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ORIGINAL ARTICLE

PREDATORY ABILITY OF *MESOCYCLOPS ASPERICORNIS* ON THE LARVAE OF *CULEX QUINQUEFASCIATUS* UNDER MONOCROTOPHOS POLLUTED CONDITION

[1]V. Nathiya, [1]P. Saravana Bhavan, [*1]K. Murugan, [1]C. Panneerselvam, [1]U. Suresh, [1]C. Vadivalagan, [2]K. Pugazhendy and [3]Jiang-Shiou Hwang

[1]Department of Zoology, Bharathiar University, Coimbatore- 641029, India.
[2]Department of Zoology, Annamalai University, Annamalainagar-608002, India.
[3]Institute of Marine Biology, National Taiwan Ocean University, Keelung 202-24, Taiwan.

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ABSTRACT

In the present study, a biological approach with *Mesocyclops aspericornis* was adopted for determining its predatory ability on the larvae of *Culex quinquefasciatus* under unpolluted, and polluted conditions with a synthetic organic insecticide, monocrotophos. The 24 hr LC_{50} value of monocrotophos to different instar larvae of *C. quinquefasciatus* was ranged between 3.045 - 9.357 ppm (3.045, 4.227, 6.232 and 9.357 ppm for I, II, III and IV Instar respectively). In this study, monocrotophos polluted condition (1 ppb of commercial grade as such) was created artificially in the laboratory. The predatory ability of *M. aspericornis* against the larval instars of *C. quinquefasciatus* in normal (unpolluted) condition was ranged between 66 – 2.8 % at 5 days duration (66, 42.2, 9.6 and 2.8% for I, II, III and IV instar respectively). The predatory ability of *M. aspericornis* against the larval instars of *C. quinquefasciatus* under monocrotophos polluted condition was ranged between 83.6 – 13.2% at 5 days (83.6, 61, 22 and 13.2% for I, II, III and IV instar respectively). *C. quinquefasciatus* larval mortality rate against monocrotophos was instar dependent and toxic dose dependent. I-instar was more susceptible to monocrotophos, followed by other instars. Actually, the toxicity of monocrotophos to *M. aspericornis* showed some resistance. The predatory ability of *M. aspericornis* against *C. quinquefasciatus* was instar dependent and toxic condition dependent. This ability was higher against the I-instar larvae over other instars. The overall predatory ability of *M. aspericornis* against *C. quinquefasciatus* was higher under monocrotophos polluted condition than that of the unpolluted environmental conditions. This suggests that the survival of *M. aspericornis* was higher in polluted environment and under such an environment its predatory ability was also enhanced as this species is pollution indicator.

Keywords: *Culex quinquefasciatus*, Monocrotophos, *Mesocyclops aspericornis*, Toxicity, Predation.

1.INDRODUCTION

Arthropoda have dangerous vectors of important pathogens and parasites, which may hit as epidemics or pandemics in the increasing world population of humans and animals. Mosquitoes (Diptera: Culicidae) represent a key threat for millions of organisms worldwide, since they act as vectors for malaria, dengue, yellow fever, West Nile virus, Japanese encephalitis, and filariasis (Mehlhorn et al. 2012). Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. More than 1.4 billion people in 73 countries are living in areas where lymphatic filariasis is transmitted and are at risk of being infected. Globally, an estimated 25 million men suffer with genital disease and over 15 million people are afflicted with lymphoedema (WHO 2014). Currently, the main control tool against *Culex* larvae is represented by treatments with organophosphates and insect growth regulators. Monocrotophos (phosphoric acid dimethyl (1-methyl-3(methylamino)-3-oxon-propenyl ester), commonly known as Azodrin, is one of the organophosphorus insecticides extensively used in agriculture, animal husbandry and public health (Rao 2004; Ray *et al.* 1985; Theurkar et al. 2014). Monocrotophos is recognized as an acutely hazardous pesticide with human health risk. Monocrotophos is toxic to humans and continuous exposure leads to poisoning by inhibition of acetylcholine esterase mechanism (Luch 2005; Farmer 2004; Denissenko et al. 1996; Brookes and Lawley 1964; Perera et al. 1992).

Corresponding* author: ***Dr. K. Murugan,* *Department of Zoology, Bharathiar University, Coimbatore-641029, India*

Insecticides affect the aquatic organisms, especially fishes. However, the use of chemical insecticides has been greatly impeded due to development of physiological resistance in the vectors, environmental pollution resulting in bio-amplification of food chain contamination and harmful effects on beneficial non-target animals. However, the utilization of synthetic organic insecticides cannot be completely avoided. Its application is very common in agriculture and public health practices.

Biological and/or environmental management methods can be used to reduce mosquito vector population without harming environment and the biodiversity (Kamareddine 2012). War et al. (2011) studied on larvicidal property of neem oil and monocrotophos at different concentrations against fourth instar larvae of *Pericallia ricini* (Fab.) revealed that neem oil was highly effective. The ingestion of these crystals by mosquito larvae rapidly leads to the formation of pores, cell lysis, septicemia and finally larva death (Gill et al. 1992; De Maagd et al. 1999). Adequate emphasis need to be given to search for biocontrol agents, and the possibility of applying several control measures simultaneously to achieve 100% control of vectors (Marten, et al., 1993; Murugan et al., 2011). **Ex**amples are odonate young instars, water bugs, tadpoles, fishes, crabs and copepods (Bowatte et al. 2013; Kalimuthu et al. 2014). Copepods, the small aquatic crustacean are omnivorous and can prey on immature mosquitoes, especially first-instar larvae, but rarely on later stages (Hurlbut 1938; Riviere and Thirel 1981; Marten et al. 1989; Williamson 1999). In particular, several species of copepods, such as *Mesocyclops aspericornis*, *M. guangxiensis*, *M. longisetus* and *M. thermocyclopoides* have been reported as potential biological control agents against *Aedes* mosquitoes (Rawlins et al. 1997; Jekow et al. 1998; Manrique-Saide et al. 1998; Schaper, 1999; Locantoni et al. 2006). Operationally, the use of copepod predators against Culicidae larvae in urban and semi-urban habitats is not expensive and requires little labor for colony maintenance (Soumare and Cilek 2011; Chitra et al. 2013; Kalimuthu et al. 2014).

Therefore, the present study was designed to evaluate the mosquito larvicidal effects of monocrotophos against *C. quinquefasciatus* and to check the predatory efficiency of the cyclopoid crustacean *M. aspericornis* against *C. quinquefasciatus* larval instars under normal (unpolluted) and monocrotophos contaminated water environment created under laboratory conditions.

2.MATERIALS AND METHODS

Rearing of *C. quinquefasciatus*

Eggs of *C. quinquefasciatus* were collected from local breeding habitats in Coimbatore (India) using an "O" type brush (Dinesh et al. 2015). Eggs were transferred to laboratory conditions [27 ± 2°C, 75-85% R.H., 14:10 (L:D) photoperiod] and placed in 18 x 13 x 4 cm plastic containers containing 500 mL of tap water until hatching. Larvae were reared in the plastic containers described above, and fed daily with a mixture of crushed dog biscuits and hydrolyzed yeast at 3:1 ratio (w:w). Water was renewed once in two days. The breeding medium was checked daily and dead individuals were removed. Breeding containers were kept closed with muslin cloth to prevent contamination by foreign mosquitoes. Pupae were collected daily from culture containers and transferred to glass beakers containing 500 mL of water. Each glass beaker contained about 50 mosquito pupae and was placed in a mosquito cage (90 x 90 x 90 cm) for adult emergence. Plastic frames and chiffon walls composed each cage. Both sexes were continuously provided with 10% (w:v) glucose solution on cotton wicks. The cotton was always kept moist with the solution and changed every day. Female mosquitoes were also allowed to feed on a rabbit host. The shaved dorsal side of the rabbit was positioned on the top of the mosquito cage in contact with the cage screen overnight. To collect eggs, an egg trap lined with filter paper containing water was placed in the cage.

Toxicity of monocrotophos against *C. quinquefasciatus*

Commercial formulation of Monocrotophos (Jeyakrishna Pesticide Limited, Salem) were purchased from the local market and utilized for the experiment. Following the methods reported in Suresh et al. (2015), 25 *C. quinquefasciatus* larvae (I, II, III or IV instar) were placed in a 500-mL glass beaker filled with 249 mL of dechlorinated water and 1 mL of the desired concentration monocrotophos was added (1.56, 3.125, 6.25, 12.5 and 25.0 ppm). Larval food (0.5 mg) was provided for each tested concentration. Each concentration was replicated three times against all instars. In control treatments, 25 larvae or pupae were transferred in 250 mL of dechlorinated water. Percentage mortality at 24 hr was calculated as follows:

Percentage mortality = Number of dead individuals / Number of treated individuals X 100.

Predation of *M. aspericornis* against *C. quinquefasciatus* in unpolluted condition

In this experiment, the predation efficiency of *M. aspericornis* adults was assessed against *C. quinquefasciatus* larvae. For each instar, 100 mosquitoes larvae in each instar were introduced, with 10 copepods, in a 500-mL glass beaker containing 250 mL of dechlorinated water. Mosquito larvae were replaced daily with new ones. For each mosquito instar, four replicates were conducted. Control was 250 mL of dechlorinated water without copepods. All beakers were checked after 1, 2, 3, 4 and 5 hours and the number of prey consumed by copepods was recorded. Predatory efficiency at 5 days duration was calculated using the following formula:

Predatory efficiency = [(Number of consumed mosquitoes / Number of predators) / total number of mosquitoes] X100.

Predatory efficiency of *M. aspericornis* in monocrotophos polluted condition

In this experiment, the predation efficiency of *M. aspericornis* adults was assessed against *C. quinquefasciatus* larvae in monocrotophos polluted condition. For each instar, 100 mosquitoes were introduced with 10 copepods in a 500-mL glass beaker filled with 250 mL of dechlorinated water containing 1 ppb monocrotophos (5 hr LC_{50} of monocrotophos to I-instar larvae of *C. quinefasciatus* was 3.125 ppm, its $1/3^{rd}$ concentration was around 1.0 ppm, and

its lowest concentration was opted as polluted environment for survival of *M. aspericornis*. The predatory efficiency of *M. aspericornis* against *C. quinefasciatus* larvae was assessed essentially as described previously.

The average mosquito mortality data were subjected to probit analysis. LC_{50} and LC_{90}, were calculated using the method by Finney (1971). Data were analyzed by *t* test using the SPSS Statistical Software Package version 17.0. A probability level of $P < 0.05$ was used for the significance of differences between values.

3.RESULTS AND DISCUSSION

Toxicity of monocrotophos against *C. quinquefasciatus*

Monocrotophos was toxic to the larval instars (I-V) of *C. quinquefasciatus* in laboratory experiments. The 5 hr LC_{50} of monocrotophos were 3.045 ppm (I instar), 4.227 (II instar), 6.232 (III instar), and 19.357 (IV instar) (Table 1). For instance, Ooi *et al*. (1975) found the trunk injection of monocrotophos may be employed to assist the tachinid parasite, *Bessa remota* to control the coconut caterpillar, *Artona catoxantha*. Further, Sahayaraj and Amalraj (2006) have reported that synthetic pesticide monocrotophos and neem oil combination was found to be very effective in reducing the defoliator infestation in groundnut. Recently, Anbu Radhika and Sahayaraj (2014) studied that the synergistic effect of monocrotophos with neem oil or commercial neem-based pesticide, pungam oil and biosilver nanoparticles on feeding, survival, growth and development of *Spodoptera litura*

Predation of M. aspericornis **against *C. quinquefasciatus***

M. aspericornis adults actively predate *C. quinquefasciatus* young larval instars in unpolluted condition. Predation efficiency of *M.* aspericornis against *C. quinquefasciatus* larvae was 66% (I) and 42.2% (II). Predation against late-instar larvae of *C. quinquefasciatus* was minimal. The predatory efficiency per copepod per day was 6.6, 4.22, 0.96 and 0.28 larvae for *C. quinquefasciatus* (I, II, III and IV, respectively) (Table 2). Similarly, the predatory efficiency of a single adult copepod of *M. thermocyclopoides* was 6.5, 4.6, 0.76, and 0.14 *C. quinquefasciatus* larvae per day (I, II, III and IV instar, respectively), while it was 8.7, 5.9, 1.2 and 0.36 larvae day (I, II, III and IV instar, respectively) after treatment with *Solanum xanthocarpum* fruit extract (Mahesh Kumar et al. 2012). Further, Vasugi et al. (2013) reported that the combined actions of plant extract and predatory copepod to effectively control mosquito population and reduce the dengue transmitting diseases.

Under monocrotophos polluted condition, the predatory efficiency of *M. aspericornis* increased (Table 2). Actually, the predatory ability of *M. aspericornis* against the larval instars of *C. quinquefasciatus* under monocrotophos polluted condition was ranged between 83.6 – 13.2% at 5 days (83.6, 61, 22 and 13.2% for I, II, III and IV instar, respectively). The percentage predatory efficiency of copepod on *C. quinquefasciatus* larvae was increased under polluted condition when compared with unpolluted condition. I and II instars were much preferred when compared with the later ones. It is suggested that the percentage predatory efficiency of copepod in monocrotophos polluted environment may be decreased when the pollution level is increased.

Table 1. Toxicity test for monocrotophos against the larvae of filarial vector, *C. quinquefasciatus*

Target instar	Concentration of monocrotophos and 24 hr mortality of mosquito larvae					LC_{50} (LC_{90})	95% Confidence Limit LC_{50} (LC_{90})		Regression equation	x^2
	1.56 ppm	3.12 ppm	6.25 ppm	12.5 ppm	25.0 ppm		LCL	UCL		
I	41.2±2.58	54.2±1.30	64.6±1.14	82.4±1.51	100±0.00	3.045 (14.525)	1.524 (12.527)	4.241 (17.602)	y=0.340+ 0.112*x*	2.268
II	35.4±2.07	48.2±2.38	60.2±1.48	78.2±1.64	95.4±2.19	4.227 (18.891)	2.525 (16.387)	5.651 (22.614)	y=0.369+ 0.087*x*	2.318
III	30.6±1.81	40.2±1.92	55.2±2.58	72.6±2.40	89.0±1.58	6.232 (23.656)	4.446 (20.484)	7.835 (28.413)	y=1.458+ 0.074*x*	4.585
IV	26.0±1.58	33.4±1.94	45.0±1.22	66.0±2.91	80.0±1.00	9.357 (29.693)	4.849 (22.028)	14.103 (51.318)	y=0.590+ 0.063*x*	5.411

Mortality rates are means ± SD of three replicates; LC_{50} = Lethal concentration that kills 50% of the exposed organisms; LC_{90} = Lethal concentration that kills 90% of the exposed organisms; LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; All the values are significantly different ($P<0.05$).

Table 2: Predatory efficiency of copepod, *M. aspericornis* on the larvae of filarial vector, *C. quinquefasciatus* in unpolluted and monocrotophos polluted condition.

Treatment	Targeted instar	Number of consumed preys					Total predation (Nos.)	Percentage predation	Consumed preys per copepod per day (n)
		Day 1	Day 2	Day 3	Day 4	Day 5			
Control (unpolluted)	I	83±1.58	73±2.73	70±1.22	64±2.00	40±1.00	**330**	66.0	6.6
	II	61±1.87	50±1.58	43±2.23	37±2.00	30±1.58	211	42.2	4.22
	III	19±2.34	12±1.87	9±1.22	5±1.73	3±1.41	48	9.6	0.96
	IV	6±0.70	4±1.58	3±1.22	2±1.00	1±0.70	14	2.8	0.28
Momocrotophos (polluted)	I	95±2.54	90±2.12	83±1.58	78±1.22	72±2.34	418	83.6	8.36
	II	76±2.34	64±2.73	60±1.58	55±1.87	50±1.22	305	61	6.1
	III	30±2.54	25±2.0	21±1.58	18±1.22	16±1.0	110	22	2.2
	IV	23±2.34	18±2.23	13±1.87	8±1.41	4±1.22	66	13.2	1.32

Predation rates are mean ±SD of four replicates (10 copepods vs. 100 mosquitoes per replicate); All the values are significantly different ($P<0.05$).

This part needs to be studied and clarified. To the best of our knowledge, no evidences are available about how monocrotophos affect the predatory efficiency of copepods on young instars of mosquitoes. It has been reported that *M. aspericornis* copepods showed greater predation against mosquito larvae, when we introduced *B. thuringiensis*, and some plant extracts as bio-control tool in the environment (Nam et al. 2000; Mahesh Kumar et al. 2012; Murugan et al. 2002, 2011, 2015a,b).

C. quinquefasciatus larval mortality rate against monocrotophos was instar dependent and toxic dose dependent. I-instar was more susceptible to monocrotophos, followed by other instars. Actually, the toxicity of monocrotophos to *M. aspericornis* showed some resistance, as copepods are pollution indicators. The predatory ability of *M. aspericornis* against *C. quinquefasciatus* was instar dependent and toxic condition dependent. This ability was higher against the I-instar larvae followed by the II-instar and others. The overall predatory ability of *M. aspericornis* against *C. quinquefasciatus* was higher under monocrotophos polluted condition than that of the unpolluted environmental conditions. This suggests that the survival of *M. aspericornis* was higher in polluted environment and under such an environment its predatory ability was also enhanced as this species is pollution indicator.

4.CONCLUSION

This study showed that monocrotophos was effective in control of *C. quinquefasciatus* under laboratory conditions. Our results pointed out that monocrotophos can be employed at very low dose without causing any detrimental effects on the predation of *M. aspericornis* against *C. quinquefasciatus*. Therefore, over contamination of natural environment could be monitored to protect the natural enemies, such as copepods to check the larval population of mosquitoes.

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