological control of *Cx. quinquefasciatus* were impressive [17]. The dragonfly larvae of *Trithemis annulata scortecii* were intense and active predators when used to control mosquito larvae, especially *Anopheles pharoensis*, in irrigation channels in Gezira Province, Sudan [18]. Bay [13] reported that dragonfly larvae are known to prey heavily on bottom feeder mosquitoes like *Aedes* larvae. Sebastian *et al*. [19] found that complete elimination of all *Ae. aegypti* larvae and pupae were achieved between day 4 and 9 depending on the density of aquatic stages of mosquitoes present per container when dragonfly larva, *Labellula sp*., was used, Sebastian *et al*. [20] conducted a pilot field study, involving periodic augmentative release of predaceous larvae of a dragonfly, *Crocothemis servilia*, to suppress *Ae. aegypti* during the rainy season in Yangon, Myanmar. Chatterjee *et al*. [21] found that significant decrease in *An. subpictus* larval density in dipper samples was observed 15 days after the introduction of *Brachytron pretense* dragonfly larvae in concrete tanks under field conditions in India. Similarly, the larvae of 5 odonate species *Aeshna flavifrons*, *Coenagrion kashmirum*, *Ischnura forcipata*, *Rhinocypha ignipennis* and *Sympetrum durum* in semifield conditions in West Bengal, India, significantly lowered the mosquito larval density in dipper samples after 15 days from the introduction, followed by a significant increase of larval mosquito density after 15 days from the withdrawal of the larva [22]. These results [18, 19, 20 21, 22] are suggestive of the use of odonate larvae as potential biological agent in regulating the larval population of mosquito vectors. Breene *et al*. [23] found no mosquito larvae in the gut of the larvae of the damselfly *Enallagma civile.* Biology, colonization and potential of *Toxorhynchites* mosquitoes as a biological control agent of vector mosquitoes are fully covered by Collins and Blackwell [24] while Garcia [25] discussed the difficulties associated with such methodologies which prevent more widespread utilization of arthropod predators. In addition to *Toxorhynchites* mosquitoes, the predaceous characters of *Culex* (Subgenus *Lutzia*) mosquitoes were reviewed by Pal and Ramalingam [26]. Lacey ad Orr [27] limited their discussion to insect predators that are used as biological control agents in integrated vector control to *Notonecta* and *Toxorhynchites* species. Mogi [28] reviewed insects and invertebrate predators based on adult, egg, larval and pupal mosquito predation beside possibilities of using such predators for mosquito control. Quiroz-Martinez *et al*. [29] discussed the arthropods (insects, mites and spiders) that prey on mosquito larvae and considerations for the success of these predators in mosquitoes' biological control programs.

The present article reports the predation on mosquito, particularly larvae using three predacious insects viz. Odonata, Hemiptera and larval predacious mosquito *Lutzia tigripes* in the laboratory by feeding on *Culex* larvae. The effectiveness of three are quite similar but regarding the field released and adaptability to different habitat the

2. Materials and Methods

Two predacious larval forms of insects and one mosquito larva known for its predacious habits were selected for the study. They were identified in accordance with the available references and expertise. For feeding *Culex* larvae were collected from surrounding habitat. For easy observation and feeding small washing dishes were used for the experiments. The mosquito larvae were counted before putting into and duration and stage of the insect were thoroughly recorded throughout the experiment.

To find out the insect consuming larvae significantly more than others Turkey's HSD for 3 means and 2 means were calculated.

Fig 1: The predacious immature insects used in the present study (Odonata-A, Hemiprtera –B and *Lutzia tigripes* mosquito predating on the *Culex* larva-C). *Laccotrephes ruber* predates on the Hemipteran and Odonata nymphs (D) neutralizing the ill effect of the pathogens.

3. Results

The predacious insects and larvae, in the present study effectively consumed or killed the mosquito larvae. Above all these insects were lastly killed by *Laccotrephes ruber* predates on the Hemipteran and Odonata nymphs neutralizing the ill effect of the pathogens (fig. 1). The Odonata and *Lt. tigripes* consumed whole of the mosquito larvae while the hemipteran larvae killed the mosquito larvae by sucking fluids. The efficacy of the predators is shown in figure 2. The efficacy is tested statistically comparing the three insects.

3.1 Statistical results

To find out the insect consuming larvae significantly more than others Turkey's HSD for 3 means and 2 means were calculated and was found to be 61.19 and 48.79. Comparing the differences between the means, all means were found to be significantly difficult. The insect consuming larvae significantly than others was Odonata. In the same way, to find out the instar of the larvae at which insects consume them in significant amount Turkey's HSD was calculated for 4, 3 and 2 means and they were 79.77, 70.66 and 56.34 respectively. Comparing the differences in the means with these values, it was found that insects consume larvae at 4th instar significantly more than the larvae at other stages.

Table 1: 24-hour observation on consumption pattern of three different insects viz., Odonata, Hemiptera and Dipteran on different stages (instars) of mosquito larvae.

Instar of Insects	Odonata				Hemiptera				Diptera			
	Replication				Replication				Replication			
	1	2	3	4	1	2	3	4	1	2	3	4
1st												
2nd												
3rd												
4th												

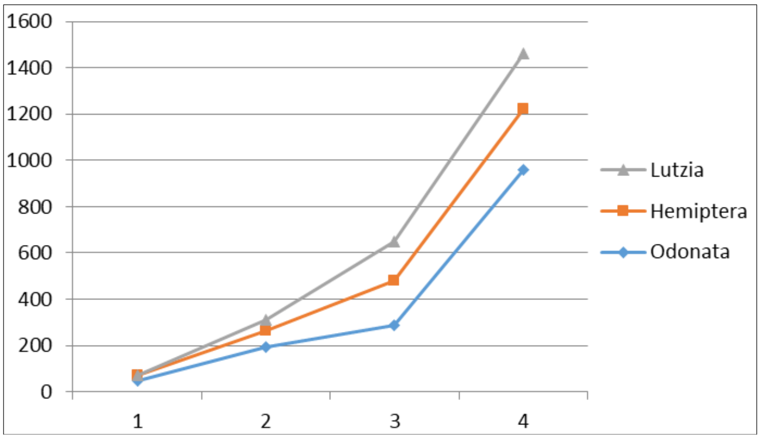

Fig 2: The comparative efficiencies of three predacious insects and data were extracted from Table 1 (Data not shown).

Analysis of variance two-way classification with 4 observations per cell:
The null hypotheses to be tested are:
H01: the different types of insects consume larvae equally.
H02: there is no significant difference in the consumption of larvae in respect of different instars
H03: there are no significant influences of the different types of insects on different instars of larvae.
For testing the above three hypotheses we use analysis of variance two-way classification with four observations per cell and its ANOVA was given as below:

ANOVA of two-way classification
Interaction SS= Between Groups SS – (SS Instar + SS Insects)
= 1972506.67- (1164912.00+426834.67)
= 1972506.67- 1591746.67
= 380760.00
or
Interaction SS = $\{(360^2/4 + 288^2/4 + 0^2/4 + \ldots\ldots\ldots\ldots\ldots + 1896^2/4)\}$ – CF – SS Instar + SS Insect)

Two-way ANOVA

Source of variation	SS	df	MS	F	P value
Insects	426834.67	2	213417.34	67.08*	<0.05
Instars	1164912.00	3	388304.00	122.06**	<0.01
Interaction	380760.00	6	63460.00	19.95**	<0.01
Within groups	114528.00	36	3181.33		

Total 2087034.67 – (4X3X4) -1
= 48-1
=47
* Significant at 5% level of significance
** Significant at 1% level of significance

4. Discussion

Stav *et al*. [30] reported that the predaceous dragonfly larvae of *Anax imperator* produced 52% reduction in *Cs. longiareolata* oviposition in outdoor artificial pools. Odonata larval stages are large that requires large space and might polyphagous and might be in efficient for mosquito control besides they prefer somewhat clean non polluted aquatic habitat. Hemipteran are of sucking type, slow consumer and whole larvae are not consuming so pathogens might be present on the mosquitoe larvae. This made somewhat deficient as biocontrol agent despite beig able to live in both polluted and non-polluted aquatic habitats. *Lutzia* on other hand having chewing type and voracious eater. Short instar periods of 2.5 days for each moulting in *Lutzia* against 4 to 6 days of other two larval stages seems to be no effect on the efficacy on predating the mosquito larvae (Fig. 2).

Although these are successful examples of predators, there are difficulties associated with rearing; colonization and handling which are obstacles to a more widespread utilization of predaceous aquatic insects [25]. The second difficulty is polyphagy that has advantages and disadvantges [31]. An advantage is that these predators can survive when mosquito larvae are rare or absent, while a disadvantage is that they may not reduce mosquito larvae due to availability of alternative preys. The third difficulty is the presence of other invertebrates and vertebrate predators that may reduce the abundance of the predaceous insects [32]. The fourth difficulty is predators may interfere through chemical or other cues; for instances the hydrophilid *Tropisternus lateralis* [33] and the phantom midge *Chaoborus albatus* [34], avoid laying eggs in pools with fish. The fifth difficulty is the avoidance by mosquitoes of water containing invertebrate predators such as backswimmers and dragonflies and makes predator's impact more complicated. Lee [35] found that mosquito larvae are consumed more than pupae by predators and assumed that this was due to the inclination of pupae to exhibit rapid tumbling action when startled. Contrarily, both bugs of family belostomatidae (Order: Hemiptera) and *Toxorhynchites* mosquito larvae have an advantage over the other aquatic predaceous insects that restrict their prey selection to the larval instars only. This is worthy of note, in particular for mosquito vectors of diseases, since pupal reduction directly reduces mosquito emergence and subsequent disease transmission.

Introduction of predatory mosquito species is one of the targeted approaches for control of immature forms of mosquitoes. *Lutzia (Metalutzia) fuscana* is one of such species of mosquito whose larvae is reported to feed upon vector species larvae i.e *Anopheles*, *Aedes* and *Culex* species in several parts of India [36, 37]. Its predatory habit was found to be excellent yet non-targeted in the *mesic* environmental conditions due to availability of several outdoor breeding habitats. Stable breeding of *Lutzia* (Metalutzia) *fuscana* has been first time reported from this arid zone [38]. The genus of predatory mosquito *Lutzia fuscana* has been elevated from *Culex* to *Lutzia* (earlier *Lutzia* was subgenus) earlier *Culex (Lutzia) fuscanus* (Wiedmann, 1820) is now *Lutzia* (Metalutzia) *fuscana* (Wiedmann) [39].

5. Conclusions

To find out the insect consuming larvae significantly more than others Turkey's HSD for 3 means and 2 means are calculated and is found to be 61.19 and 48.79. Comparing the differences between the means, all means are found to be significantly different. The insect consuming larvae significantly than others is Odonata.

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The three larvae for managing or even controlling the mosquito larvae, *Lutzia* might be the most efficient/effective candidate releasing in wild to control for it is inhabitable to the any habitats whether polluted or non-polluted but the Odonata and Hemiptera prefer somewhat non polluted aquatic habitats. The main focus of the study is to select a bio agent that has habitat boundary or constrain and nature of aquatic habitat. Hence the efficient candidate for controlling the mosquitoes of a particular habitat will be to utilize natures' best weapon against the mosquitoes that *Lutzia tigripes.*

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