How to Cite:
Gandhi, G., Ravi Shankar, V., & Bhanu Prakash, K. (2023). Anopheles 3rd instar larvae predation by local cyclopoid copepod, Mesocyclops scrassus to prevent malaria in Telangana State. *International Journal of Health Sciences*, *7*(S1), 951–959. https://doi.org/10.53730/ijhs.v7nS1.14300

Anopheles 3rd instar larvae predation by local cyclopoid copepod, *Mesocyclops scrassus* to prevent malaria in Telangana State

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Abstract---*Mesocyclopus scrassus* is an insect predatory with an environmentally favourable profile. Although malaria is in control in India, there is a possible risk of spread from malarial infected people coming from other endemic countries. The probability of mosquito vectors in several parts of India, necessitates the identification of effective and innovative control methods. The present study focused on identifying an effective biological control method for the 3^{rd} instar of anopheline larvae using *Mesocyclops scrassus* under laboratory and field conditions to prevent malaria. So, we investigated its potency against *Anopheles* larvae.

Keywords---*Anopheles* larvae, biological control, copepods *(Mesocyclops scrassus),* predator capacity.

Introduction

In 2018, 228 million malaria cases were reported globally, nineteen countries in Sub-Saharan Africa [WHO Geneva.2015] and India carried almost 85% of the global malaria burden, *Plasmodium falciparum* is the most widespread malaria causative agent in the WHO South-East Asia Region (50%) [WHO, 2019]. In 2018, there were an estimated 4, 05,000 deaths from the world, compared with 4, 16,000 estimated deaths in 2017, and 5, 85,000 in 2010 [WHO, 2019]. There was

Manuscript submitted: 09 Jan 2023, Manuscript revised: 18 March 2023, Accepted for publication: 27 April 2023

an assessment that malaria positive cases in India turned down by 28% in 2018 compared with 2017 and 24% depletion in 2016-2017.

There were 6,737,000 malarial positive cases and a total of 9620 deaths in India in 2018, down from 9,348,000 positive cases and 16,310 deaths in 2017, according to the WHO record. [WHO, 2019]. *Anopheles culicifacies* (Diptera: Culicidae) a common vector of both *falciparum* and *vivax* malaria in India. These are two types of parasites of human malaria, which are commonly reported from India [NVBDCP, 2012]. Treatment with antimalaria drugs and vector control are integrated methods for malaria control. However, the use of antimalarial drugs over a long period has contributed to the development of drug-resistant malarial parasites in endemic areas [WHO Geneva, 1992]. In this, work focused on, vector control has played an important role in the reduction of malaria. Different methods available in vector control have been used throughout history, includes chemical and biological methods [Chitra T, et al, 2013]. Continuously using chemicals for vector control can affect non-target organisms, increase environmental pollutions, and develop resistance power in mosquitoes and ill-effects on humans and other aquatic animals. However, they are safe if used at the recommended chemical dosages. Therefore, to overcome these problems, emphasis has been placed recently on eco-friendly, cost-free, viable and innovative methodologies for vector control [Samanmali, et al., 2018].

Biological control methods are used in these works that have been used to control mosquitoes from many years. The WHO has recommended larvivorous fish, aquatic bugs, nematodes, dragonflies, beetle larvae, certain fly larvae, and cyclopoid copepods, in many parts of the world since 1940's [Udayanga L, et al, 2019]. The bacteria *Bacillus thuringiensis var israelensis (Bti*) has been in the Indian market for several years and is one of the most successful biological control agents currently used. *Bacillus sphaericus* is also used either alone or in combination with *Bacillus thuringiensis var israelensis (Bti)*.

Cyclopoid copepods are one of the crustacean's family, these are mosquito larval predators [Chitra T, et al, 2013]. The use of copepods for *dengue* vector larval control was first recognized by Riviere RF Riviere [Riviere RF, et al., 1981]. He observed that the number of *Aedes aegypti* and *Aedes polynesiensis* larvae highly reduced in ovitraps that contained *Mesocyclops aspericorni*s, accidentally introduced with creek water. Marten followed by Similar observations were reported independently [Marten GG, 1984] with *M. aspericornis* for *Aedes albopictus* larvae in household water sources in Hawaii. Since then, copepods have proved to be particularly effective at reducing the *Aedes sp.* production from household water storage tanks and other storage container breeding habitats that have water for long periods. The use of copepods in *Aedes* container habitats has been responsible for virtually all published instances of mosquito eradication in recent year in the world [Kay BH, et al, 2005]. However, the use of copepods to control anopheline mosquito larvae has not been sufficiently tested in Telangana in India. Therefore, the present study focused on identifying and observing effective biological control agents (only for larvae) for anopheline mosquito larvae using carnivorous copepods under laboratory and field conditions to prevent malaria in the Telangana state.

Material & Methods

Field collection of copepods

Copepods were collected from the margins of slow-moving waterways, small lakes, rivers, edges of local ponds, ditches and edges of other stagnating water sources to establish a culture. They were collected by dipping a standard mosquito dipper (Enamel bowl) flying pan or ladle should be immersed in the breeding places at an angle of 45° [NVBDCP 2016] in water, particularly near submerged vegetation. After dipping, 2/3 of the water in the container was slowly filtered through a zooplankton net. A larval net consists of a ring of iron frame of 25 cm in diameter with nylon/muslin cloth net measuring about 10 cm long [Lahiru Udayanga, et al, 2019]. The net was inverted over a container with clean dechlorinated water and the filtrate with copepods was washed using a squirt bottle into the containers.

Identification of field collected copepods in the laboratory

Some individual live copepods were preserved from selected sites under most circumstances, ethanol is the preservative of choice for copepod. In concentrations ranging from 70 to 75%. Dead copepods were placed individually on the microscopic glass slide with a minimum amount of water and identified under a light stranded microscope with an objective (10×) using perfect keys.

Identification of predatory efficacy of copepods in the laboratory

The predatory efficacy of copepod on *Anopheles culicifacies* larvae was estimated under laboratory conditions. About 150 newly hatched mosquito larvae were placed in 100–200 ml sterile culture plate wells. The containers were filled with dechlorinated water and 15 adult copepods were introduced. Different species of copepods were introduced to separate sterile culture plates. The larvae remaining in each container and dead larvae were observed at 4 h intervals until 24 h at 27 °C, under constant laboratory lighting conditions. Count the all larvae first, recount and we know how many larvae have been eaten by copepod after 24h Finally, *Anopheles culicifacies* larvae were counted using a magnifying glass (90 mm) every 24 h for 4 days after which the number of larvae consumed was replenished in each of the containers to maintain the prey density for another interval. The Predatory efficiency of a copepod was calculated by the formula below [NVBDCP 2015].

$$Predatory\,efficiency = \frac{No.of\ prey/No.of\ predator\ introduced}{Total\ no.of\ prey\ introduced}\ X\,100$$

This experiment was repeated 15 times to identify the best species for effective larval control.

Identification of the predatory efficacy of copepods in the field

Identification of the study area: The identification of predatory efficacy of carnivorous copepods in the field study area was selected in the District of

Khammam, Telangana State, India. Khammam was selected due to this time of a greater number of rivers, bedrock pools with high surveillance of anopheline mosquitoes including *Anopheles culicifacies*. These larval prone areas identified through Geographical Information System and Remote Sensing were selected for intervention with copepods.

Pre- field monitoring

Each larval breeding site in the Khammam, Telangana State area was monitored from May to July 2020 as a primary case study for mosquito larvae. Anopheline larvae sample were collected from each site by National Vector Borne Diseases Control Programme (NVBDCP) standard dipper. The same method was repeated more than three times in each larval prone area. From each breeding sites, approximately 10 dips were collected depending on the size of the breeding sites. The volume of each dip was around 200–250 ml. The number of dips and anopheline larval species positive dips was recorded. Among the positive dips, a number of different larval stages were recorded. Collected *Anopheles culicifacies* mosquito larvae were reared until the adults emerged. Emerged adults were identified to the species level by using an achromatic magnifying lens (10×) following the taxonomic keys prepared by IVM, India [15].

Intervention

A total of 30 adults of two copepod species, namely *M. scrassus* and *C. varicans* in this observation *M. scrassus* proved to be effective, Anopheline larval controlling agents in the laboratory, were introduced separately into 15 sites with 20 gravid females of each species for one site. Three sites and four days were maintained as a control without intervention with copepods.

Post-intervention

Survival of the introduced copepods in the respective water login sites was evaluated (in terms of number) before and after 24, 48, 72 and 96hrs. The abundance of the *Anopheles culicifacies* mosquito larvae in each site were monitored after the introduction of copepods once a fortnight. For the collection of mosquito larvae, water samples, were systematically taken by dipping every site. From the collected water samples anopheline mosquito larval counts were made. Water samples were (5 dips/ site) poured away slowly through a zooplankton net and the copepods retained on the mesh were counted. Counted copepods and mosquito larvae were re-introduced into the respective breeding sites. The percentage of larval reduction was calculated by the following formula [14, 15]

$$\% \, Reduction = 100 \, \frac{C1 \, x \, T2}{T1 \, x \, C2} \; X \, 100$$

Where,
C_1 = Pre-treatment immature density in control habitats, C_2 = Post-treatment immature density in control habitats, T_1 = Pre-treatment immature density in treated habitats, T_2 = Post-treatment immature density in treated habitats.

Data analysis

The numbers of *Anopheles* larvae predated by the *M. scrassus* and *C. varicans* were analysed with ANOVA to evaluate the predatory efficacy of copepod species, and the most effective copepod species identified.

Results

Field collection of copepods the copepod species, namely *M. scrassus*, and *C. varicans* were collected from lakes, streams small local ponds and rivers. The species collected and their locations are given in the table below:1.

Table 1. Collected copepod species, GIS locations and GPS point

No of Copepod species	Location	GPS points
M. scrassus(15), *C. varicans*(15)	Khammam river	17° 14' 50.1108'' N 80° 9' 5.1948'' E
	Palair lake (near pond also)	15°19′N 80°06′E 15.317°N 80.100°E
	Wayra river (near diches, ponds).	17.210009°N 80.376660°E
	Lakaram lakes and local ponds	17°14′53.6″N80°09′32.5″E

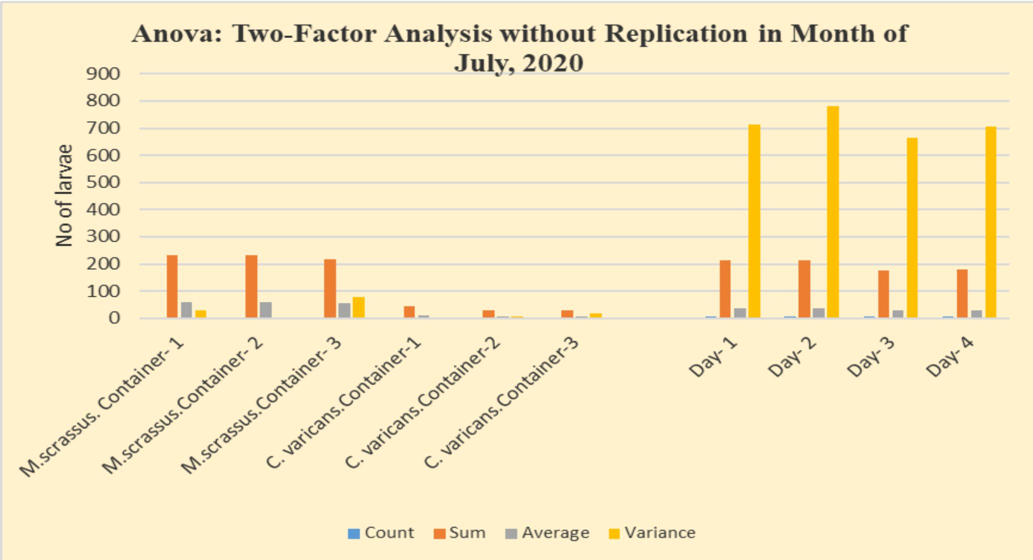
Table 2. Predatory efficacy of two copepod species on the larvae of An. Culicifacies in Month of July.2020

Species	No of copepod introduction		Day-1	Day-2	Day-3	Day-4	Predatory efficiency of single copepod/day
M.scrassus	15	Container-1	61	63	58	51	58.50%
		Container-2	59	60	57	56	58%
		Container-3	60	61	42	55	54%
C. varicans	15	Container-1	13	11	11	9	11%
		Container-2	9	10	6	4	6.75%
		Container-3	12	10	3	4	8%

Table No. 2.1. Anova: Two-Factor Analysis without Replication in Month of July, 2020

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
M.scrassus. Container-1	4	233	58.25	27.58333
M.scrassus. Container-2	4	232	58	3.333333
M.scrassus. Container- 3	4	218	54.5	76.33333
C. varicans. Container-1	4	44	11	2.666667
C. varicans. Container-2	4	29	7.25	7.583333
C. varicans. Container-3	4	29	7.25	19.58333
Day- 1	6	214	35.66667	712.6667

Day- 2	6	215	35.83333	781.3667
Day- 3	6	177	29.5	664.3
Day- 4	6	179	29.83333	706.9667



Graph No. 1. Anova: Two-Factor Analysis without Replication in Month of July, 2020

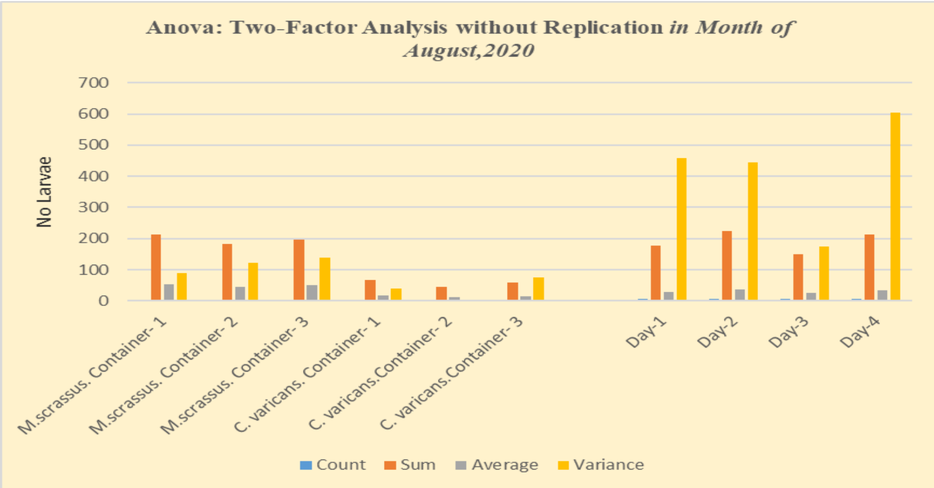
Table 3. Predatory efficacy of two copepod species on the larvae of *An. Culicifacies* in Month of August, 2020

Species	No of copepod introduction		Day -1	Day -2	Day -3	Day -4	Predatory efficiency of single copepod/day
M.scrassus	*15*	Container-1	52	64	41	55	53.00%
		Container-2	52	42	32	57	46.75%
		Container-3	42	58	37	61	49.50%
C. varicans	*15*	Container-1	10	25	17	14	16.40%
		Container-2	14	9	12	10	11.25%
		Container-3	7	27	11	15	15%

Table. 3.1. Anova: Two-Factor Analysis without Replication in Month of August, 2020

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
M.scrassus Container- 1	4	212	53	90
M.scrassus Container- 2	4	183	45.75	122.9167
M.scrassus Container- 3	4	198	49.5	139
C. varicans Container- 1	4	66	16.5	40.33333
C. varicans Container- 2	4	45	11.25	4.916667
C. varicans Container- 3	4	60	15	74.66667
Day-1	6	177	29.5	459.1
Day-2	6	225	37.5	444.3

Day-3	6	150	25	175.6
Day-4	6	212	35.33333	605.0667



Graph No.2. Anova: Two-Factor Analysis without Replication in Month of August, 2020

A total of 30 adults of two copepod species, *M. scrassus* proved to be effective anopheline larval controlling agents in the laboratory, three containers and four days were maintained with copepods and show the result were shown in tabular forms. Prior to the intervention larval population of *Anopheles* mosquito was higher in all the study sites. The breeding capacity and survival rate were high for all the two copepods species at all the above study sites. In the container or lab conditions and field sites with *M. scrassus* indicated the lowest abundance of mosquito larvae. Therefore, it can be suggested that, *M. scrassus* has the highest degree of predatory efficacy of *Anopheles culicifacies* than *C. Varicans to* control the malaria and mosquito borne diseases in the Telangana State.

Discussion

The current study was going the effectiveness of two locally available crustacean organism of copepods, namely *M. scrassus* and *C. varicans,* collected from different sites in the district as a biological control method of *Anopheles* vector**,** *Mesocyclops scrassus was* found in Khammam District*, C. varicans* are common species in the districts of Khammam. Out of the two predators, *Mesocyclops scrassus* copepod species showed significant effects against the *anopheles larvae* of the malaria vector, in both the laboratory and field conditions.

Studies before this have reported *Mesocyclops sp.* Such as *M. thermocyclopoides* [Benelli, et al, 2016], *M. aspericornis* [Lahari Udayanga, et al, 2019], as efficient biological control agents of 1st and 2nd instar mosquito larvae. In this study the survival rate of *Mesocyclops scrassus* was inferior, but their predatory efficacy was very high level in both the laboratory and field levels. Another species such as *C. varicans* was a weak predator while showing a high survival rate in the field and laboratory. *Anopheles culicifacies* mosquitoes, which is considering as the primary vector of malaria in India, breeds in natural streams, containers, ditches, lakes, rivers, in wet areas, or during high rainfall season [S. S. Sahu, et al., 2017 & WHO 2003]**.** Although earlier studies World Health Organization have recognized the high larvivorous potential of Guppy and Gambusia affinis, this species recommended to larvivorous fish as an effective biological controlling method for *Anopheles* mosquito control [Pernia, et al., 2007] limitations in the body size and depth of such breeding places often reduce the survival of the introduced fish. Therefore, *Mesocyclops scrassus* that can easily survive even in the small pools, ponds, containers and lakes also could be introduced for control of anopheline mosquito larvae.

The current study focused on the 3rd instar of the larval stage, it was observed that *M. scrassus* is a more perfect predator than the other species in the laboratory and field conditions. Copepods are successful predators of the 3rd instar stage in *anopheles* mosquito larvae than the 4th instar. Because of the swimming methods, based on body size and shape of the copepods, the predatory capacity on the 4th instar *anopheles mosquito* larvae was close to zero. Therefore, under field conditions, many circumstances required to be evaluated, such as predatory efficacy survival rates, physical parameters and chemical parameters of water, reproductive function and food sources.

Therefore, some useful parameters should be followed before the use of copepods for mosquito larval controls such as copepod species, area, temperature, habitat type, how long the population lasts in that kind of local water login in the objects and the number of copepods in the objects. The use of synthetic chemicals for mosquito larval control and adult control increases damage to the total environment like water, soil, air, effect to the terrestrial and aquatic animals. The biological control method of adult mosquito and larvae is an efficient method and eco-friendly than using chemical insecticides.

Conclusion

These study findings suggest that copepods can be employed for the biological control of anopheline larvae. Among the two copepod species which were used to evaluate predatory efficacy on anopheline mosquito larvae, *M. scrassus* showed the high-level predacious capacity under laboratory and field conditions. Thus, larvivorous fish and copepods can also be utilised as a perfect agent in Integrated Vector Management. It is pivotal to identify local copepod species that thrive under the conditions prevalent in mosquito larval hotspots in different areas of the state for use in the control of mosquitoes. Anopheline mosquito control using copepods should be used in addition to other biological methods that were already introduced in India on 1st and 2nd Instar larval stage.

Acknowledgements

The study was supported by SR & BGNR Govt Degree and PG College, Khammam. “Functional diversity of ecosystems”.

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