Article

Predatory habits of *Lutzia* (Metalutzia) *fuscana* (Wiedmann) (Diptera: Culicidae) in the arid environments of Jodhpur, western Rajasthan, India

Himmat Singh, **Robin Marwal**, **Anusha Mishra**, **Karam Vir Singh**
Desert Medicine Research Centre, New Pali Road, Jodhpur, India -342005
E-mail: hspawar@rediffmail.com

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Abstract
The stable breeding of *Lutzia* (Metalutzia) *fuscana* was recorded form different locations of Indian Desert the "*Thar*" for the first time. The species being predatory in its larval form was investigated for evaluation of its biological control aspect in the desert setup where breeding sites and prey species are limited. Though its predatory habit is established yet using it as biological controlling agent was not found promising due to untargeted approach due to unlimited outdoor breeding places in sub-humid climatic conditions in rest of India. Whereas in desert due to limited water sources, mosquito vectors share the available breeding niche this increases possibility of targeted biological control using predatory species. Laboratory experiments on predatory habit of *Lutzia* (Metalutzia) *fuscana* showed that it preferred *Aedes aegypti* larvae most (88.5%), *Anopheles stephensi* (47.5%) and *Culex quinquefasciatus* larvae (39.0%). Average consumption of daily larvae is 18.89 larvae/day. If colonized properly and released in controlled conditions they can be useful in controlling of socially protected and unattended breeding containers resulting reduction in mosquito population.

Keywords *Lutzia* (Metalutzia) *fuscana*; predatory; biological control; desert.

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1 Introduction

Vetorborne disease burden has increased considerably worldwide in recent decades, globally cases have increased from few millions to several billions per year (WHO Report, 2010). During the year 2010 - 2011 in India alone the vector-borne disease cases were about 781603 including 1390 deaths which included 745599 malaria cases with 233 deaths, 14047 cases with 93 deaths of dengue, 14820 cases of chickungunya and 7137 cases with 1064 deaths reported due to Japanese encephalitis (National Vectorborne Disease Control Program Report, 2011).

One of the effective methods of control over vector-borne transmission is through reduction in density of vector population through various chemical means but now it is known that continuous use of chemicals for control is associated with the emergence of insecticide resistance, this has necessitated exploring and opting for new alternate methods.

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 (Chandra et al., 2008) bacteria (Dua et al., 1993; Kumar et al., 1995; Indranil et al., 1997, Shukla et al., 1997; Biswas et al., 1997), fungi (Chandrahas and Rajagopalan, 1979; Roberts and Strand, 1977, Poopathi and Tyagi, 2006) and predatory mosquitoes of subgenera *Mucidus* (Mattingly, 1961), *Culex fuscanus* (Ikeshoji, 1966; Panicker et al., 1982) *Toxorhynchites* spp. to control *Aedes aegypti and Culex quinquefasciatus* larvae (Gerberg and Visser, 1978; Focks et al., 1982; Sempala, 1983; Ramalingam and Ramakrishnan, 1971; Mogi and Chan, 1996). 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 (Geetha, et al. 1982; Panicker et al., 1982). 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 (Singh et al., 2013).

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) (Tanaka, 2003). We have also used the same in present communication.

The present study is a laboratory-based study conducted in Vector Biology Laboratory of Desert Medicine Research Centre, Jodhpur, Rajasthan. We envisage to explore the predatory species preference and potential of *Lutzia* (Metalutzia) *fuscana* and possibility of its being utilized in mosquito control in the limited outdoor breeding habitats and in community containers which are socio-culturally protected for cattle drinking, looking into the predatory habits of *Lutzia* (Metalutzia) *fuscana* larvae, laboratory experiments were carried out on feeding capacity and preference on immature stages of Culex, Anopheles and Aedes species. The results are presented through this communication.

2 Material and Methods

Larval collection were carried out at seven selected study areas of urban and peri-urban localities of Jodhpur city (Jhalamand, Khema ka Kunwa, Mandore, Nagori Gate, Salawas and Nehru Park) during 2010-11 on monthly basis. Collections were carried out at various domestic and peri-domestic water storing containers, cemented tanks, ponds, ditches, gardens and green complexes with parks, stored/ stagnated water, community tanks for cattle drinking and artificial fountains in the parks.

Larvae were collected using white enamel bowls (4 cm diameter) with classical dipping method. The larvae collections were kept in white enamel tray (20 x 15 x 3 cm) in laboratory at 26- 27C^o

Three main breeding species of mosquito vectors in the region i.e. *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*, were used for determining the feeding preference of *Lutzia* (Metalutzia) *fuscana* (other sympatric mosquitoes species of seasonal occurrence and of less significance as disease vectors were not included in the study). However, for the daily consumption of larvae in predatory activity and feeding preference on different stages of mosquito larvae, laboratory reared *Aedes aegypti* larvae were used. Only early IVth stage larvae of predatory species were included in the present experiments to avoid any bias of the different size and age of larvae.

For estimating species preference, equal number (50 larvae of IVth Instar) of larvae each of *Aedes*, *Culex* and *Anopheles* were provided to larvae of *Lutzia* (Metalutzia) *fuscana* larvae by keeping them in one container enamel tray (20 x 15 x 3 cm) for feeding with equal opportunity. The observations were made after 24hrs of each experiment. After 24 hrs *Lutzia* (Metalutzia) *fuscana* larvae were taken out of the tray and transferred to the fresh tray, the remaining prey species larvae in the tray were separated and counted. The experiment was repeated again with fresh set of 150 larvae (50 of each species) with the available larvae of predator in the following day. The experiment was run for four days.

To estimate feeding preference of fourth stage larvae of *Lutzia* (Metalutzia) *fuscana* on different instars i.e. I,II,III,IV and pupae of *Aedes aegypti*, 50 larvae (10 larvae of each larval stage of *Aedes aegypti*) were provided to predatory larvae in enamel trays (10 x 5 x 1.5 cm). Experiments were conducted in duplicates (Experiment I and II) each day and repeated for five days. Different instars of *Aedes*, *Culex* and *Anopheles* larvae used to feed the larvae of *Lutzia* (Metalutzia) *fuscana* were laboratory reared and were obtained from the insectary of Vector Biology Laboratory, Desert Medicine Research Centre, Jodhpur.

Pupae were excluded from the experiments except for larval stage preference studies. During the experiment in insectary the larvae were maintained under optimum temperature condition of 26-27 ^{o}C. Observations were made after 24hrs of each day of experiment using visual counting method by separating out the predatory larvae from the experimental trays. Average weight of IVth instar *Ae. aegypti* was calculated by weighting 25 larvae separately on electronic balance for biomass calculation. Chi square (χ^2) was used as test of significance and calculated on the actual values.

3 Results

Lutzia (Metalutzia) *fuscana* were recorded form seven locations, six from urban localities (Jhalamand, Nagauri Gate, Khema ka kunwa, Mandore, Nehru park and Jalori Gate) and one from rural locality (Salawas). Nine species of mosquito alongwith one species of predator were collected during field survey. Occurrence of *Culex quinquefasiatus* (20.4%), *Anopheles stephensi* (20.07%) and *Aedes aegypti* (17.5%) were higher than other occurring species (Table 1).

Table 1 Species composition of study sites and larval collection habitat.

Study Sites (Larvae Collection Sites)	*An. stephensi*	*An. culicifacies*	*An. subpictus*	*An. pulchirrimus*	*An. annularis*	*Aedes aegypti*	*Aedes vittatus*	*Aedes albopictus*	*Culex quinquefasciatus*	*Lutzia* (Metalutzia) *fuscana*	Habitat
NEHRU PARK	25.0	0	5.6	0	0	55.6	2.8	0	5.6	5.6	Fountain water , *Azadirachta indica* tree shade
JHALAMAND	31.6	13.2	15.8	0	2.6	18.4	13.2	0	2.6	2.6	Extra domiciliary cement tanks, with shade of *Prosopis julifora* tree
KHEMA KA KUAN	25.0	8.3	25.0	0	0	8.3	0	0	16.7	16.7	Cement tank under *Ficus religosa* tree shade
MANDOR	34.2	13.2	2.6	5.3	2.6	7.9	5.3	10.5	13.2	5.3	Fountain stream under *Ficus religosa* tree shade
NAGAURI GATE	10.1	3.0	5.1	2.0	2.0	12.1	18.2	2.0	37.4	8.1	Cemented Cattle tank, *Ficus religosa* tree shade
JALORI GATE	13.5	16.2	13.5	5.4	5.4	10.8	8.1	8.1	13.5	5.4	Cement tank, *Ficus religosa* tree shade
SALAWAS	21.4	14.3	14.3	0	0	7.1	7.1	0	28.6	7.1	Cemented Cattle tank, *Prosopis juliflora* tree shade
Total	**20.1**	**8.0**	**8.8**	**2.2**	**2.2**	**17.5**	**10.9**	**3.3**	**20.4**	**6.6**	

*Values in percent.

The breeding of *Lutzia* (Metalutzia) *fuscana* were found maximum in Cemented tanks which were found under shades of trees like *Ficus religosa* and *Prosopis juliflora* (Table 1).

The predatory larvae of *Lutzia* (Metalutzia) *fuscana* were found from July to March except three months from April to June in these locations (Fig. 1). It is observed that high temperature and low RH of summer did not support outdoor breeding and in addition to this nearly drying of the water bodies and other water collection results in reduction of prey community during these months. After onset of monsoon the predatory mosquito larvae population were observed to appear again and its density increases with increase in the density of prey mosquito species belonging to genera *Aedes, Anopheles* and *Culex* (Fig. 1).

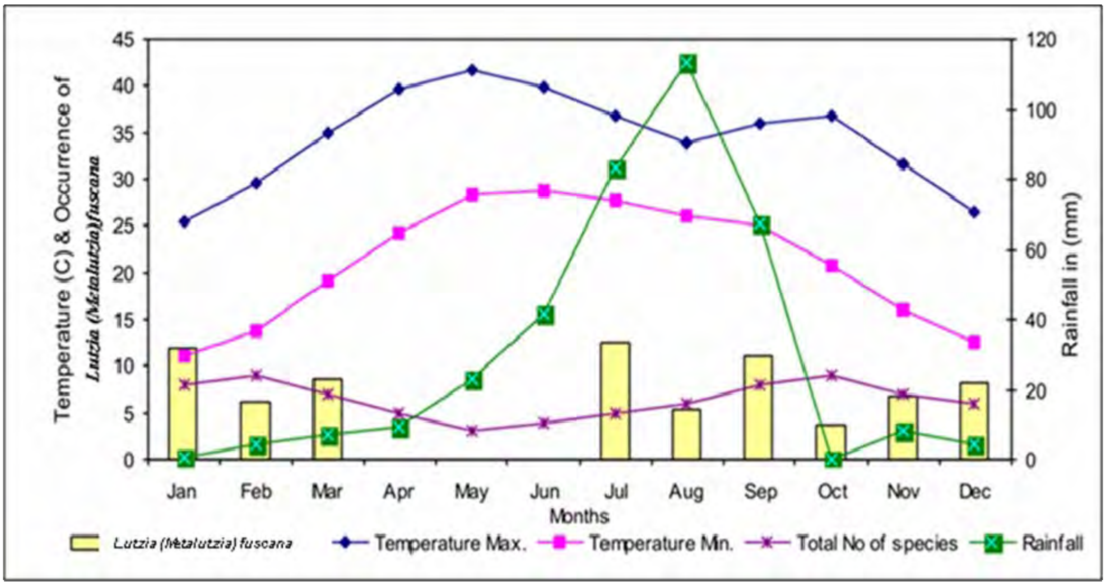

Fig. 1 Monthly occurrence of *Lutzia* (Metalutzia) *fuscana* with respect to rainfall and temperature (Singh et al., 2013).

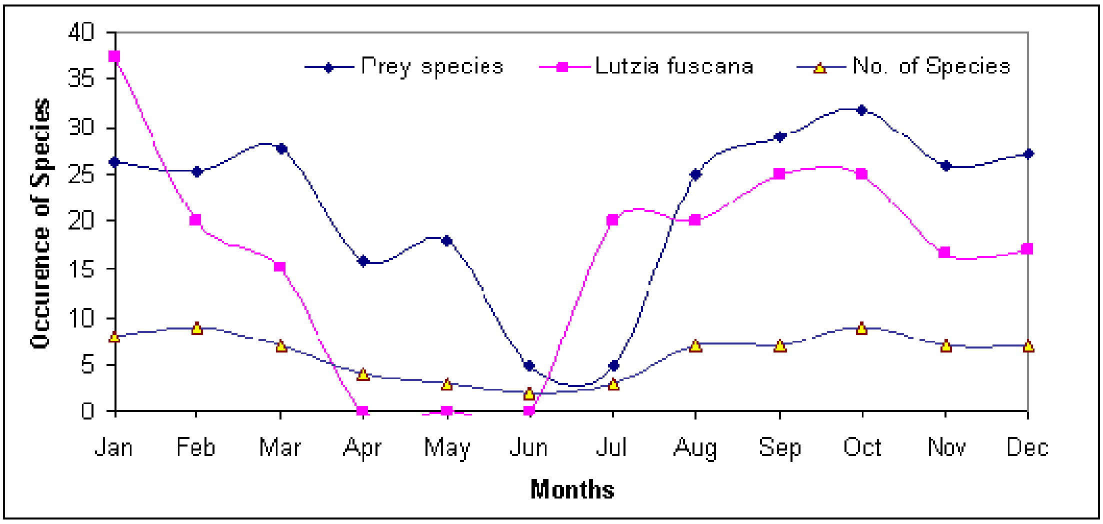

Fig. 2 Monthly occurrence of *Lutzia* (Metalutzia) *fuscana* with other prey species.

The occurrence of *Lutzia* (Metalutzia) *fuscana* is also associated with density of prey population in breeding sites, frequency of occurrence of prey species during the hotter months of June and July was found very low (Fig. 2).

The larvae of predator shared niche with *Ae. aegypti, Ae. albopictus, Ae. vittatus, An. stephensi, An. culicifaces, An. subpictus* and *Cx. quinquefaciatus larvae*. The predatory larvae mainly found in peri-domestic cemented containers which were under shades of large trees like Peepal (*Ficus religosa*), Neem (*Azadirachta indica*) and Kikar (*Prosopis juliflora*).

Occurrence of predatory larvae species was found high with *Cx. quinquefaciatus* (11time out of 13 times when predator was found in the cement tanks) followed by *Ae. aegypti* (10 times out of 13) and *Ae. vittatus* (9 out of 13 times in cement tanks) though breeding of *An. stephensi* was high in cement tanks but the occurrence with *Lutzia* (Metalutzia) *fuscana* was very low (4 out of 13 observations of predator species) (Table 2).

Table 2 Showing details of occurrence of different species larvae with *Lutzia (*Metalutzia*) fuscana*.

Breeding sites	*An. stephensi*	*An. culicifacies*	*An. subpictus*	*An. pulchirrimus*	*An. annularis*	*Ae.aegypti*	*Ae. vittatus*	*Ae. albopictus*	*Cx quniquefasiatus*	*Lutzia fuscanus*	*No. of Obs.*
cement tanks	22 (4)*	3 (0)	10 (0)	0 (0)	1 (0)	28 (10)	21 (9)	0 (0)	29 (11)	13	**47**
Fountains	24 (3)	7 (3)	9 (1)	1 (0)	5 (0)	2 (1)	1 (0)	3 (0)	11 (2)	3	**32**
River bed	2 (0)	2 (0)	0 (0)	0 (0)	1 (0)	2 (0)	0 (0)	0 (0)	2 (0)	0	**3**
Stone quarry	5 (0)	4 (0)	3 (0)	0 (0)	0 (0)	2 (0)	2 (0)	0 (0)	3 (0)	0	**7**
Pond	0 (0.0)	0 (0)	0 (0)	0 (0)	1 (0)	1 (1)	0 (0)	0 (0)	1 (1)	1	**1**
Water pit	1 (0.0)	0 (0)	1 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	1 (0)	0	**2**

*Figures in parenthesis are occurrence of species with *Lutzia* (Metalutzia) *fuscana* larvae in same breeding spot

The result showed that *Lutzia* (Metalutzia) *fuscana* preferred *Ae. aegypti* larvae the most (50.6%, χ^2= 31.20 , p≥ 0.001 at df =2) which was found significantly higher than *An. stephensi* larvae (27.1%, χ^2 = 4.02 at df =2) and *Cx. quinquefasciatus* (22.3%, χ^2= 12.8 , p≥ 0.001 at df =2) (Table 3).

When single predator (IVth stage) larvae was given the choice to feed on different stages of larvae of *Ae. aegypti* (50 larvae, 10 of each instar i.e. I,II, III, IV and pupae). The preference of feeding was on fourth instar larvae (45.5%, χ^2= 54.33, p≥ 0.001 at df = 4), followed by third instar (33.5%, χ^2= 15.29, p≥ 0.01) and the least preferred larvae were Ist instar (1.8%, χ^2 = 27.66, p≥ 0.01 at df = 4). Although pupae were not consumed by predator larvae but were found dead may be due to attack by predator causing mortality 2.99 % (Table 4).

Average consumption of larvae by predator (IVth Instar) per day was found to be about 18.4 larvae (using IVth instar larvae of *Ae. aegypti* as they were most preferred) (Table 5). On the other hand using mixture of equal number of three species larvae (*Aedes*, *Anopheles* and *Culex*) the average consumption per day was about 18.4 larvae (Table 3) which was almost similar to the consumption using single species larvae feeding experiment (Table 5).

Predatory larvae consumed about 37.21 mg biomass per day (calculated using mean daily consumption multiplied by the average weight of live *Ae. aegypti* (n=25, mean weight = 1.96±0.22*)*.

Table 3 Species wise feeding preference of *Lutzia* (Metalutzia) *fuscana* on mosquito vector species.

	No of predatory larvae (IV - Instar)	150 larvae provided, 50 larvae (IVth instar) of each species in each experiment						Total consumption
		Ae. aegypti	% Preference	*An. stephensi*	% Preference	*Cx. quinquefasiatus*	% Preference	
Exp-1	4	43	55.1	15	19.2	20	25.6	78
Exp-2	6	48	42.9	40	35.7	24	21.4	112
Exp-3	5	48	61.5	13	16.7	17	21.8	78
Exp-4	4	38	46.3	27	32.9	17	20.7	82
Total	19	177	50.6	95	27.1	78	22.3	350
Chi Square (X^2) at df=2 (Calculated on Actual values)		31.2**		4.02		12.81*	Avg. consumption/larvae-350/19=18.4/perday	

** Significant at p<0.001; * Significant at p< 0.01

Table 4 Estimation of predatory preference of *Lutzia* (Metalutzia) *fuscana* on different larval stages of *Ae. aegypti* larvae.

		No of Larvae of *Ae. aegypti*	Instars-wise comnsuption (10 larvae of each instars)					
			Ist	IInd	IIIrd	IVth	Pupae	Larvae consumed
DAY 1	EX. I	50	0	1	7	6	0	14
	Ex II	50	0	0	5	10	1	16
DAY 2	EX. I	50	0	4	7	8	0	19
	Ex II	50	0	2	4	7	0	13
DAY 3	EX. I	50	1	2	5	7	1	16
	Ex II	50	0	4	7	8	0	19
DAY 4	EX. I	50	1	3	7	8	1	20
	Ex II	50	0	5	4	6	1	16
DAY 5	EX. I	50	0	4	6	8	0	18
	Ex II	50	1	2	4	8	1	16
Total		**500**	**3 (1.8)**	**27(16.17)**	**56 (33.53)**	**76 (45.51)**	**5 (2.99)**	**167 (33.4)**
Chi Square (X^2) df=4			**27.66****	**1.59**	**15.02***	**43.08****	**28.06****	

Values in parenthesis are percent consumption; ** Significant at p<0.001; * Significant at p< 0.01

Table 5 Feeding capacity estimation of *Lutzia* (Metalutzia) *fuscana* on IV instar larvae of *Ae. aegypti*.

Exp.	**No. of** *Lt. fuscana* **IVth instar larvae**	**No. of *Aedes* larvae provided**	**No. of larvae Cons-umed**	**No. of larvae left**	**No. of larvae pupated**	**Average Consumption per Larvae**
Day 1	8	150	150	0	0	18.75
Day 2	6	150	119	30	1	19.83
Day 3	4	150	76	64	10	19
Day 4	2	150	36	97	17	18
Total	20	600	381	191	28	19.05

4 Discussion

There have been several reports on its feeding behavior (Jin et al., 2006). The predatory activity of these larvae was observed during entomological surveys in rest of the places other than the desert part of India. This is also a first report of presence of this species in the desert scenario. Presence of stable population of *Lutzia* (Metalutzia) *fuscana* is indicator of stability of vector species (*Aedes, Anopheles* and *Culex* species) on which its larvae prey upon in this desert climate.

Except for three months (April to June) it is found throughout the year with stable reemerging population which also signifies the stability of this predatory mosquito in this fragile and hostile climate of desert.

It was interesting to note that *Lutzia* (Metalutzia) *fuscana* preferred *Ae. aegypti* over *Cx. quinquefasciatus* as reported in other part of India (Ikeshoji, 1966; Panicker et al. 1982; Singh et al. 1984; Thangam and Kathiresan, 1996; Mariappan et al., 1997; Yanovisk, 2001), This changed preference of feeding of this predator becomes very important for bio-control of *Ae. Aegypti* in this part of western Rajasthan as *Ae aegypti* is major vector for Dengue and DHF. The possible change in the feeding preference might be due to niche sharing by both prey and predator species (*Aedes aegypti* and *Lutzia* (Metalutzia) *fuscana*). A study by Shrama et al. (2008) on surveillance of *Aedes aegypti* in Jodhpur showed cement tanks as the most important breeding containers for *Aedes* breeding. Our study revealed that *Lutzia* (Metalutzia) *fuscana* also prefer to breed in the same types of cement containers which are pri-domestic placed under shades of big trees for cattle drinking The second reason may be due to limited availability of conducive outdoor breeding containers might bound the mosquito species to share the common niches. Similar observations on the *Aedes* preference is recently reported in Sri-lanka (Sinnathamby et al., 2013).

Lutzia (Metalutzia) *fuscana* is container and tree hole breeder (Belkin, 1962; Jackson, 1953, Tanaka, 2003; Rattanarithikul et al., 2005) and breeds in the containers where other mosquitoes are breeding as they serves food for this mosquito larvae, though cannibalism is obligatory (Hopkins, 1952; Edwards, 1941). Our preliminary observation seems to be promising for biological control in important mosquito breeding containers in part of western Rajasthan.

The study by Jackson (1953) in west Nigeria showed that the density of *Lutzia* (Metalutzia) *fuscana* larvae is dependent on the density of other larvae available i. e. one larvae of *Lutzia* (Metalutzia) *fuscana* per 60 to 90 other larvae of either species of *Aedes, Anopheles* or *Culex* and this was found to be true in this part of desert also.

Results on feeding behavior shows that single IVth instar predator larvae of *Lutzia* (Metalutzia) *fuscana* can eat an average of 18.8 larvae per day of IVth instar larvae of *Aedes aegypti* and the average biomass consumption was 37.21 mg/day which was found considerably lower in contrast to the consumption in sub humid areas of south east coast of India where biomass consumption was reported 76.0 mg/day (Thangam and Kathiresan, 1996). Present study have shown that in case of western Rajasthan where water resources are limited but socio cultural practices of water storing have stabilize mosquito breeding of vectors of dengue and malaria. In addition to this in recent past increased supply of water through Indira Gandhi Canal water to city have increased overall ambience of Jodhpur city and has resulted in creation of gardens and increased larger trees might have attracted this tree hole / container breeder predatory mosquitoes to this part of desert, on the other hand due to limited breeding sources available, the sharing of species might have changed the preference of *Lutzia* (Metalutzia) *fuscana* from *Cx. quinquefasciatus* (Thangam and Kathiresan, 1996) to *Ae. aegypti* in this part of desert.

The dynamics of species occurrence in the desert of *Lutzia* (Metalutzia) *fuscana* is correlated with other species occurrence during the year it can be a tool for focused biological control if colonized properly and released in controlled conditions. They can cover a lot of unattended key breeding containers and resulting reduction in mosquito population (Hopkins, 1952; Edwards, 1941) this biological control method seems more suitable and focused in this setting of desert condition . Although some reports show that there may be lack of interaction between larvae of mosquito vectors and their natural enemies and/or lower predator survivorship in certain habitats, particularly shallow water pools and cement tanks (Das et al., 2006) and urban environments such as temporal habitats (Carlson et al., 2004). A study by Jin et al. (2006) through gut content analysis of *Lutzia* (Metalutzia) *fuscana* showed chironomidae larvae 78.6% and only 2.5% larvae of mosquitoes in province of China whereas in this arid part of India where chironomids are not common in appearance, as arid climate do not support much to them, the possibility of prey choice increases towards mosquito larvae in the desert part due to less habitat availability the species interaction may occur more frequently

It was observed that ability of larvae consumption increases as larvae grew up and passes stages up to IVth instar larvae (Appawu et al., 2000). It was also interesting to note that predatory larvae attacks on mostly on its equal sized prey larvae by attacking them at the joint of head and thorax (Jin et al., 2006).

Ikeshoji (1966) used larvae of *Lutzia* (Metalutzia) *fuscana* to control *Cx quinquefasciatus* larvae in small ditches in simulated field conditions.

Introduction of such predatory species to those public tanks which are filled for cattle and other animals to drink water and community do not accept use of insecticide in these key can prove to be very useful. Secondly in desert there are limited water resources and very less available breeding containers as a result of that species of mosquito show niche sharing which might have changed this predatory species feeding preference from *Culex* to *Aedes* species. Therefore the predatory species becomes more targeted in absence of large outdoor breeding sources.

Environmental methods and biological control are alternatives to chemical control and are key components of the integrated strategy which may go hand in hand to the National Vector Control Program. The successful implementation of these organisms depends on an in-depth understanding of the ecology of both the targeted species and the biological controlling predators.

Introduction of prey comes under environmental management strategies that can reduce or eliminate vector breeding through use of biological controls that target and reduce vector larvae without generating the ecological impacts of chemical use. The approach is cost-effective, ecological balanced and sustainable for vector control if used in this type of climatic condition where mostly outdoor breeder larvae are restricted in pockets.

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