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Role of the predator *Anisops sardea* (Hemiptera: Notonectidae) in control mosquito *Culex pipiens molestus* (Diptera: Culicidae) population

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Abstract
The presence of the predators can alter the prey niche in the community. We evaluated the influence of the predator, *Anisops sardea* on mosquito *Culex pipiens molestus* population by predation, and non-consumptive effect by life-cycle prolongation in predator presence and residual kairomones. The predation depends on prey density, individual *A. sardea* predated 16.0-19.7 and 24.7-29.3 3rd larvae/ day at the densities 50 and 100 larvae / liter respectively, with clearance rate 0.19 and 1.1 for those densities. The predatory impact along four days within 8 hours daytime ranged between 0.98-1.20. The non-contact presence of the predator folded the larval stadium from 11.9 in contract to 23.7 days while it increased to 16.0 days at residual kiromone, the pupal stadium in control 5.8 days, then increased to 7.8 days in the two treatments. Thus, release of notonectid *A. sardea* will effectively control *C. pipiens molestus* by predation and besides decrease number of generations through immature stages extension. *A. Sardea* can be actively used as essential factor in integrated vector control.

Keywords: *Anisops sardea, Culex pipiens molestus,* biocontrol, predation, metamorphosis

1. Introduction

Mosquitoes are well known as annoying pests, the annoyance of the mosquitoes comes from biting feeding habit and tones produced by beating of their wings. Also, mosquitoes have been transmitted many fatal disease agents as malaria, filariasis and arboviruses such as Zika virus [1].

Today, because of insecticide resistance raising in vector population and magnified insecticides toxicity in food chains, more attention paid for aquatic entomophagous insects as one of the effective alternatives for mosquito control [2, 3, 4, 5]. Aquatic mosquito predators have direct role in immature predation and sub lethal effect through physiological and oviposition deterrence [2, 3, 6]. Among aquatic hemipteran predators, many species of Notonectidae are important predators of mosquito larvae with potential role in the biological control of mosquitoes [5].

The mosquito predator, *Anisops sardea* H.S. (Hemiptera: Notonectidae) is a smaller bodied backswimmer, it is very common in temporary pools and permanent water bodies [7]. Laboratory based predatory experiments were revealed that *A. sardea* have a high predation rate against larval mosquitoes [8].

Daily predation of the mosquito larvae was varied between the notonectid species and the larval instar mosquito larvae; *Notonecta undulate* was efficiently preyed the second instar of the mosquito larvae [9]. *A. boavieri* fed on 2-34 mosquito larvae/ day [10], and *N. sellata* consumed 8-30 larvae every day and fed on early mosquito instars with higher rate than on late instars [6]. A mean predation rate of 16 mosquito larvae/ day for *Anisops* spp., [11]. While Shaalan and Canyon (2009) [2] found that *Anisops* spp. predated 25 first or 13 fourth instar larvae/ day. Also, predation rates were affected by prey density [5], the predation rate of *A. sardea* in variable prey densities exhibited a linear rise to plateau curve and it can be used as biological agent against the larvae forms of *C. quinquefasciatus* in temporary breeding clear water [12].

The presence of predators or their cues can have significant effect on the prey population [13, 14].

The predator *Toxorhynchits rutilus* presence was increased time of *Aedes triseriatus* Metamorphosis [15]. The predator *N. sellata* presence was altered the life history of *Culex pipiens* by extended developmental time [6]. Also, the presence of the naiads of damselfly, *Ischnura evansi* relay life cycle of *Culex pipiens molestus* from 20.8 days in control up to 30.0 days [16]. The objective of the present study is to evaluate the predation efficiency of *A. sardea* as one of the abundant and common larvivorous predator in mosquito natural habitat. Also, investigate its direct and indirect effect on *C. pipiens molestus* metamorphosis.

2. Materials and Methods

2.1 Predator colony

Colony of the predator, *Anisops sardea* H.S. were established by collecting them with a hand-held net from natural mosquito habitat the temporary pond after rainfall season in October, 2018, Located in Mosul University Park, Mosul, Iraq (36° 20' 06" N 43° 0.7' 08" E). In the laboratory, the predator were kept in 60×30×40 cm aquarium and mainly fed with mosquito larvae and less with chironomid larvae.

2.2 Prey-mosquito colony

The immature stages of the mosquito, *Culex pipiens molestus* were collected from stagnant pool after rainfall season. The immatures were kept in plastic containers with their clean breeding water for obtaining F1 generation, which their new hatched larvae fed on biscuit and yeast, the water was changed every 4 days [17]. The developed pupae were separated in proper cage for adult emergence. Firstly, the newly emerged adults were fed on 15% honey solution and juicy fruits for 3 days, then adult females were fed nightly on a naked chest pigeon. The four instars of the larval stage were continuously available for the experiments. The colony was maintained in the laboratory at 22-28 ºC temperature.

2.3 Experiment 1

50 or 100 third instar *C. pipiens molestus* larvae were introduced as prey for the individual predator *A. sardea* in enamel tray filled with liter aged water for 3 days with 3 replications for each density. The prey consumption was noted every 24 hours, and the prey density remains at the experimental beginning after every 24 hours. The data analyzed by the following equation to obtain the clearance rate (CR) after Gillbert and Burns (1999) [18]:

$$CR = \frac{Vx\ \ln(Pc - PE)}{TN}$$

Where, CR- clearance rate, V-volume of water (in liters), Pc- prey at start of the experiment, PE- prey left after T time in days.

2.4 Experiment 2

Predatory potential was worked using 500 ml beaker. Prey third instar larvae and adult predator (in ratio 20:1) or without predator (control), for 5 replications for four serial days. For every day the experiment starting at 8.00 AM and ending at 4.00 PM, and noted at 2 hours interval. After every 2 hours interval the consumed larvae were calculated and at 4.00 PM the predator separated alone in another beaker and returned again next morning 8.00 to the same beaker, then fresh prey larvae added to the beakers to complete the initial prey-predator ratio. The method adopted by Aditya *et al*. (2006) [19] and Jacob *et al*. (2017) [20] were used to calculate the predatory impact.

$$PI = \frac{\sum_{n=1}^{8} PE}{T}$$

Where, PI is the predatory impact (number of prey larvae consumed or killed/ hour); PE is the number of prey eaten; T is time in hours.

2.5 Experiment 3

The direct and indirect influence of the predator *A. sardea* on the life cycle of the mosquito, *C. pipiens molestus* from larval hatching till adult emergence were tested. Within direct effect, 10 adult predator individuals and newly hatched larvae were transferred into plastic container filled with 2 liters water, to avoid interference between the larval prey and their predator, nylon mesh which permeable for water only separates them and prevents predation, the dead predators were replaced by fresh another. Whereas indirect influence of the predator by its kairomones, by putting a newly hatched 50 larvae in container were 10 predators in its water at prior time lasting for a week. Clean water without predators or their kairomones for control treatment. 25 stages of each larvae and pupae were noted to determine the development time.

2.6 Statistical analysis

Mean and standard deviation (±SD) assessment by using JMP software [21]. Duncan's test at P = 0.05 was applied for mean separation.

3. Results and Discussion

3.1 Predation and habitat clearance

Fig. 1 showed that, the daily predation of the backswimmer *Anisops sardea* was affected by the mosquito prey density in case constant water volume, the average of consumed larvae (Table 1) at the 50 larvae/ predator density was 18.1 larvae, but if the density 100 larvae, the predation increased to 27.1 larvae consumed every day. There is no significant alternation between the three days of the experiment in number of predated larvae/ day. Also, along the three days of the experiment, at 50 prey density the daily clearance ranged between 2.77-2.99 with clearance rate 0.79, while the daily clearance ranged between 3.21-3.38 and clearance rate 1.1 in case density 100 prey/ predator.

In comparison with other studies, the clearance rate parameter of the mosquito larvae by aquatic insect predators depends on the predator species and its developmental stage; one of Aditya *et al*. (2006) [22] results, the clearance rate of *C. Quinquefasciatus* larvae by *Toxorhynchytis Splendens* larvae and the beetle, *Rhantus sikkimensis* was about 2.22 and 1.59 respectively. The CR in Manibhai (2014) [23] study was 1.29 for predated *Culex* spp. by naiad odonate *Bradinopyga geminate*. While Jacob *et al*. (2017) [20] found the CR of Odonata naiads about 1.65 for prey *Aedes aeygpti* third instar, The clearance rate of 1st and 2nd instars *C. pipiens molestus* 0.63 which predated by the damselfly, *Ischnura evansi* [16]. The present results coincide with Gurumoorthy *et al*. (2013) [24]. as stated the predation rate increases with density of the prey.

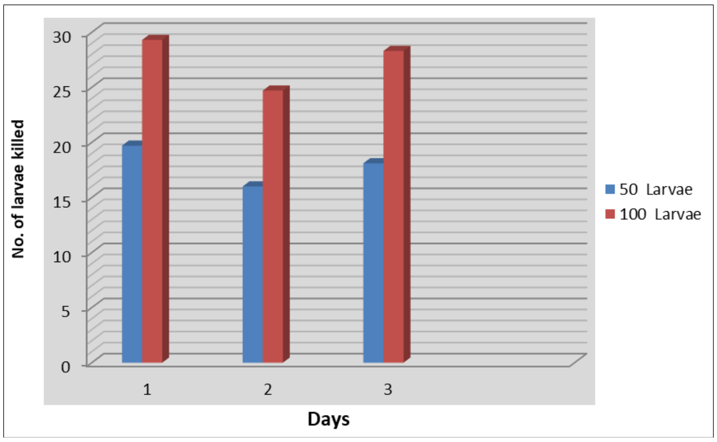

Fig 1: Predation of *Anisops sardea* third insar larvae of C*ulex pipiens molestus* at two prey densities

Table 1: Clearance rate of the mosquito, *Culex pipens molestus* habitat after three days of exposure to the predator, *Anisops sardea*

Day	Consumed prey at Density		Daily Clearance		Daily Rate	
	50	100	50	100	50	100
1st day	14.7 ± 0.6	29.3 ± 1.2	2.99	3.38		
2nd day	16.0± 1.0	24.7 ± 0.6	2.77	3.21	0.97	1.10
3rd day	18.1 ± 1.9	27.4 ± 2.4	2. 93	3.34		
Mean	18.1 ± 1.9	27.4 ± 2.4				

3.2 predatory potential of ***Anisops sardea***

Table 2 is illustrates the predator *A. sardea* has not peak prey number consumed every two hours which divided on 8 hours at daytime and that continued for four days of the experiment. The number of consumed third instar larvae (PI) for every day ranged between 0.98-1.20 larvae. Other studies concluded that the predatory impact of the predatory depends on its taxon [24, 25, 20].

The notonectid *A. sardea* is abundant and common as native in Asia and Africa [26]. So, the studies on biological control of mosquitoes with aquatic predators focused on predation efficiency of *A. sardea.* This predator killed heavily *Cluex* mosquito in the laboratory [27]. Mondal *et al*. (2014) [12] have suggested that *A. sardea* can be changed the community structure and used as biological control for *C. quinquefasciatus* in temporary clear water.

Table 2: Predatory potential of *Anisops sardea* against third instar larvae of *Culex pipiens molestus*

Day	Eaten prey between 8.00 AM – 4.00 PM				Predatory Impact
	10.00	12.00	2.00	4.00	
1st day	1.6 ± 0.49	2.0± 0.63	2.0 ± 0.71	2.2 ± 0.83	0.98
2nd day	2.4 ± 0.54	2.2 ± 0.45	1.8 ± 0.84	2.4 ± 0.55	1.1
3rd day	2.4 ± 0.55	2.0 ± 0.71	2.4 ± 0.55	2.0 ± 0.71	1.1
4th day	1.8 ± 0.45	2.4 ± 0.55	2.4 ± 0.55	2.0 ± 0.71	1.2

3.3 Sub lethal effect of life cycle

In addition to mortality as result of larvae predation, the predator *A. sardea* indirectly interfered with the prey *C. pipiens molestus* population abundance, through significant extension of the prey life cycle. The indirect effect of *A. sardea* on developed larvae and pupae were represented by residual cues (kairomone) lasting after a week in same water of developed larvae and pupae later, or predator-prey presence together till development was completed.

Table 3 is reveal significant prolongation of treated immature stages with predator alone or with its kairomones. In comparison with controlled larval stage 11.9 days, it was found high significant differences for larval development time 16.0 and 23.7 days for predator residual cues and predator presence respectively. On the otherwise, controlled pupae developed within 5.8 days, treated pupae developed after 7.8 days without not differences in pupal stadium whether predator presence or predator residual cues treatments.

Time extension of metamorphosis is one of the pest control strategy. In previous studies, the presence of predators were prolonged mosquito metamorphosis [15, 16]. *A. sardea* was decreased fecundity by oviposition avoidance in its habitat [28, 6]. *Culex* mosquitoes can be detected residual cues of *Notoneta* sp., so not oviposited their eggs [9]. In the present study, it was found the predator residual kairomone effected on the prey metamorphosis by life cycle elongation.

Table 3: Effect of the predator *Anisops sardea* presence and its residual cues on life history of *Culex pipiens molestus*

Developmental stage	Duration time (days)		Control
	Predator residual cues	Predator with prey	
Larva	$16.0^{d} \pm 0.7$	$23.7^{a} \pm 0.5$	$11.9^{g} \pm 2.4$
Pupa	$7.8^{a} \pm 1.2$	$7.8^{a} \pm 0.7$	$5.8^{c} \pm 1.6$

- Values are in mean and standard deviation.
- Horizontal different letters significant with Duncan's test ($P < 0.05$)

4. Conclusion

From the view of integrated vector control strategy, larvicides have variable control trails to cut-off the vector life-cycle. Predation is natural feeding habitat of aquatic life and can be used as potential tool in prey control.

We found that *Anisops sardea* abundant in natural habitats of the mosquito *Culex pipiens molestus.* Because of its aggressive hunting behavior and high predation efficiency of this predator in comparison with other mosquito predators, *A. sardea* can candidate to control mosquito population dynamics in urban environments.

Also, predation role was enhanced in this study by non-consumption impact on the population structure, the not contact presence of *A. sardea* and its residual kairomone were significantly prolonged the life cycle, by delaying the immature stages in the two treatments. The number of generations were decreased as result of generation time extension. In addition, prey-predator combination increases risk of predation as result of larvae and pupa stadia prolongation.

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