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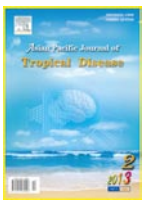

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# Laboratory and field efficacy of *Pedalium murex* and predatory copepod, *Mesocyclops longisetus* on rural malaria vector, *Anopheles culicifacies*

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PEER REVIEW

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**Comments**

The research work is very much important to the society to control morbidity and other defects caused by mosquitoes and also other insect vectors. This work reports a novel approach for the control of vector mosquitoes.
(Details on Page 117)

ABSTRACT

**Objective:** To test the potentiality of the leaf extract of *Pedalium murex* (*P. murex*) and predatory copepod *Mesocyclops longisetus* (*M. longisetus*) in individual and combination in controlling the rural malarial vector, *Anopheles culicifacies* (*An. culicifacies*) in laboratory and field studies. **Methods:** *P. murex* leaves were collected from in and around Erode, Tamilnadu, India. The active compounds were extracted with 300 mL of methanol for 8 h in a Soxhlet apparatus. Laboratory studies on larvicidal and pupicidal effects of methanolic extract of *P. murex* tested against the rural malarial vector, *An. culicifacies* were significant. **Results:** Evaluated lethal concentrations ($LC_{50}$) of *P. murex* extract were 2.68, 3.60, 4.50, 6.44 and 7.60 mg/L for I, II, III, IV and pupae of *An. culicifacies*, respectively. Predatory copepod, *M. longisetus* was examined for their predatory efficacy against the malarial vector, *An. culicifacies*. *M. longisetus* showed effective predation on the early instar (47% and 36% on I and II instar) when compared with the later ones (3% and 1% on III and IV instar). Predatory efficacy of *M. longisetus* was increased (70% and 45% on I and II instar) when the application was along with the *P. murex* extract. **Conclusions:** Predator survival test showed that the methanolic extract of *P. murex* is non-toxic to the predatory copepod, *M. longisetus*. Experiments were also conducted to evaluate the efficacy of methanolic extract of *P. murex* and *M. longisetus* in the direct breeding sites (paddy fields) of *An. culicifacies*. Reduction in larval density was very high and sustained for a long time in combined treatment of *P. murex* and *M. longisetus*.

KEYWORDS

*Anopheles culicifacies*, *Pedalium murex*, *Mesocyclops longisetus*, Larvicidal, Predatory efficacy

## 1. Introduction

Malaria and other vector-borne diseases contribute to the major disease burden in India. Mosquitoes are vectors of several diseases like malaria, filariasis, dengue fever, yellow fever, *etc.*, causing serious health problems to human beings. Malaria is one of the world's major public health concerns and contributes to 216 millon clinical cases and under a millon deaths each year, about two thirds of the confirmed cases of malaria in Southeast Asia are reported from India[1].

*Anopheles culicifacies sensu lato* (*s.l.*) is well established as the major vector of both falciparum and vivax malaria[2]. Earlier studies unequivocally incriminated *Anopheles culicifacies* (*An. culicifacies*) as the major malaria vector, responsible for transmission of 65% of malaria cases in India[3]. *An. culicifacies* exists as a complex of five sibling species provisionally designated as A, B3, C4, D5 and

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E6. These sibling species are reported to have various biological differences, *viz.* their distribution, response to insecticides[4], host preferences and vector carrying capacity[5]. *An. culicifacies* A and C are primary vectors whereas species B has very little role, if at all, in the transmission of malaria[6].

The outbreaks of malaria and other mosquito transmitted diseases are due to the increase in breeding sites in today's throwaway society, creating healthy environment for mosquito larvae. Control of mosquito immature's using synthetic insecticides creates multifarious problems like environmental pollution, insecticide resistance and toxic hazards to humans. Globally there have been conscientious efforts to overcome these problems and great emphasis has been placed recently on eco-friendly and economically viable methodologies for pest control. In recent years, biocontrol using predatory copepods and plant products have received much attention as potent bioactive compounds against various species of mosquitoes[7,8].

*Pedalium murex* (*P. murex*) commonly known to the world as "Large caltrops". It is commonly found in Deccan and in some parts of Ceylon and Gujarat. It is about 15 to 40 cm in height, having four angled spiny brownish colour fruits. The fruits are rich in polyphenolics (flavonoids and phenolics), glycosides like sapogenin (diosgenin 0.06%) and soluble proteins (20.14 mg/g)[9,10]. It has also been evaluated for its analgesic and antipyretic activities. Flavanoid, an important constituent of *P. murex*, has been reported for its antioxidant activities. Several other plants containing antioxidant properties exhibited nephroprotective activity against gentamicin and cisplatin[10].

The petroleum ether extract of *P. murex* is effective against Japanese encephalitis vector *Culex quinquefasciatus*[11]. The aqueous extract of the whole plant has been found to possess analgesic and anti-inflammatory properties[12]. Extensive phytochemical investigations on the plant have revealed the presence of Pedalitin and Pedalin (major flavanoids) along with Diosmetin, Dinatin, Dinatin-7-glucoronide, Quercetin, Quercimeritin, and Quercetin-7-glucorhamnoside[13]. Copepods are predatory crustaceans. It is known that copepods prey on mosquito larvae but active research on the predatory copepods was carried out from 1980s[14-16]. Since then, few species of predatory copepods was found to be potential in controlling mosquitoes at their larval stage. Recent and earlier reports on *Mesocyclops thermocyclopoides*, against *Aedes aegypti* (*Ae. aegypti*)[17], *Mesocyclops longisetus* (*M. longisetus*) and *Macrocyclops albidus* against *Anopheles* spp. and *Culex quinquefasciatus*[18], *Mauremys guangxiensis* against *Ae. aegypti*[19] states that copepods are effective mosquito control agents and are practical for large-scale use.

*M. longisetus* is common enough in irrigation water to stock the fields when they are flooded. *M. longisetus* is the one among the largest copepod species and a correspondingly aggressive predator[20].

Laboratory and field studies were carried out in the present study to test the potentiality of the leaf extract of *P. murex* and predatory copepod *M. longisetus* in individual and combination in controlling the rural malarial vector, *An. culicifacies*.

## 2. Materials and methods

### 2.1. Plant collection and extracts preparation

Leaves of *P. murex* (Pedaliaceae) were collected from in and around Erode (11° 20′ N and 77° 43′ E), Tamilnadu, India. The plants were authentified at Botanical Survey of India, and the specimens were deposited at Zoology Department, Bharathiar University, Coimbatore (11° 1′ 6″ N, 76° 58′ 21″ E), India. Leaves of *P. murex* washed with tap water, shade dried at room temperature and powdered by an electrical blender. The active compounds were extracted with 300ml of methanol for 8hrs in a soxhlet apparatus[21]. The crude extracts were evaporated to dryness in rotary vacuum evaporator and diluted to different concentrations for bioefficacy study.

### 2.2. Mosquito rearing

The eggs of *An. culicifacies* were collected from from paddy field in Erode (77° 42.5″ N and 77° 44.5″ E), Tamil Nadu, India. These were returned to the laboratory and transferred (the same aliquot numbers of eggs) to 18 cm×13 cm×4 cm enamel trays containing 500 mL of water, where they were allowed to hatch.

Mosquito larvae were reared at (27±2) °C and 75%-85% RH in a 14:10 (L:D) photoperiod. Larvae were fed 5g ground dog biscuit and brewers yeast daily in 3:1 ratio. Pupae were collected and transferred to plastic containers with 500 mL of water. The container was placed inside a screened cage (90 cm×90 cm×90 cm) to retain emerging adults, for which 100 mL/L sucrose in water solution was available *ad libitum*. On Day 5 post- emergence, the mosquitoes were provided access to a rabbit host for blood feeding. The shaved dorsal side of the rabbit was positioned on the top of the mosquito cage in contact with the cage screen (using a cloth sling to hold the rabbit) and held in this position overnight. Glass Petri dishes lined with filter paper and containing 50 mL of water were subsequently placed inside the cage for oviposition by female mosquitoes.

### 2.3. Collection, identification and rearing of copepod

Copepod was collected from the pond in Muthannankulam, Coimbatore (11° 1′ 6″ N, 76° 58′ 21″ E), Tamil Nadu, India during early morning before sun rise using standard plankton mesh net with 100 μm mesh net. Collected copepod in 200 mL of plastic bottle were detached and transferred to the laboratory cultured following Kosiyachinda *et al*[22]. The

predatory nature and the rate of predation on mosquito larva were observed under a stereomicroscope. The morphological and taxonomic characters of copepod were identified using Triocular stereo microscope 10×. Copepods in the laboratory was identified as *M. longisetus* based on distribution of feathered and non-feathered outgrowths on the antennule, the presence aesthetascs, spinules by the method of Van de Velde and Holynska[23,24]. Copepod specimens preserved in 80% ethyl alcohol and were deposited at Zoology Department, Bharathiar University, Coimbatore, India. Copepod was cultured, a system based on algae, protozoans such as *paramecium*, chilomonas, wheat seed and some lettuce particles are cultivated in laboratory in 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. Male and female copepod species from the colonies were separated using medicine dropper under a stereomicroscope. The copepods in container are covered with net cloth and gravid isofemale lines were pooled. The females continue to produce multiple batches of egg sacs. Each container should yield approximately 1 500-2 000 adult copepods. The density of *Paramecium caudataum* sp. in the culture is assessed before giving food to the copepods. Females live and reproduce for several months.

### 2.4. Larvicidal and pupicidal bioassay

A laboratory colony of *An. culicifacies* larvae and pupae were used for the larvicidal and pupicidal activity. Hundred numbers of 1st, 2nd, 3rd, 4th instar larvae and pupae were kept in glass beaker containing 250 mL of dechlorinated water with desired concentrations of *P. murex* methanolic leaf extract. Larval food was given for the tested larvae. For each tested concentration, 2 to 5 trials were made and each trial consists of three replicates. The control was set up by mixing acetone with dechlorinated water. Mortality was corrected by using Abbott's formula[25].

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/pupae treated}} \times 100$$

The values of $LC_{50}$, $LC_{90}$ and their 95% confidence limit of upper confidence limit (UCL) and lower confidence limit (LCL) and *Chi*-square values were calculated by using probit analysis[26].

### 2.5. Copepod on larval instars predatory efficiency test

Predatory efficacy of copepod on *An. culicifacies* larvae was assessed by placing single adult copepod in tissue culture plate wells (35 mm diameter, 18 mm depth) with newly hatched first instar larvae in the laboratory condition. The predatory nature and the rate of predatory efficiency of adult *M. longisetus* on mosquito larvae were observed under a stereomicroscope. Hundred numbers of mosquito larvae (I to IV instars each) and twenty numbers of adult copepod were introduced individually into the 500 mL glass beaker containing 250 mL of dechlorinated water and observe for whole day at (27±1) °C. The copepod attacked and killed *An. culicifacies* larvae were observed under microscope. The numbers of dead larvae were counted at every 24 h for 3 d. The glass beakers checked without treatment served as control. 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. Predatory efficiency of a copepod was calculated by the following formula,

$$\text{Predatory efficency} = \frac{\text{Number of prey / Number of predator introduced}}{\text{Total number of prey introduced}} \times 100$$

### 2.6. Predatory efficiency test after the treatment of P. murex on larval instar

Hundred numbers of mosquito larvae (I to IV instars each) and 20 numbers of adult copepods were introduced into glass beaker containing 250 mL of dechlorinated water with *P. murex* methanolic leaf extract. The various stages of copepod such as nauplius, copepodite and adult copepod were used to test the predatory efficacy after the addition of *P. murex*. 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. The number of prey consumed by the predator was checked and recorded at every 24 h for 3 d. Predatory efficiency of a copepod was calculated by the following formula.

$$\text{Predatory efficency} = \frac{\text{Number of prey / Number of predator introduced}}{\text{Total number of prey introduced}} \times 100$$

### 2.7. Predator survival test

The predatory survival test was analyzed to ascertain the survivability and safety of copepod after the treatment of *P. murex* extracts. The effect of *P. murex* extracts was tested against non-target predator, *M. longisetus* which was released into disposable bowl containing 250 mL dechlorinated water with different concentrations of plant extract. Five replicates were performed for each concentration along with untreated controls. *M. longisetus* was observed for mortality, abnormalities, survival and swimming activity after 24 h treatment of plant extract. The exposed predator was observed for a week, after the treatment of different concentration of *P. murex* extracts in order to observe suitability of interaction for mosquito control. $LC_{50}$ values were obtained by probit

analysis and suitability index (SI) or predator safety factor (PSF) was calculated by the following formula[27],

$$SI/PSF=\frac{LC_{50}\text{ of non target organism}}{LC_{50}\text{ of target vector organism}}\times 100$$

SI/PSF=$LC_{50}$ of non target organism/$LC_{50}$ of target vector organism×100.

Non-target organism indicates copepod; Target organism indicates mosquito; SI/PSF indicated that less value is lethal and higher value is susceptible.

### 2.8. Field trial

Field trials were conducted in different places to test the outdoor efficacy of plant extract and copepod in individual and in combination. For the field trial the concentration of plant extracts and natural predator (copepod) required for each treatment was determined by calculating the total surface area of the water in each habitat and based on laboratory $LC_{50}$ values (10 times). Field applications of the plant extracts were done with the help of a knapsack sprayer and Predatory efficiency of copepod was analyzed by introducing 10 numbers of adult copepod into the field. Dipper sampling and counting of larvae and copepod monitored the densities before and after 24, 48 and 72 h treatment. Surviving larvae were collected from all habitats and brought to the laboratory for recording and species identification to find any other species was present. The percentage of larval reduction was calculated by the following formula:

Percentage reduction=C-T/C×100

where, C-the total number of mosquitoes in control, T-the total number of mosquitoes in treatment.

### 2.9. Statistical analysis

The data from bioassay were subjected to statistical analysis. The SPSS software package was computing all the data including confidential limits, *Chi*-square values and mean of the sample. $P<0.05$ is regarded as significant.

## 3. Results

Table 1 illustrates the effect of methanolic extract of *P. murex* on larval and pupal stages of *An. culicifacies*. Mortality was dose dependent and also the early instars were much susceptible than the later ones. Percentage mortality was 30% at concentration 0.5 mg/L whereas, it was been increased to 78% at 8.0 mg/L against the I instar larvae and percentage mortality was 19% at 0.5 mg/L and increased up to 58% at 8.0 mg/L against the IV instar larvae.

Predatory efficiency of *M. longisetus* against different larval instars of *An. culicifacies* is given in Table 2. Predation was high on I and II stage larvae and very low on III and IV instar larvae when compared with earlier stages. This shows the killing capacity of *M. longisetus* was high in I and II instars than the III and IV instars of *An. culicifacies*.

Effect of methanolic leaf extract of *P. murex* on the copepod *M. longisetus* was tested to know the predator survival in biopesticide treatments which is further applied for combined treatment and also to test the effect of plant extracts on non-target organisms (Table 3). Fifty number of copepod were introduced into different concentrations of methanolic leaf extract of *P. murex* and observed to evaluate the percentage of survival. The survival rate was increased as the concentration of *P. murex* was decreased.

**Table 1**

Effect of methanolic extract of *P. murex* on larval and pupal stages of the malarial vector, *An. culicifacies*.

| Larval &pupal stage | Larval and pupal mortality (%) Concentration (%) | | | | | Values of $LC_{50}$ ($LC_{90}$) | 95% Confidence limit | | *Chi*-square value ($\chi^2$) |
|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 | | $LC_{50}$ LCL-UCL (mg/L) | $LC_{90}$ LCL-UCL (mg/L) | |
| I | 30.0±0.8$^{a}$ | 37.0±1.2$^{b}$ | 46.0±1.2$^{c}$ | 70.0±1.5$^{d}$ | 78.0±2.2$^{e}$ | 2.68 (9.95) | 0.32-4.73 | 6.86-23.34 | 7.507 |
| II | 25.0±0.8$^{a}$ | 34.0±1.3$^{b}$ | 41.0±1.8$^{c}$ | 63.0±0.6$^{d}$ | 71.0±1.8$^{e}$ | 3.60 (11.68) | 1.46-6.74 | 7.86-31.23 | 7.423 |
| III | 22.0±2.6$^{a}$ | 30.0±3.4$^{b}$ | 40.0±2.8$^{c}$ | 55.0±1.4$^{d}$ | 65.0±1.5$^{e}$ | 4.50 (13.42) | 2.64-8.58 | 9.05-34.87 | 5.997 |
| IV | 19.0±1.4$^{a}$ | 23.0±1.8$^{ab}$ | 35.0±3.2$^{b}$ | 46.0±2.4$^{c}$ | 53.0±2.6$^{d}$ | 6.44 (17.23) | 4.09-21.33 | 10.74-79.22 | 6.152 |
| Pupal | 18.0±1.6$^{a}$ | 25.0±1.1$^{b}$ | 36.0±2.1$^{d}$ | 41.0±2.5$^{e}$ | 48.0±0.4$^{f}$ | 7.60 (20.83) | 4.60-221.55 | 11.93-886.71 | 5.952 |

LCL-95% Lower confidential limit; UCL-95% Upper confidential limit; within a column means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range test.

**Table 2**

Predatory efficiency of copepod *M. longisetus* on the malarial vector *An. culicifacies*.

| Larval stage | No. of copepod introduced | Predation Duration Day 1 | Day 2 | Day 3 | Total predation | Predation (%) | Predatory efficiency of a single copepod per day |
|---|---|---|---|---|---|---|---|
| I | 10 | 55.0±2.2$^{a}$ | 47.0±1.9$^{a}$ | 38.0±1.5$^{a}$ | 140.0 | 47.0±6.9 | 4.7 |
| II | 10 | 46.0±2.3$^{b}$ | 36.0±1.6$^{b}$ | 25.0±3.1$^{b}$ | 107.0 | 36.0±8.6 | 3.6 |
| III | 10 | 5.0±0.8$^{c}$ | 3.0±0.8$^{c}$ | 1.5±0.5$^{c}$ | 9.5 | 3.0±1.4 | 0.3 |
| IV | 10 | 1.0±0.8$^{cd}$ | 1.0±0.2$^{c}$ | 0.5±0.5$^{c}$ | 2.5 | 1.0±0.2 | 0.1 |

Within a row means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range test.

The 100% survival rate was recorded during the treatment at 10 mg/L concentration of plant extract. This shows the concentration below 10 mg/L is non-toxic to non-target organisms.

**Table 3**
Effect of methanolic extract of *P. murex* on the copepod *M. longisetus*.

| Concentration of MEPM (%) | Number of copepods | | Survival rate (%) |
|---|---|---|---|
| | Introduced | Survived | |
| 30 | 50 | 38 | 76 |
| 25 | 50 | 43 | 86 |
| 20 | 50 | 45 | 90 |
| 15 | 50 | 49 | 98 |
| 10 | 50 | 50 | 100 |

MEPM-methanolic extract of *P. murex*.

Table 4 provides the predatory efficiency of *M. longisetus* against different larval instars of *An. culicifacies* after treatment with methanolic leaf extract of *P. murex*. Predatory efficiency of copepod on mosquitoes treated with methanolic extract of *P. murex* were high, compared to the predatory efficiency on untreated mosquito larvae. The instars I and II were much preferred by copepods when compared to the late instars.

Percentage of predation of copepod on mosquitoes treated with methanolic extract of *P. murex* was 70%, 45%, 6% and 1% on I, II, III and IV instars of *An. culicifacies*, respectively. Predatory efficiency of a single copepod per day on *P. murex* treated larvae was 7.0 and 4.5 numbers of mosquito larvae of I and II instars, respectively. The effect of methanolic extract of *P. murex* at the actual breeding sites of *An. culicifacies* is shown in Table 5.

It was observed that only *An. culicifacies* were found and there was no mixed population. The total numbers of larvae observed before treatment/experiment were 341 individuals. Larval mortality was observed at 24, 48 and 72 h after treatment of methanolic extract of *P. murex*. Table 6 shows the predatory efficacy of copepod, *M. longisetus* at the direct breeding sites of *An. culicifacies*. No mixed populations other than *An. culicifacies* larvae were observed. Before treatment the number of total larval count was enumerated by dipping methods and recorded. Percentage reduction in larval density was observed at 24, 48 and 72 h after introduction of *M. longisetus*.

**Table 5**
Field trial by using methanolic extract of *P. murex* on the larvae of *An. culicifacies*.

| S. No. of field trial | % Larval density before treatment | % Larval density after treatment | | |
|---|---|---|---|---|
| | | 24 h | 48 h | 72 h |
| 1. | 60.0 | 40.0 | 38.0 | 35.0 |
| 2. | 65.0 | 43.0 | 39.0 | 37.0 |
| 3. | 48.0 | 26.0 | 25.0 | 24.0 |
| 4. | 56.0 | 39.0 | 34.0 | 32.0 |
| 5. | 50.0 | 30.0 | 28.0 | 26.0 |
| 6. | 62.0 | 20.0 | 22.0 | 24.0 |
| Average | 56.8 | 33.0 | 31.0 | 29.6 |
| % Reduction | 23.4 | 41.9 | 45.4 | 47.8 |

**Table 6**
Field trial by using copepod, *M. longisetus* on larvae of *An. culicifacies*.

| S. No. of field trial | % Larval density before treatment | % Larval density after treatment | | |
|---|---|---|---|---|
| | | 24 h | 48 h | 72 h |
| 1. | 55.0 | 44.0 | 38.0 | 32.0 |
| 2. | 60.0 | 42.0 | 40.0 | 36.0 |
| 3. | 42.0 | 32.0 | 28.0 | 24.0 |
| 4. | 35.0 | 22.0 | 20.0 | 18.0 |
| 5. | 30.0 | 16.0 | 15.0 | 12.0 |
| 6. | 38.0 | 23.0 | 22.0 | 20.0 |
| Average | 43.3 | 29.8 | 27.1 | 23.6 |
| % Reduction | nil | 31.1 | 37.3 | 45.3 |

Field trial of *M. longisetus* after treatment with methanolic leaf extract of *P. murex* at the breeding sites is shown in Table 7. It was observed that only *An. culicifacies* larvae were found and there was no mixed population. Before treatment the number of total larval count was enumerated by dipping methods and recorded.

**Table 7**
Field trial using methanolic extract of *P. murex* with the *M. longisetus* on the larvae of *An. culicifacies*.

| S. No. of field trial | % Larval density before treatment | % Larval density after treatment | | |
|---|---|---|---|---|
| | | 24 h | 48 h | 72 h |
| 1. | 53.0 | 12.0 | 10.0 | 8.0 |
| 2. | 48.0 | 10.0 | 9.0 | 7.0 |
| 3. | 47.0 | 10.0 | 8.0 | 5.0 |
| 4. | 50.0 | 11.0 | 9.0 | 6.0 |
| 5. | 62.0 | 21.0 | 17.0 | 15.0 |
| 6. | 56.0 | 15.0 | 13.0 | 10.0 |
| Average | 52.6 | 13.1 | 11.0 | 8.5 |
| % Reduction | nil | 75.0 | 79.1 | 83.0 |

**Table 4**
Predatory efficiency of copepod *M. longisetus* on the malaria vector *An. culicifacies* after treatment with *P. murex*.

| Larval stage | No. of copepod introduced | Predation duration | | | | Total predation | Predation (%) | Predatory efficiency of a single copepod per day |
|---|---|---|---|---|---|---|---|---|
| | | Control | Day 1 | Day 2 | Day 3 | | | |
| I | 10 | 0 | 76.0±1.6$^{a}$ | 71.0±1.9$^{a}$ | 64.0±1.9$^{a}$ | 70 | 211 | 7.0 |
| II | 10 | 0 | 56.0±1.8$^{b}$ | 42.0±1.1$^{b}$ | 36.0±1.5$^{b}$ | 45 | 134 | 4.5 |
| III | 10 | 0 | 8.0±1.1$^{c}$ | 6.0±0.8$^{c}$ | 5.0±0.8$^{c}$ | 6 | 19 | 0.6 |
| IV | 10 | 0 | 2.0±0.7$^{cd}$ | 1.2±0.8$^{cd}$ | 1.0±0.7$^{cd}$ | 1 | 4 | 0.1 |

Within a row means followed by the same letter(s) are not significantly different at 5% level by Duncan's multiple range test.

## 4. Discussion

The selected control agents leaf extract of *P. murex* and predatory copepod, *M. longisetus* showed significant effect against the rural malarial vector, *An. culicifacies* in both laboratory and field environments. Methanol extract of *P. murex* have brought out toxicity on different larval stages and pupae of *An. culicifacies*. At various concentrations larvicidal and pupicidal activity of *P. murex* was significant when compared to the control mortality. High mortality recorded after the treatment of *P. murex* may be due to the presence of active chemical compounds, which might had entered the digestive tract and disturbed the digestive enzymes of mosquito larvae. Saponins and tannins present in *P. murex* extract are reported to have insecticidal activity. Furthermore tannin combine with protein inhibit the enzyme activity and reduce the availability of protein in haemolymph of the insect[28]. The varying median lethal concentrations for different larval stages and pupae are due to the susceptibility of the earlier stages than the later ones. The results are favorably supported by the larvicidal and pupicidal activity of *Cassia fistula* against *Culex quinquefasciatus* and *Anopheles stephensi* (*An. stephensi*[29], larvicidal activity of methanolic extract of *Senna alata* and microbial insecticide, *Bacillus sphericus*, against the polyphagous crop pest, *Spodoptera litura* (Fab.), and malarial vector, *An. stephensi* and larvicidal activities of *Cynodon dactylon, Aloe vera, Hemidesmus indicus* and *Coleus amboinicus* against *An. stephensi*, *Culex quinquefasciatus* and *Ae. aegypti*[8,30].

Biological control of mosquito larvae with predators and other biocontrol agents would be a more effective and eco-friendly approach than the use of synthetic chemicals and reduce concomitant damage of insecticide applications to the environment[31]. Few *Mesocyclops* spp. such as, *Mesocyclops thermocyclopoides*[17], *Mesocyclops aspericornis*[7], *Macrocyclops albidus*[32], *M. longisetus* and *Macrocyclops albidus*[18], were reported as an antagonist of mosquito larvae. In the present study assays has been conducted to test the predatory efficiency of copepod, *M. longisetus* on *An. culicifacies*.

The predator *M. longisetus* showed effective prey consumption on first and second instars in greater numbers than third and fourth instars. The active movements and large size of the later instars may have reduced the predation rate of the copepods. Though there was little consumption of the late instars, punctures and injures to late instars of mosquitoes lead to constrained development and death. As a support reports from earlier work states that, cyclopoid copepods have been extensively used as biocontrol agents in the South-East Asian countries for container breeding mosquito species like *Ae. aegypti*[33]. It is known for its wide distribution and predatory efficiency against several species of mosquito larvae. As a support to the present study Murugan *et al.* tested the predatory efficiency of copepods on dengue vector, *Ae. aegypti*. The percentage of predatory efficiency of copepod was 6.80%[7]. Kumar *et al.* stated that with earlier instars of both *An. stephensi* and *Culex quinquefasciatus*, the larval consumption rates of *M. thermocyclopoides* increased significantly with increasing prey densities[31]. Chansang *et al.* reported that the copepod, maintained larval numbers at a low level as compared with those of the control[34].

Predatory copepods are significant in controlling first and second instars of mosquitoes but are not effective against the late instars; hence, a combined approach using plant based larvicide to increase the predatory efficiency of copepods against the late instars was also done. When *M. longisetus* and *P. murex* extract are applied together, the larvicide can produce an immediate kill of all larval stages including the later stages, and copepods can take over as the larvicide diminishes in effectiveness[20]. The active compounds present in *P. murex* might have affected the development and active movement of mosquito larvae, which increased the predatory efficacy of copepod on the early and also late instars.

*P. murex* was also tested against copepods to estimate the effect of the plant extract on the survival and development of the *M. longisetus*. The results suggest that the extract of *P. murex* was non-toxic to the copepods. The survival rate and development of copepod was optimum even in higher concentrations used for the combined predatory efficacy test. Similar investigations have also been done using *Mesocyclops* spp. in conjunction with other controlling methods and resulting in eradication mosquitoes[35-38]. Larvivorous copepods such as *Macrocyclops albidus*, *M. longisetus* and *Mesocyclops aspericornis* are highly effective for controlling *Aedes* sp. larvae in discarded tires[39,40].

The selected plant extract, *P. murex* and copepods were also tested in the direct breeding sites of *An. culicifacies* (paddy fields). The results also proved that the plant extract and copepods were effective in controlling the rural malaria vector in paddy field. The relatively low sensitivity of cyclopoids to plant extracts suggests that the plant extract is probably compatible with cyclopoids as field trials in paddy fields showed that the introduction of copepods along with plant extracts can eliminate *An. culicifacies* larvae. Combined treatment showed greater reduction in larval density when compared with individual treatment. Larval reduction was comparatively higher in the breeding sites with copepod and the plant extract where more than 80% larval mortality was observed.

In conclusion, the findings of the study clearly demonstrated that markedly significant larvicidal and pupicidal effects even at low concentrations of botanical and combination of predatory cyclopoids on *An. culicifacies*. This integrated application could be useful as an alternative for synthetic insecticides. *P. murex* and *M. longisetus* are promising in mosquito control and also safe for the non target organisms. These agents should preferentially to be

applied in mosquito control strategies to reduce the mosquito populations and prevent the malaria.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### *Background*

Malaria is a major public health problem in India, accounting for sizeable morbidity, mortality and economic loss. Apart from preventive measures, early diagnosis and complete treatment are the important modalities that have been adopted to contain the disease. According to the World Malaria Report 2012, over 70% of India's population face the risk of malaria infection. Around 31 crore people face the "highest risk" of getting infected. India has over 10 crore suspected malaria cases but only 15.9 lakh could be confirmed in 2010.

### *Research frontiers*

*An. culicifacies*, the major rural malarial vector was shown not much interest by many other scientist in southern India, has been taken into account. Novel combination of copepod *M. longisetus*, and plant extract in controlling mosquito larvae is interesting.

### *Related reports*

Number of scientists have worked on the predatory efficacy of copepods in controlling mosquito larva (Marten and his group in 1994; Hwang *et al*., 2006). Regarding mosquito control using plant products, the author and their group are well know for their in India. I appreciate the author for further more efforts.

### *Innovations & breakthroughs*

Control of rural malarial vector is an important aspect. Using copepod as a natural enemy without causing any percentage of destruction to environment and combining plants are very much important to the society.

### *Applications*

1. Malarial awareness programs.
2. Product development for mosquito control.
3. Eco-friendly approaches.

### *Peer review*

The research work is very much important to the society to control morbidity and other defects caused by mosquitoes and also other insect vectors. This work reports a novel approach for the control of vector mosquitoes.

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**Retraction notice**

Retraction notice to "Prevalence of intestinal parasitic infestation in Ma′an governorate, Jordan", [*Asian Pac J Trop Dis* 2011; 1(2): 110-112], Available online 1 June 2011.

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Reason: This article has been retracted at the request of the Editor.

This paper was published in error prior to the author having proofread and given consent to the final version. The editor and publisher regret any inconvenience caused.