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Effectiveness of Copepod, *Acanthocyclops vernalis* on Dengue Fever Victor, *Aedes aegypti* under Laboratory Conditions in Jeddah, Saudi Arabia

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Abstract

***A. aegypti*, the primary carrier for dengue viruses that cause dengue fever and are widespread over large areas of the tropics and subtropics. The copepod, *Acanthocyclops vernalis* was collected from a pond located in Hail, a city in northwestern Saudi Arabia. *A. aegypti* colonies established from Laboratory of Public Health Pests, Jeddah Municipality, Jeddah, Saudi Arabia. In the laboratory, the predatory capacity of copepod predator *A. vernalis* tested on first and second instars of *Aedes aegypti*. A single adult female of *A. vernalis* was tested against 50 larvae of *A. aegypti* in case the presence and absence of an alternative food. Several doses of *A. vernalis* (10, 15, 20 and 25 adults) against 100 larvae of mosquito were tested also in case the presence and absence of an alternative food under laboratory conditions.**

Keywords

Copepods, *Acanthocyclops vernalis*, Dengue Fever, *Aedes aegypti*, Biological Control

1. Introduction

Mosquitoes (Diptera: Culicidae) cause more human suffering more than any other organism. Mosquitoes can be

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an annoying as well as serious problem in human domain. Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti*, is classified as a major global health threat by the WHO [1]. Mosquitoes impose serious threat to public health as they transmit several life threatening diseases to over two billion people in the tropics [2]. A total of 463 *A. aegypti* adult mosquitoes were identified in Al-Madinah Al Munawwarah, Saudi Arabia, which are abundant all seasons and peak in April [3]. [4] observed significant increases in dengue fever incidence, in Makkah-Saudi Arabia, among males in general the study period and among those 25 - 44 years of age old in particular.

A. aegypti, the primary carrier for viruses that cause dengue fever, dengue hemorrhagic fever and yellow fever are widespread over large areas of the tropics and subtropics and is reported to infect more than 100 million people every year in more than 110 countries in the tropics [5]. Indeed, the use of insecticides remains the first line of defence against mosquitoes in the short-term still depends on the use of conventional insecticides [6] [7]. The indiscriminate use of chemical insecticides has given rise to many well known and serious problems, such as the risk of developing insect resistance and insecticidal residual for humans and the environment [8]. Insecticide resistance in mosquitoes is a global problem and several surveys have shown that such resistance is wide spread and increasing [5] [9]. These problems coupled with the high cost of chemical pesticides have stimulated the search for biologically based alternatives. Therefore, copepod species are known to be predators of mosquito larvae supply [10].

Various species of *Mesocyclops*, *Macrocyclops*, *Megacyclops* and *Acanthocyclops* have been tested in a variety of *Aedes*-breeding habitats [11]-[16] with promising results. Since 2011, Alshammari and Abdelmageed [17] recorded and establishing the presence of predacious copepods *Acanthocyclops vernalis* (Fischer, 1853) in Hail city, Saudi Arabia and they concluded that this organism can be applied as predator for *A. aegypti* to control dengue disease which is widespread in Jeddah, Saudi Arabia, and its neighboring areas. Thus laboratory trials were condicted to evaluate the efficacy of the predaceous cyclopoid *A. vernalis* on controlling *A. aegypti* mosquitoes.

2. Materials and Methods

2.1. Rearing of *A. aegypti*

A. aegypti colonies established from Laboratory of Public Health Pests, Jeddah Municipality, Jeddah, KSA (generation > F75) and maintained under a 14:10 (light:dark) and water and maintained at 28˚C ± 2˚C and 70% - 80% humidity were used. Mosquitoes were reared using standard conditions [18] to generate similar-sized individuals. Adult mosquitoes were housed in 0.5 liter cardboard cages (Instawares, Kennesaw, GA) with mesh screening on top, provided a 10% sugar solution. A hand-held aspirator was used to access mosquitoes through a plugged hole in the side of the cage. Females of *A. aegypti* were starved of sugar 24 h prior to feeding on pigeon, each cage 27 - 95 mosquitoes/cage. Live pigeons are commonly used to maintain *A. aegypti* in colony then replace the pigeons every three months, this is artificial feeding methods are often used in our laboratory. Experiments may require using mosquitoes that have completed more than one gonotrophic cycle and the reproductive effects of these feeding regimens are currently unknown.

2.2. Mosquito Oviposition

Four days after the first blood feeding (mosquitoes 9 d old), 50 ml of tap water and filter paper placed vertically in case *A. aegypti* to each oviposition cup and mosquitoes were allowed to oviposit overnight. We and others have observed this time period to be sufficient for *A. aegypti* to complete the gonotrophic cycle prior to oviposition [19]. Five days post-blood feeding, all adult mosquitoes were aspirated from cages and transferred according to treatment group to new cages containing an empty 100 ml plastic cup affixed to the bottom. As previously described, the plastic cup was used later to contain an oviposition substrate.

2.3. Collection of *A. vernalis*

The copepod, *A. vernalis* was collected from a pond in Hail district during early morning before sun rise. To collect the copepod from the pond, standard plankton mesh net of 100 µm mesh was used. Collected copepod in 200 mL of plastic bottle were detached and transferred to the laboratory. Copepods were identified according to [20]-[22]. Individual gravid females of *A. vernalis* were placed in containers containing 200 ml of dechlorinated tap water, and added protozoan and algae as food. Copepod larvae were placed in containers with 2000 ml of dechlorinated water and incubated at 26˚C ± 2˚C to adult stage.

2.4. Culture of *A. vernalis* under Laboratory Conditions

A. vernalis was cultured, a system based on algae, protozoans such as paramecium, chilomonas, wheat seed and some lettuce particles are cultivated in laboratory in 30 litres fish tank. Protozoans serve as excellent food and provide support for adult copepod in dechlorinated water to culture more number of copepods for the experiment. *Paramecium* sp. prepared side by side from boiled rice straw water extract and commercial powdered fish foods used as food to the copepod. Copepod was cultured in dechlorinated water where temperature during the culture was kept at 28.8˚C with pH 7.0. Male and female copepod species from the colonies were separated using medicine dropper under a stereomicroscope. The copepods in container were covered with net cloth and gravid female lines were pooled. The females continued to produce multiple batches of egg sacs. Each container yields approximately 1500 - 2000 adult copepods [23].

2.5. *Acanthocyclops vernalis* Efficiency Test

Predatory capacity of *A. vernalis* on first and second instar larvae of *A. aegypti* was determined. A single *A. vernalis* adult female was placed with 50 first and second instar mosquito larvae (24 h old) in a 250 ml plastic container (8 × 8 × 5 cm) containing 100 ml of dechlorinated tap water at 26˚C. This experiment was conducted over three days, and number of surviving larvae on each day was recorded. The predatory nature and the rate of predatory efficiency of adult *A. vernalis* on mosquito larvae were observed under a stereomicroscope. Fifty larvae of each mosquito species and one adult female copepod were introduced in containers containing 100 ml of dechlorinated tap water.

While, designed another experiment to determine the number of adult copepods needed to consume a large number of first and second instars of *A. aegypti* larvae. Several doses of *A. vernalis* of 10, 15, 20 and 25 adults were introduced to 100 numbers of mosquito larvae into the 500 mL glass beaker containing 250 mL of dechlorinated water and observed for whole day. The copepod attacked and killed *A. aegypti* larvae were observed under microscope. The numbers of dead larvae were counted at three days. The glass beakers were checked without control treatment on first day, second day and third day and the number of prey consumed by the predator was checked and recorded. The mosquito larvae were replaced daily with the new ones. The experiment was held up with 4 trials and each trial consisted of four replicates.

2.6. Statistical Analysis

Mortality percentage of mosquito larvae were compared between treatments with ANOVA test using SAS program [24] statistical software. Multiple comparisons were tested using Tukey HSD test.

3. Results and Discussion

Results in **Table 1** show that predatory efficiency of single adult copepod, *A. vernalis* against 1st and 2nd larvae, *A. aegypti* in case the presence and absence of an alternative food under laboratory conditions, tests were carried out in beakers by introducing *A. aegypti* 1st and 2nd instar larvae by keeping single adult of *A. vernalis* and constant for 24, 48 and 72 hours.

The percent mortality of 1st and 2nd larvae, *A. aegypti* reached 39.3%, 47.3% and 49.6% after 24, 48 and 72 hours in case absence of an alternative food, respectively, whereas 19.8, 23.8 and 37.5% of mortality were recorded in case of the presence of an alternative food, respectively (**Table 1**).

Statistical analysis between *A. aegypti* alone, *A. aegypti* + food and control had significant effects (LSD = 0.788, 0.6857, 0.9204 and 0.2401) as results of using single adult copepod, *A . vernalis* against 1st and 2nd larvae after 24, 48, 27 and average mortality %, respectively (**Table 1**).

In Viet Nam, under laboratory conditions, *M. aspericornis* consumed a mean of 23.75 L and killed a mean of 13.43 within 24 hours, while *M. ogunnus* consumed a mean of 8.481 and killed a mean of 7.54. *Acanthocyclops* is one of the most important genera of Cyclopoida utilized for the control of *A. aegypti* larvae. *Avanthocyclops* species can effectively reduce the number of *A. aegypti* larvae in both laboratory and natural settings [13]. [25] studied the predatory effectiveness of two vernal-pool copepods, *Acanthocyclops vernalis* (Fischer) and *Diacy-*

Table 1. Predatory efficiency using single adult copepod *A. vernalis* against 1st and 2nd larvae *Aedes aegypti* in the presence and absence of an alternative food.

	% Mortality (Mean ± Standard Deviation)			Average mortality (%)
	24 h	48 h	27 h	
A. aegypti alone	$39.3^a \pm 0.61$	$47.3^a \pm 0.47$	$49.6^a \pm 0.61$	45.4^a
A. aegypti + food	$19.8^b \pm 0.31$	$23.8^b \pm 0.36$	$37.5^b \pm 0.51$	27.1^b
Control	$0.0^c \pm 0.0$	$0.0^c \pm 0.0$	$0.0^c \pm 0.0$	0.0^c
LSD	0.788	0.6857	0.9204	0.2401
Probability	0.0001	0.0001	0.0001	0.0001

Different letters denote significant differences ($p < 0.05$) between means.

clops bicuspidatus thomasi (Forbes) against cohabiting larvae of *Aedes canadensis* (Theobold) and *Aedes stimulans* (Walker) in laboratory bioassays. They concluded *A. vernalis* did feed on early instars, but its effectiveness was significantly influenced by the presence of alternate food, the size of the container, and the size and age of the larval prey. Results strongly suggest that in a comparatively unrestricted natural vernal-pool habitat where an abundance of other food sources are available, neither copepod species plays a major role in reducing larval mosquito populations. According to [26], research efforts should be increased in this field in addition to plant extract. [27] reveals that plant extract of *Aloe vera* could serve as a potential larvicidal agents against the dengue vector *A. aegypti*.

A comparative study between different concentrations (10, 15, 20 and 25 adults) of *A. vernalis* were introduced to 100 numbers of mosquito larvae into the 500 mL glass beaker in case the presence and absence of an alternative food under laboratory conditions are shown in **Table 2**.

The efficiency of *A. vernalis* to feed on mosquito larvae in the presence and absence of an alternative food is shown in **Table 2**. The percent reduction of *A. aegypti* larvae consumed by *A. vernalis* of concentration 10 adults:100 larvae of *A. aegypti* were 61.6%, 66.2% and 68.5% after 24, 48 and 72 hours in case absence of an alternative food, respectively (**Table 2**). While were 78.9%, 81.0% and 83.9% after 24, 48 and 72 hours with concentration 15 adults: 100 larvae. They were 86.5%, 87.5% and 88.1% after 24, 48 and 72 hours with concentration 20 adults: 100 larvae. On the other hand, concentration 25 adults:100 larvae gave 94.4%, 96.6% and 97.7% after 24, 48 and 72 hours.

Statistical analysis between different concentrations (10, 15, 20 and 25 adults) of adult copepod, *A. vernalis* against 1st and 2nd larvae of *A. aegypti* had significant effects (LSD = 1.1734, 0.8095, 0.8442 and 1.2321) after 24, 48, 27 and average mortality%, respectively (**Table 2**).

The predatory capacity of cyclopoid copepods was considered for use as a biological control agent for *A. aegypti* larvae [28]. Along with the other methods of control, such as chemical and environmental control, biological control can be classified as naturalistic control, which is a broad term for the control of mosquito larvae by predation. The importance of naturalistic measures in the control of *A. aegypti* has been well emphasized in recent years. One of the best methods of successfully combating mosquitoes on an extensive scale could be the biological control methods [13] [29]. The larvae showed damage mainly at the anal segment, the siphon and the abdomen; only three attacks to the head were observed. The size of the siphon might be of importance in determining whether or not a copepod will attack a mosquito larva [30].

The results of cage-stimulated experiments conducted by [11] [31] [32] showed that *M. guangxiesis* and *M. aspericorns* eliminated all mosquito larvae produced by 25 pairs of *A. aegypti* in 3-litre tins placed in screen cages that were inoculated by 50 gravid female *cyclopoids* six weeks after the start of the experiment. Fifty copepods each of *M. longuisetus* and *M. aspericornis* killed all *A. aegypti* larvae in 15-litre earthenware pots placed in cages within three weeks. The difference between the results of the present study and those of related studies is due to the larger size of the container and large volume of water (100 litres) which we used in our experiment and also experiments by [28], in addition to the species used. In such a large volume of water, the frequency of encounter between the predator and the prey is greatly reduced. In small-sized containers with a small volume of water, the chance of encounter is high. To compensate for the level of reduced encounters, it will be desirable to stock large numbers of predators, or augment the number of predators by timely inoculations. The

Table 2. Predatory efficiency of different concentrations of the adult copepod *Acanthocyclops vernalis* against 1st and 2nd larvae *Aedes aegypti* (100 larvae) in the presence and absence of an alternative food. (a) The absence of an alternative food; (b) The presence of an alternative food.

(a)

Concentrations of *A . vernalis*	%Mortality (Mean ± Standard Deviation)			Average mortality (%)
	24 h	48 h	27 h	
10	$61.6^{d} \pm 1.20$	$66.2^{d} \pm 0.68$	$68.5^{d} \pm 0.26$	65.4^{d}
15	$78.9^{c} \pm 0.61$	$81.0^{c} \pm 0.57$	$83.9^{c} \pm 0.36$	81.3^{c}
20	$86.5^{b} \pm 0.35$	$87.5^{b} \pm 0.40$	$88.1^{b} \pm 0.42$	87.4^{b}
25	$94.4^{a} \pm 0.38$	$96.6^{a} \pm 0.21$	$97.7^{a} \pm 0.23$	96.2^{a}
Control	$0.0^{e} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{e} \pm 0.0$	0.0^{e}
LSD	1.1734	0.8095	0.8442	1.2321
Probability	0.0001	0.0001	0.0001	0.0001

Different letters denote significant differences ($p < 0.05$) between means.

(a)

Concentrations of *A . vernalis*	%Mortality (Mean ± Standard Deviation)			Average mortality (%)
	24 h	48 h	27 h	
10	$51.4^{d} \pm 1.15$	$53.4^{d} \pm 0.30$	$55.2^{d} \pm 0.15$	53.3^{d}
15	$62.7^{c} \pm 0.40$	$65.4^{c} \pm 0.35$	$69.3^{c} \pm 0.21$	65.8^{c}
20	$71.2^{b} \pm 0.67$	$73.5^{b} \pm 0.35$	$77.0^{b} \pm 0.50$	73.9^{b}
25	$81.5^{a} \pm 0.25$	$85.3^{a} \pm 0.32$	$88.2^{a} \pm 0.31$	85.0^{a}
Control	$0.0^{e} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{e} \pm 0.0$	0.0^{e}
LSD	1.1506	0.539	0.5231	0.479
Probability	0.0001	0.0001	0.0001	0.0001

Different letters denote significant differences ($p < 0.05$) between means.

predatory capacity of two local populations of *Mesocyclops aspericornis* (Daday) and *Mesocyclops ogunnus* species were evaluated, in the Philippines, as a biological control agent for *Aedes aegypti* (L) mosquitoes by [33]. They found that *M. aspericornis* females were good biological control agents, for they destroyed/consumed about two-thirds of the wild dengue mosquito larvae population.

A comparative study between different concentrations (10, 15, 20 and 25 adults) of *A. vernalis* were introduced to 100 numbers of mosquito larvae into the 500 mL glass beaker in case the presence and absence of an alternative food under laboratory conditions are shown in **Table 2**. The efficiency of *A. vernalis* to feed on mosquito larvae in the presence of an alternative food is shown in **Table 2**. The percent reduction of *A. aegypti* larvae consumed by *A. vernalis* of concentration 10 adults:100 larvae of *A. Aegypti*, with an alternative food were 51.4%, 53.4% and 55.2% after 24, 48 and 72 hours, respectively (**Table 2**). While, mortality were 62.7, 65.4 and 69.3% after 24, 48 and 72 hours with concentration 15 adults: 100 larvae. They were 71.2, 73.5 and 77.0% after 24, 48 and 72 hours with concentration 20 adults: 100 larvae. On the other hand, concentration 25 adults: 100 larvae gave mortality 81.5, 85.3 and 88.2% after 24, 48 and 72 hours. [23] reported that the predatory copepod fed on 39% and 25% in I and III instar larvae of mosquito, and in combined treatment of *D. elata* and copepod maximum control of mosquito larval states and at 83%, 80%, 75% and 53% in I, II, III and IV instars, respectively.

Statistical analysis between different concentrations (10, 15, 20 and 25 adults) of adult copepod, *A. vernalis* against 1st and 2nd larvae of *A. aegypti* had significant effects (LSD = 1.1506, 0.539, 0.5231 and 0.479) after 24, 48, 27 and average mortality%, respectively (**Table 2**).

In laboratory bioassays tested the predatory capacity of the copepod *Mesocyclops annulatus* on *A. aegypti* and *Culex pipiens* larvae. *M. annulatus* caused 51.6% and 52.3% mortality of 50 first instar larvae of *A. aegypti* and *Cx. Pipiens*, respectively, in a 72 h test period. When alternative food was added to the containers, mortality rates declined to 16% and 10.3% for *A. aegypti* and *Cx. Pipiens*, respectively [13] [34]. [35] found that copepod predation significantly biased mosquito sex ratios toward females, and that both the males and females emerging from copepod-containing recipients were significantly larger than control insects.

4. Conclusion

In conclusion, the results of our study suggested that the predatory copepod *A. vernalis* can be used as a bio-control agent for the management of the mosquito vector *A. aegypti* larvae. These results were carried out in containers. More research is suggested in field to apply these results.

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