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Influence of Antagonistic Crustaceans on Population of *Aedes aegypti* Larvae under Simulated Conditions

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Original Research Article

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Abstract: Dengue and chikungunya are two mosquito-borne viral ailments transmitted by the same species of mosquito, *Aedes aegypti* and these ailments are of great public health concern in country like India. Thus, the present study was aimed to check the influence of antagonistic crustaceans on population of *Aedes* mosquitoes. Based on our laboratory experiments, the best ratio of antagonistic crustaceans (Cyclopoid copepod, *Mesocyclops aspericornis* and cladoceran, *Daphnia magna*) was chosen against *Aedes aegypti* larvae to test the influence of these crustaceans under simulated conditions. During the study it was observed that overall time taken for the larvae to convert into adult was 14.82±2.65 ranging from 14.0-21.0 days i.e. 4 days longer than the control sets. L1 larval stage was more prone to mortality i.e. 42.35±0.69 than other larval stages. Total adult emergence was significantly reduced i.e. 52.30±1.57 (31.65±0.69 males and 20.65±0.88 females). Significant reduction in body size of both male and female was observed i.e. 3.79±0.16 mm (control 4.44±0.07 mm) and female size significantly reduced to 4.17±0.15 mm when compared with control sets i.e. 5.48±0.08 mm. Life span of males was reduced from 15.35±1.20 to 9.33±0.33 days and in case of females longevity was reduced from 22.00±1.15 to 11.67±0.66 days.
Keywords: Antagonistic, Cladoceran, Cyclopoid copepod**,** *Mesocyclops aspericornis, Daphnia magna*.

INTRODUCTION

Dengue, one of the mosquito borne sicknesses, has created havoc to all over world. Globally, 950 species of *Aedes* are reported and to which India contributes 115 species and out of these two species i.e. *Ae. aegypti* and *Ae. albopictus* are major vectors for the transmission of dengue infection from the infected to healthy persons [1]. Dengue is endemic in all states and union territories of India and a total of 99, 913 dengue cases and 220 deaths in 2016 were reported in 35 states and Union territories of India as reported by WHO [2]. The incidences of vector-borne diseases are increasing alarmingly due to many factors including uncontrolled urban developments that support breeding of vector mosquitoes. Bio-control of mosquito larvae as suggested by Waage and Greathead [3] with predators and competitors becomes more convenient and mitigates the requirement for frequent chemical use. Recent studies have demonstrated the deterrent effects of predators (like cyclopoid copepods) and competitors (like cladocerans) on mosquito populations, either by direct feeding on larvae or by altering their oviposition process and thus affected their overall fitness. Thus predators and competitors are highly effective in controlling the growth of populations of mosquito larvae and can be used as bio- control agents against mosquito larvae [4-6].

MATERIALS AND METHODS

Pure cultures of *M. aspericornis* and *D. magna* (crustaceans) especially females were taken starved for 24 hours and after that transferred into plastic tubs containing pond water for providing them simulated conditions. Food was given on the day of the start of the experiment i.e, 0 day and on 4th day of the experiment (yeast stock solution) along with some leaf litters. Based on our previous laboratory experiments, best ratio i.e. 1:1:2 (*Aedes: Mesocyclops: Daphnia*) was taken out to test the influence of antagonistic crustaceans on *Ae. aegypti* larvae under simulated conditions. Three replicates were performed for each test and control set (without *Mesocylops* and *Daphnia* having larvae only) was also run simultaneously.

STATISTICAL ANALYSIS

Data was statistically analyzed with the help of SPSS statistical software version 16 by comparing antagonistic crustaceans treated trials with that of control sets by using Kruskal Wallis test.

RESULTS

Influence of effective ratio of mixed crustaceans against *Ae. aegypti* larvae under simulated conditions

On developmental period of *Aedes* larvae

In control sets, duration of larval stages i.e. L1, L2, L3, L4 and pupal to adult stage was 1.66±0.23, 1.83±0.26, 3.66±0.47, 4.00±0.40 and 2.00±0.50 days respectively (Table 1). No significant delay was observed in development period of all larval stages in the treated set (1:1:2). However conversion of L1 larval stage till adult took 14-21 days which was significantly different when compared with the control sets i.e. 11-16.5 respectively (Table 1).

Table-1: Influence of mixed crustaceans on developmental period of *Aedes aegypti* larvae

Treatment	Duration of developmental period (Range in days)					
	L1-L2	L2-L3	L3-L4	L4-Pupa	Pupa-Adult	Total
Control	1.66±0.23 (1-2)	1.83±0.26 (2-3)	3.66±0.47 (3-4)	4.00±0.40 (3-5)	2.00±0.50 (2-2.5)	13.15±1.86 (11.0-16.5)
1:1:2 (A:M:D)	1.66±0.23* (2-3)	2.00±0.50* (3-4)	4.00±0.47* (4-6)	4.66±0.81* (3-5)	2.50±0.64* (2-3)	14.82±2.65* (14.0-21.0)

A=*Aedes*, M=*Mesocyclops aspericornis*, D=*Daphnia magna*
Values are Mean±S.D
*superscript indicate significant difference ($p<0.05$) by using Kruskal Wallis test

On mortality of developmental stages of *Aedes* larvae

Significant larval mortality was observed in the treatment set @1:1:2. L1 larval stage was found to be more prone to mortality (average 42.35±0.69) followed by L2 larval stage with average percent mortality of 21.±65±1.94. Average percent mortality was 11.65±0.66 and 6.65±0.33 in case of L3 and L4 larval stages. Least percent mortality i.e. 3.30±0.60 was observed in case of pupae as shown in table 2.

Table-2: Influence of mixed crustaceans on mortality of developmental stages of *Aedes aegypti* larvae

Treatment	Percent Mortality (Mean±S.D)				
	L1 stage	L2 stage	L3 stage	L4 stage	Pupa
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1:1:2 (A: M: D)	42.35±0.69*	21.65±1.94*	11.65±0.66*	6.65±0.33*	3.30±0.60*

A=*Aedes*, M=*Mesocyclops aspericornis*, D=*Daphnia magna*
*superscripts indicate significant difference ($p<0.05$) by using Kruskal Wallis test

On emergence of *Aedes* mosquitoes

Average adult emergence was 100% out of which 51.65±1.52 were males and 48.38 were females recorded in the control sets. Whereas significant reduction in case of both males and females i.e. 31.65±0.69 and 20.65±0.88 (total adult emergence 52.3%) was observed in treated set 1:1:2 (Fig 1).

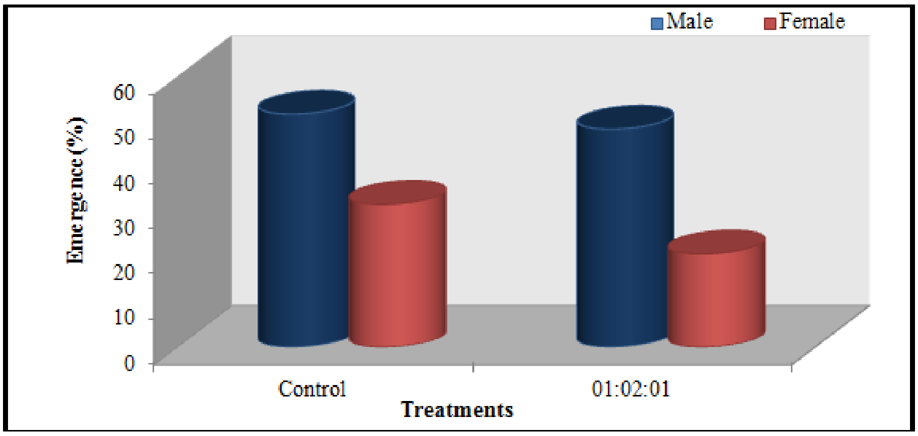

Fig-1: Influence of antagonistic crustaceans on emergence of *Aedes* mosquitoes

On body size of emerged male *Aedes* mosquitoes

Average total body length of emerged males was found to be 4.44±0.07mm (proboscis 0.86±0.05 mm, abdomen 1.72±0.01 mm and wing 1.84±0.02 mm) in control sets (Table 3). However significant reduction in body size of emerged male mosquitoes was recorded in the treated set @ 1:1:2. The size of proboscis was 0.60±0.04 mm, abdomen 1.49±0.08 mm and wing 1.70±0.04 mm (average total 3.79±0.16 mm) recorded respectively (Table 3).

On body size of emerged female *Aedes* mosquitoes

Average total body size of emerged females i.e. 5.48±0.08 mm was recorded in control sets having proboscis, abdomen and wing size of 1.35±0.02 mm, 1.89±0.01mm and 2.24±0.05mm respectively. Significant reduction in the proboscis, abdomen and wing size of emerged females was observed i.e. 0.70±0.03mm, 1.61±0.03 mm and 1.86±0.09 mm respectively in the treated sets when compared with that of control sets. Overall average body size of emerged females was also significantly reduced to 4.17±0.15 mm respectively as shown in table 3.

Table-3: Influence of mixed crustaceans on body size of emerged *Aedes aegypti*

Treatment	Body length of emerged males (mm)			
	Proboscis	Abdomen	Wing	Total body length
Control	0.88±0.05	1.72±0.01	1.84±0.02	4.44±0.07
1:1:2 (A:M:D)	0.60±0.04*	1.49±0.08*	1.70±0.04*	3.79±0.16*
	Body length of emerged females (mm)			
Control	1.35±0.02	1.89±0.01	2.24±0.05	5.48±0.08
1:1:2 (A:M:D)	0.70±0.03*	1.61±0.03*	1.86±0.09*	4.17±0.15*

Values are Mean±S.D

*-superscript indicate significant difference ($p<0.05$) by using Kruskal Wallis test.

On longevity of adult *Aedes* mosquitoes

Average life span of males was 15.33±1.20 days (ranging from 10-15 days) and in males it was 22.00±1.15 days (ranging from 14-25 days) respectively in control sets. However it was observed that in treated set @ 1:1:2, average longevity of males was significantly reduced to 9.33±0.33 days (ranging from 5-10 days). Whereas longevity of females was also significantly reduced to 11.67±0.66 days (ranging from 7-12 days) respectively (Fig 2).

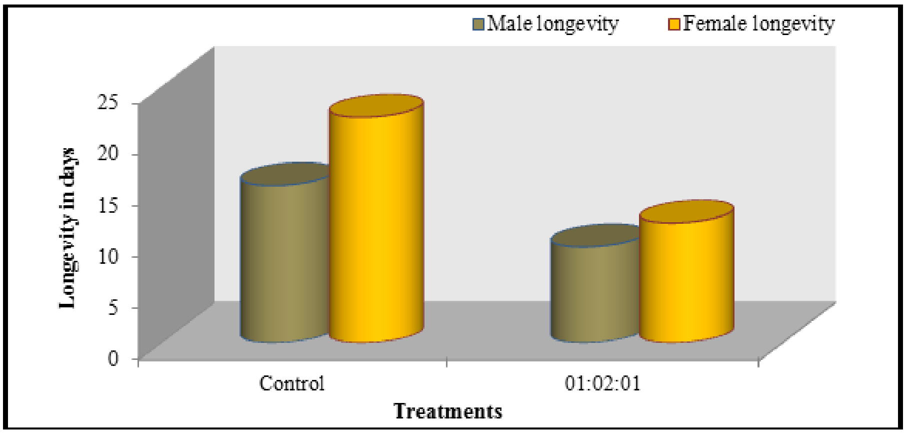

Fig-2: Influence of antagonistic crustaceans on longevity of *Aedes* mosquitoes

DISCUSSION

Previous experiments conducted on *Anopheles quadrimaculatus* by Knight *et al.* [7] showed that both predators and competitors greatly increased the time it took for larvae to grow to adulthood. Thus, causing longer generation time slows down the rate at which mosquito populations can increase. Researches like Juliano and Gravel [8], reported that in the presence of predation, mosquitoes spent more time motionless, hide themselves from predators which leads to longer generation time. They observed that larvae took four days longer in predator treatment and larvae in the competitor treatment took seven days longer than larvae in the control treatment. There was a considerable amount of variation in time of emergence among individuals within treatments. Some of this was likely due to the fact that male and female mosquitoes have different development times [9, 10]. However Holt [11], showed that there were direct effects of mosquito predators on mosquito competitors as the effects of predators on mosquitoes can be amplified in the presence of competitors through "apparent competition". Similar results were also taken by Dye [12], he observed that overcrowding of mosquito larval habitats generally results in retarded growth, high mortality, small and non-uniform sized adults with decreased fecundity.

Thus biological control, using natural enemies of *Aedes*, appears to be an alternative approach to the systematic failure of insecticidal usage [13].

CONCLUSION

During the present study, it was observed that when mixed crustaceans were exposed to *Aedes* larvae under simulated conditions, resulted in delayed developmental period, higher mortality of the larvae, less emergence of adults, smaller body size of emerged adults, reduced longevity of the *Aedes* mosquitoes which influence the overall *Aedes* mosquito fitness. Thus biological control, using natural enemies of *Aedes*, appears to be an alternative approach.

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