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The effect of cladoceran, *Daphnia magna*, on the growth and pupation of *Aedes aegypti* L. mosquito larvae

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Abstract

Background: Dengue and chikungunya are two mosquito-borne viruses transmitted by the mosquito, *Aedes aegypti*, and are responsible for great public health concerns in India. The present study tested the effect of *Daphnia magna,* a freshwater cladoceran, on the growth and pupation of *Ae. aegypti* larvae.

Results: *Ae. aegypti* third instar larvae and adult, *D. magna*, were introduced in the ratios 1:1, 1:2 and 1:3 into beakers and placed in an illuminated BOD incubator at a temperature of 26 ± 1 °C. The delay in the duration of larval stages (the time taken from third instar stage until their emergence as adults) was measured. The emergence of adults was delayed for 3–4 days in the treatments where the ratio of *D. magna* was thrice the number of *Ae. aegypti* larvae. The L3 larval stage was found to be more prone to mortality than later instars, with 51.66% larval mortality. The sex ratio of males to females emerged was only 5:5 in the 1:3 ratio. Body size of both males and females was significantly reduced in all the treatment sets in the ratios: 1:1, 1:2 and 1:3 when compared with the control set. Longevity of adult was also reduced from 8–15 to 4–7 days in the case of males, and in the case of females it was reduced from 14–25 to 5–9 days in the treatment set when *Daphnia* was introduced thrice the number of *Aedes* larvae.

Conclusions: Our study provides evidence that *D. magna* affects the growth and pupation of *Aedes* larvae and consequentially the life history parameters that affect the fitness of the population.

Keywords: *Daphnia magna*, Growth, Life history parameters, Longevity, Mosquito population, *Aedes aegypti*

Background

Mosquitoes are responsible for transmitting ailments approximately to 700 million people every year (Kessler & Guerin, 2008; Taubes, 2000). Globally, there were 96 million instances of dengue infections and India alone accounted 34% (22–24 million) in 2010. Dengue is endemic in all states and union territories (UTs) of India, and a total of 99, 913 dengue cases and 220 deaths in 2016 were reported in 35 states and UTs of India as reported by WHO (2017). Mosquitoes are a vital part in the study of disease transmission as they cause lethal ailments (Shinde, 2010). This aggravating situation of rising cases focuses towards the wasteful mosquito control operations. *Aedes aegypti* transmits the greater part of dengue infections, and vector control is the principal technique for combating this ailment for which no vaccine or therapeutant at this moment exists (Phillips, 2008). Biotic interaction such as competition is known to affect mosquito larval development. Competitors such as crustaceans are common in all kinds of pond waters and can quickly colonize new biotopes (Williams, 2006). As filter feeders, they consume similar food resources as of mosquito larvae which are also filter feeders. Williams (2006) found that crustaceans were dominated by cladocera (*Daphnia* spp.), common in all kinds of freshwater waterbodies. Zooplankton like cladocerans can fulfil all the requirements necessary for the bio-control

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of mosquitoes as they can easily adapt to the breeding sites of mosquitoes, their close interaction with mosquito larval population and widespread occurrence in fields. These characters of cladocerans make them potential contenders against mosquitoes as suggested by Kumar and Hwang (2006). According to Duquesne et al. (2011), in semifield conditions, *D. magna* strongly affected the population of *Culex pipiens* and *Anopheles quadrimaculatus* by suppressing their oviposition, increasing time to pupation and reducing total abundance. The effect of competition and predation depends on environmental factors and presence of other species; for instance, the presence of alternate prey lowers the predation pressure on one species and on the other hand the presence of high food resources changes the competitive effect as reported by Bevins (2007). Therefore, the objective of the present study was aimed at checking the influence of the crustacean *D. magna* on *Ae. aegypti* L. mosquito population. There is still need for more information to depict the natural habitats which allows the control of mosquitoes via predators/competitors by natural means.

Methods

Adult *Daphnia*, especially females, were taken from a pure laboratory reared culture and introduced into beakers with 300 ml of dechlorinated water. Twenty third instar wild-collected *Aedes* larvae (from water samples) and *D. magna* isolated from pure culture were introduced in the ratio of 1:0, 1:1, 1:2 and 1:3 (20:0, 20:20, 20:40 and 20:60). Food was given on the day of the start of the experiment, i.e. 0 day, and on the 4th day of the experiment (5 mg yeast/larva). Three replicates were performed for each combination of mosquito larvae and cladocerans. Three replicates of control set (without *Daphnia*) were run simultaneously. All beakers were placed in an illuminated BOD incubator kept at a temperature of 26 ± 1 °C. The number of live larvae was counted every 24 h. The time taken for the development (from larva to pupa and from pupa to adult) of *Aedes* larvae in treated and control sets was calculated. Other parameters such as emerged adult body size, male-to-female ratio and adult longevity were also recorded.

Total mortality per container was scored as per the following formula:

$$\text{Larval mortality (\%)} = \frac{\text{Number of larvae damaged /dead}}{\text{Total number of mosquito larvae taken initially}} \times 100$$

Statistical analysis

Data were statistically analysed with multivariate analysis of variance by the help of SPSS statistical software version 16.

Results

The effect of Daphnia magna on developmental period of Aedes aegypti larvae

The durations of larval stages in the control treatments (having *Ae. aegypti* larvae only): L3–L4, L4 to pupa and pupa to adult, were 2.83 ± 0.84, 4.00 ± 1.00 and 1.73 ± 0.25 days, respectively. The overall duration recorded was 6.5–11.0 days. It was observed that when *Ae. aegypti* larvae were kept along with *D. magna* in the ratios: 1:1, 1:2 and 1:3, a significant delay in the developmental period of larval stages was recorded (Wilk's $\wedge = 0.00$, $F_{15,11} = 11.75$, $p < 0.001$) (Table 1). The time taken for the conversion of L3 larval stage to L4 stage was 2–4 days in the control set. This time increases as the ratio of *D. magna* increases (3–4 days, 3–5 days and 4–5 days, respectively). For the L4 stage, the pattern differs from the L3 stage. The control treatment and the ratio 1:1 (*D. magna*: *Ae. aegypti*) were similar, 3–5 days. The treatments with 1:2 and 1:3 ratios took longer to complete the L4 stage (4–5 days). The time taken for the conversion of pupal stage to the adult form was 1.5–2 days in control set, and in the 1:1 and 1:2 treatment sets. The duration of the pupal stage was longer in the treatment set with the ratio of 1:3 (2.5–3 days). The overall development was found to take longer in all the cladoceran treatments sets, but it took longest (10.5–13.0 days) in the treatment with the ratio 1:3 (Table 1). The time for conversion of L4–pupa was longer than either of the others in the control group, but was least affected by the cladoceran treatments. It was recorded

Table 1 Effect of cladoceran density on developmental period of later stages of larval *Aedes aegypti*

Treatment (*Aedes* larvae: *D. magna*)	Duration of developmental period in days (Mean ± SD)					
	L3–L4	Range	L4–pupa	Range	Pupa–adult	Range
Control (20 *Aedes* larvae)	2.83 ± 0.84	2–4	4.00 ± 1.00	3–5	1.73 ± 0.25	1.5–2.0
1:1 (20:20)	3.83 ± 0.28	3–4	4.33 ± 0.28	3–5	1.93 ± 0.51	1.5–2.5
1:2 (20:40)	4.00 ± 0.50	3–5	4.40 ± 0.50	4–5	2.33 ± 0.76	1.5–2.5
1:3 (20:60)	4.5 ± 0.86	4–5	4.66 ± 0.28	4–5	2.66 ± 0.28	2.5–3

Table 2 Effect of cladoceran density on the mortality of later stages of *Aedes aegypti* larvae

Treatment (*Aedes* larvae*: D. magna*)	L3 stage		L4 stage		Pupa	
	Mean ± SD	% Mortality	Mean ± SD	% Mortality	Mean ± SD	% Mortality
Control (20 *Aedes* larvae)	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
1:1 (20:20)	6.33 ± 1.15	31.66	1.66 ± 0.57	6.66	3.33 ± 0.57	16.66
1:2 (20:40)	8.33 ± 1.15	41.66	3.33 ± 0.57	16.66	3.33 ± 0.57	16.66
1:3 (20:60)	10.33 ± 0.57	51.66	3.33 ± 0.57	16.66	3.00 ± 0.00	15.00

Table 3 Effect of cladoceran density on sex ratio of emerged *Aedes aegypti* mosquitoes

Treatment (*Aedes larvae: D. magna*)	Adult emergence (%)	Sex ratio Male/female
Control (20 *Aedes* larvae)	100.00	31:29
1:1 (20:20)	43.33	18:8
1:2 (20:40)	25.00	10:5
1:3 (20:60)	16.66	5:5

that with the increasing number of cladocerans in the treatments there was an increase in the duration of each larval developmental stage.

The effect of Daphnia magna on the mortality of later stages of Aedes aegypti larval development

There was no mortality in control group of larvae at any larval stage, and there was mortality in the cladocerans group at all levels and at all larval stages. The mortality rate was higher in the L3 stage than in the other two groups. There was a significant increase in mortality rate (Wilk's $\wedge = 0.00$, $F_{15,11} = 11.75$, $p < 0.001$) with average per cent mortality of L3 larval stage being found to be 31.66%, 41.66% and 51.66% in the treatment sets 1:1, 1:2 and 1:3. However, per cent mortality of L4 stage was 6.66%, 16.66% and 16.66%. It was also observed that pupal stages were also prone to mortality when exposed to different ratios: 1:1, 1:2 and 1:3 of *D. magna*, having average per cent mortality of 16.66%, 16.66% and 15.00%, respectively, which were found to be statistically significant compared to the control sets (Table 2).

The effect of Daphnia magna on the sex ratio of emerged adult Aedes aegypti mosquitoes

100% of larvae emerged as adults, and the sex ratio of male/female recorded was 31:29 in the control set. In the treatment set 1:1, the sex ratio was 18:8 with 43.33% adult emergence. In the treatment set 1:2, 25% of the larvae emerged as adults with a sex ratio of 10:5.

Table 4 Effect of rearing *Aedes aegypti* larvae with *Daphnia magna* on the wing length of adult mosquitoes

Treatments (*Aedes* larvae: *D. magna*)	Wing length (mm) of emerged *Aedes* mosquitoes			
	Male		Female	
	Mean ± S.D	Range	Mean ± S.D	Range
Control (20 *Aedes* larvae)	1.88 ± 0.02	1.86–1.90	2.29 ± 0.15	2.28–2.30
1:1 (20:20)	1.60 ± 0.02	1.58–1.63	1.76 ± 0.01	1.75–1.78
1:2 (20:40)	1.68 ± 0.01	1.68–1.70	1.82 ± 0.03	1.80–1.85
1:3 (20:60)	1.59 ± 0.02	1.58–1.61	1.76 ± 0.01	1.75–1.77

However, least adult emergence (16.66%) was recorded in the treated set 1:3, with the sex ratio of 5:5 (Table 3). Similar number of females survived in all three competition treatments (8, 5 and 5), but that the number of males that survived decreased monotonically (18, 10 and 5). The males appear to be affected differently by the competition provided with *Daphnia* than were the females.

The effect of rearing larvae with Daphnia magna on the wing length of adult Aedes aegypti

Average body length (wing length) of emerged males was 1.88 ± 0.02 mm recorded in the control set ranging from 1.86 to 1.90 mm. A significant difference was recorded in all the treatment sets (Wilk's $\wedge = 0.00$, $F_{18,8.9} = 131.74$, $p < 0.001$) with average wing length of 1.60 ± 0.02 mm, 1.68 ± 0.02 mm and 1.59 ± 0.02 mm in the ratios: 1:1, 1:2 and 1:3, respectively, as compared to the control set. In the case of adult females, average wing length was also reduced: 1.76 ± 0.01 mm, 1.82 ± 0.03 mm and 110 1.76 ± 0.01 mm, respectively, as compared to the control set with females having wing lengths of 2.29 ± 0.01 mm (range 2.28–2.30) (Table 4). It is clear that rearing these larvae with *D. magna* reduces the wing length of both males and females compared to the control group. It was also observed

that the larvae reared in the ratio 1:2 group grow larger than those reared in the 1:1 and 1:3 ratio groups.

The effect of rearing mosquito larvae with Daphnia magna on the longevity of adult Aedes aegypti

The life span of both male and female was significantly reduced with increasing ratio of *D. magna* in the treatment sets (Wilk's $\wedge = 0.003$, $F_{12,21} = 8.71$, $p < 0.001$). In the control set, the average adult longevity was found to be 10 ± 4.35 days (males) and 20.33 ± 5.68 days (females). In the case of males, the average longevity was reduced to 8.66 ± 1.52 days, 7.00 ± 2.00 days and 5.33 ± 1.52 days in the respective treated sets. Similarly, the average longevity was reduced to 10.33 ± 1.52 days, 8.33 ± 1.52 days 7.00 ± 2.00 days, respectively in the case of females (Table 5).

Discussion

Our outcomes demonstrate clear evidence that in a laboratory conducted trial, *Aedes* larval development, survival and body size and longevity were most affected by increased densities of *D. magna*. The presence of interspecific competitors limits the amount of food resources, resulting in delayed development and decreased survival of mosquito larvae (Juliano and Reminger 1992). This is consistent with findings from studies that focused only on mosquitoes, which showed that under both laboratory and field conditions, an increasing density of competitors (i.e. intra or interspecific mosquito competitors) is linked to increased mortality of the mosquito species of concern, delayed maturity, reduced adult size and reduced adult longevity (Agnew et al., 2002; Braks et al., 2004; Reiskind & Lounibos, 2009; Renshaw et al., 1993; Teng & Apperson, 2000). *D. magna* were found to be effective competitors than *M. aspericornis* which being larger in size resulted in delaying the overall developmental period of *Aedes* larvae efficiently (Thakur & Kocher, 2020). Yoshika et al. (2012) had also demonstrated that intraspecific competition for food among larvae of *Ae. albopictus* had a significant reduction on the number of adults that emerged. The sizes of *Aedes* females were smaller when provided with interspecific competition as reported by Costanzo et al. (2011). Large-bodied mosquitoes may play a more important role in maintenance and amplification of mosquito-borne pathogens than smaller individuals, and a mosquito population with large average body size may have a higher vectorial capacity than a population with small average body size (Nasci, 1986). The size of adults was determined during the aquatic larval stage. Competition among larvae for food influences the size of the pupae and the adults. It was recorded that in the treatment ratio 1:2, the wing size of both male and female mosquitoes was somewhat larger as compared to 1:1 and 1:3 group which might be due to the smaller size of *D. magna* in the ratio 1:2, thus competing less efficiently with *Aedes* larvae than the other two groups. Therefore, the size of the adult female affects her success, fitness and ability to transmit diseases (Steinwascher, 2018). Wing length is known to be directly related to the body size in *Ae. aegypti* as reported by Christophers (1960). Thus, the smaller the wing length, the smaller the body size of mosquito reported. The small-sized *Ae. aegypti* females exhibited reduced blood feeding success and most likely reduced survival when compared with large adults (Nasci, 1986). The degree of intraspecific competition had a significant effect on adult longevity under low humidity conditions as reported by Reiskind & Lounibos (2009).

Table 5 Effect of cladoceran density on longevity duration of adult *Aedes aegypti* mosquitoes

Treatments (*Aedes* larvae: *D. magna*)	Adult longevity			
	Male		Female	
	Mean ± S.D	Range	Mean ± S.D	Range
Control (20 *Aedes* larvae)	10.00 ± 4.35	8–15	20.33 ± 5.68	14–25
1:1 (20:20)	8.66 ± 1.52	7–10	10.33 ± 1.52	9–12
1:2 (20:40)	7.00 ± 2.00	5–9	8.33 ± 1.52	7–10
1:3 (20:60)	5.33 ± 1.52	4–7	7.00 ± 2.00	5–9

Conclusions

The presence of *Daphnia magna* in containers along with *Aedes* mosquito larvae resulted in a longer developmental period; a reduction in the rate of adult emergence; a smaller body size of the adult mosquitoes; and a reduced longevity of male and female mosquitoes which affects the life history parameters that could alter the overall *Aedes* mosquito population.

Abbreviations

A: *Aedes* Larvae; D: *Daphnia magna*.

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Author contributions

AT carried out the study, analysed the data and drafted the manuscript. DK designed the project and was responsible for final interpretation of data. All authors read and approved the final manuscript.

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