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# Testing of synergistic effect of copepod (s) and sodium chloride on the mosquito larvae

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**Abstract**
The present research project deals on the elimination of mosquito larvae by using synergistic effect of copepods and sodium chloride on various instar larvae of *Aaegypti*. Mosquitoes are causing various diseases to human and many other organisms in world wide. Mosquito borne disease causes 3.5 billion deaths annually. People of underdeveloped countries have more risk of dengue and malaria. Dengue is a one of the major challenge of 21st century to eliminate it from the breeding sites to low the risk of dengue in population. The control of mosquito population by using pesticides and insecticides make them resistant. Biological control method is a natural way of eliminating harmful insect and pest from environment by using different predators. Biological control is using as alternative source of control mosquito which are becoming resistant to various chemicals agent. Copepods are small crustaceans which were used to control early stage of adult mosquito from water sources. Predation effect of copepods was tested for first, second, third and fourth instar of larvae with different intervals of time. The highest mortality rate after treatment of copepods was 66.66% on first and second instar larvae. The highest mortality rate after treatment of sodium chloride was 73.33% and highest mortality rate with synergistic test was 75.66%.

**Keywords:** synergistic effect of copepod (s) and sodium chloride on the mosquito larvae

## Introduction
Mosquitoes are worldwide in distribution from tropical to subtropical except the coolest place of world. There are more than 3000 thousand species of mosquitoes having 41 genera. It is a small insect belongs to family *Culicidae* and order Diptera. Mosquitoes are small in size ranges from 3mm to 5mm. The feeding behavior of mosquito depend upon it's species. Female mosquito feed on blood meal of human or cattle. Different genera of mosquito have different site for breeding. *Anopheles* are mostly found in fresh water habitats, *Culex* found in polluted water, including tanks and *Aedes* breeding sites are domestic, small water collection and water cooler and flower pot. Frogs are introduced into mosquito breeding places in pond, tanks etc., for prey on larvae and decrease rate of vector borne disease. Biological control of mosquito depends upon the type of predator used to pray it. Predators are helpful in reducing the population of mosquito from environment. Different microorganism like as bacteria, fungi, and nematodes are used to control mosquito population from environment. The use of microorganism is used after 1960 to control development of mosquito after harmful effect of synthetic compound. The toxin produced by *Bti* and *L. sphaericus* are used in control of biological means of mosquito. The larvae and adult have three body parts which can be studies under following parts that is head thorax abdomen in case of larvae of mosquito legs are absent wings and proboscis are also lacked in larva but in case of adults are of these organs are fully present.

The head of larva of mosquito is sclerotized and large the head of larvae of mosquito is broad and elongated depend on the species as in anopheles is elongated and broad in case of culex 2 broad eyes and 2 antannae and comb like parts of mouth the antannae may be variable and size depend on species from very short to quite long articulation appendages of the mandable and maxilla by which mouth parts are composed of setae is different in from and length Mosquito larvae should be killed at early time. We have to put natural enemies of mosquitoes in water bodies. Small copepods, frogs, fish, and many insects use to feed mosquito as food source. We have to use seasonal basis of control method because sometime natural enemy cannot eliminate mosquitoes. people should have awareness about mosquito borne disease

and control method to eliminate mosquito from endemic areas. Some of the species of *A. aegypti* are tolerant to salinity presence in water with osmoregulation by maintains of hemolymph. In salt resistant mosquito salt are absorbed by the excretory organ known as annals papillae. Salt tolerant mosquito of *Aedes* species are *A. detritis* and *A. compestris* resist external salt present in water and decline osmotic level. Salt tolerant mosquito have a special organ for secretion of salt from hemolymph to rectal fluid. (Patrick and Bradley *et al*., 2000) In case of *A. aegypti* anal papillae helps to maintain salt level presence in water and Malpighian tubules helps in excretion (Pagast *et al*., 1936)

**Statement of the research problem:** The statement of the research problem is reported as under:
Testing of synergistic effect of copepod (s) and sodium chloride on the mosquito larvae

**Materials and Research Methodology**

**Collection of copepods:** First of all, getting idea from the local person discussing about pond situated nearby the Gurdashpur village, 3km far away from the Lovely Professional University, Phagwara as site for collection of copepods. Copepods were collected from pond with the help of plastic dipper of size 40mm. It was collected in Plastic bottle with help of dipper and observeda small whitish appearance swimming fastly with jerky movements. After the collection of copepod from the collection site it was transferred into a clean beaker with de-chlorinated water.
Copepods were taken out from a beaker with the help of small dipper and brush for identification of female and male. A clean grease free slide was taken and copepod specimen placed over on slide with drop of water and observe at 10X and 40X under compound microscope. Female copepods were identified with the presence of gravid like structure containing eggs on both sides of bodies. For culture of copepods, female copepods were placed in container adding proper volume of de-chlorinated water and number of male was added in same container for reproduction. After set up of culture, a drop of fresh milk was added in container as copepods diet. Fig.1 show male copepod and Fig.2 show female copepod.

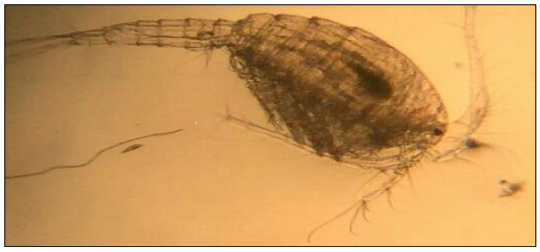

**Fig 1:** Male Copepod

**Fig 2:** Female copepod

**Preparation of NaCl Solution:** A total of 1 gram of NaCl was weighted on digital balance and dissolved in 1000ml of distilled water. A preparation of NaCl was kept as stock solution in a container for making various required concentration with the help of pippetestock solution for making various ppm concentration in a required volume of de-chlorinated water.

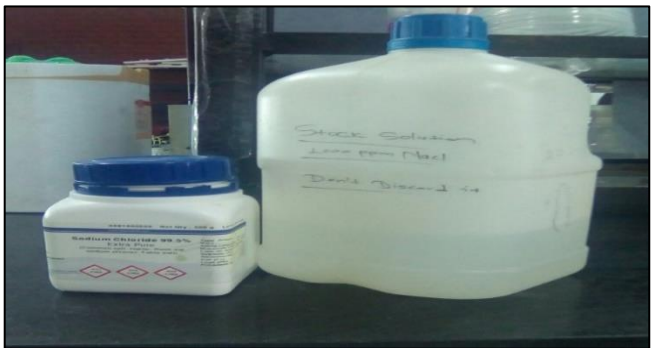

**Fig 3:** NaCl solution

**Collection of mosquito larvae:** The larvae of mosquito were collected from flower pots and ditches near Chehru. Mosquito larvae were collected in plastic bottle with open lid and kept in laboratory.

**Treatment to larvae:** The treatment of *A. aegypti* different instar larvae were set up in triplicate order adding different number of copepods tested and finally four treatment of copepods were selected as 2,4,5 and 6 in 250 ml of de-chlorinated water and 15 number of *A. aegypti* larvae were placed in beaker. After exposure of different number of copepods as predators of larvae with different interval of time as 6,12,24,48 and 72 hours to check the mortality rate. One of the control set was also run along with these treatment without adding any copepods and same number of larvae and 250ml 0f de-chlorinated water.
The treatment with different concentration of sodium chloride as 40,50,60and 70 ppm and 15 number of larvae with 250 ml of de-chlorinated water was placed in triplicate order to check the mortality rate of larvae with different interval of time as 6,12,24,48, and 72 hours. One control set was run without adding any salt concentration and same number of larvae and same volume of de-chlorinated water.
At last of treatment different number of copepods and NaCl concentration were combined together to test synergistic effect of copepods and NaCl on larvae with 15 larvae and 250 ml of de-chlorinated water. The control set of treatment also run with same intervals time without adding anything except mosquito larvae with 250 ml of de-chlorinated water.


**Fig 4:** Larvae treatment

**Analysis and interpraytation of the data**

chloride on the mosquito larvae. The main aim of this project was to elimination of mosquito population from breeding sites by using natural enemies and low risk of chemical which was known as sodium chloride or salt. Treatment of mosquito larvae was set in triplicate order with one control set using 250ml of de-chlorinated water, different number of copepods, sodium chloride concentration and last combination of both copepods and sodium chloride was test as synergistic test.

First treatment was done with different number of copepods 3,4,5 and 6 in beaker containing 250ml of de-chlorinated water with 15 larvae with different intervals of time as 6,12,24,48 and 72 hours respectively. According to (Table.1) themortalityrate with 3,4,5and 6 copepods were 46.66%,53.33%,62.22% and 66.66% with 72 hours on first and second instar larvae of *A. aegypti*. According to (Table.2) the mortality rate with 3,4,5 and 6 copepods were 44.44%, 49.00%, 55.55% and 60.00% with 72 hours on third and fourth instar larvae.

Second treatment was done with 40,50,60 and 70 ppm concentration of sodium chloride in beaker containing 250 ml 0f de-chlorinated water with 15 larvae with different intervals of time 6,12,24,48 and 72 hours respectively. According to (Table. 3) lowest mortality rate was with 40 ppm concentration of sodium chloride 48.88% and highest mortality rate was with 70 ppm concentration of sodium chloride 73.33% at 72 hours on first and second instar larvae of *A. aegypti* and (Table.4) showed lowest mortality rate with 40 ppm concentration of sodium chloride 46.66% and highest mortality rate was with70 ppm concentration of sodium chloride 64.44% at 72 hours.

Third treatment was done for testing synergistic effect of sodium chloride concentration and copepods as 20 ppm with 2 copepods, 30 ppm with 4 copepods, 40 ppm with 3 copepods and 50 ppm with 3 copepods in beaker containing 250ml de-chlorinated water with 15 larvae with different intervals of time 6, 12, 24, 48 and 72 hours respectively. According to (table. 5) lowest mortality rate with 2o ppm and 2 copepods 55.55% and highest mortality rate with 50 ppm +3 copepods 75.55% on first and second instar larvae of *A. aegypti* and (Table. 5) lowest mortality rate with 20 ppm and 2 copepods 46.66% and highest mortality rate 70 ppm and 3 copepods 68.88% on third and fourth instar larvae of *A. aegypti.* The highest rate of mortality was with synergistic effect 75.55% with 50 ppm and 3 copepods at 72 hours on first and second instar larvae of *A. aegypti* and lowest with 3 copepods 44.44% at 72 hours.

**Table 1:** Rate of mortality (%) of first and second instar larvae of *A. aegypti* treated with different number of copepods with different interval of time

| No. of Copepods | Mortality (Percentage) | | | | |
|---|---|---|---|---|---|
| | 6 hours | 12 hours | 24 hours | 48 hours | 72 hours |
| Three | 8.88%±0.99 | 20.00%±0.98 | 24.44%±1.15 | 42.44%±0.56 | 46.66%±0.98 |
| Four | 11.11%±0.99 | 26%±0.97 | 31.11±0.58 | 46.66%±0.57 | 53.33%±0.54 |
| Five | 15.55%±0.56 | 31.11%±0.55 | 40%±0.53 | 49%±0.44 | 62.33%±0.36 |
| Six | 20%±01.52 | 33.33%±1.53 | 46.66%±0.96 | 60%±0.98 | 66.66%±0.57 |
| Control | 0.00±0.00% | 0.00±0.00% | 0.00±0.00% | 0.00±0.00% | 0.00±0.00% |

Table.1 showing the mortality rate of first and second instar larvae with different number of copepods 3,4,5 and 6 with 6 hours were 8.88%,11.11%,15.55% and 20.00%. The mortality rate with 12 hours were 20.00%, 26.00%, 31.00% and 33.33%. The mortality rate with 24 hours were 24.44%,31.11%,40.00% and46.66%. The mortality rate with 48 hours were 42.22%,46.60%,53.00% and 60.00%. The mortality rate with 72 hours were 46.66% 53.33%, 62.22% and 66.66%. The lowest mortality rate was with 6 hours and highest mortality rate was with 72 hours with 3 copepods and 6 copepods.

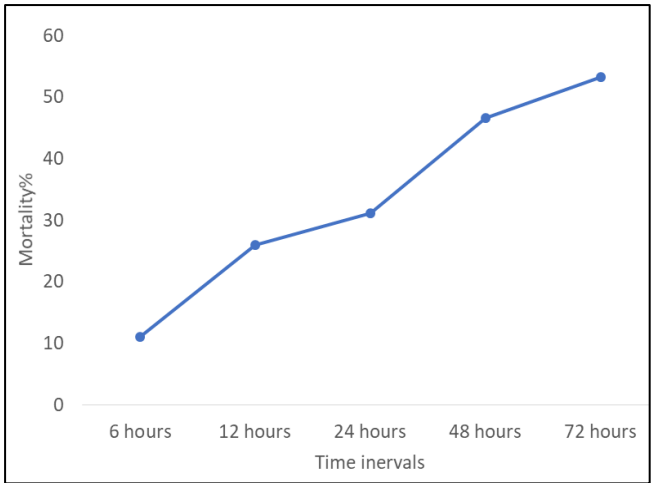


**Fig 5:** Effect of copepods (3) after exposure of first and second instar larvae of *A. aegypti* at different intervals of time

Figure.5 showing rate of mortality after treatment of (3) copepods with 6,12,24,48 and 72 hours. Mortality rate with 6 hours was 8.88%,12 hours was 20.00%,24 hours was 24.44%, 48 hours was 42.22% and 72 hours was 46.66%. The difference with rate of mortality after treatment with 6 hours and 12 hours was 11.12%, rate mortality difference with 12 hours and 24 hours was 4.44%, rate of mortality difference with 24 hours and 48 hours was 17.78% and rate of mortality difference after 48 hours and 72 hours 4.44%. (Fig.5)

**Conclusion**

The study from various test done in this project show the best result with synergistic test by used of small crustaceans' copepods and sodium chloride. It was one of the cheapest method to control mosquito larvae and eco-friendly. This test can be used for the control of mosquito which are resistant to various pesticides and insecticides. Copepods is easy to production in large mass by using food sources like as paramecium and algae in water source. Copepods has good effect on predation of mosquito larvae in water bodies. Copepods can also be use in control of A. aegypti from old tires, containers air cooler and flooded areas. The biological agent can control mosquito population from breeding site and helps in eliminating vector borne diseases from endemic areas. We know that chemical product use to control mosquito can causes various disease like respiratory diseases and these chemicals are carcinogenic to human. Instead of using these chemicals we

have to give more focus on biological control method which is cost effective.

**Reference**


1. Aksnes DL, Nejstgaard J, Soedberg E, Sornes T. Optimal control of fish on zooplankton populations. Limnology and Oceanography 2004;49:233-238. Awasthi, A. K., C. H. Wu & J. S. Hwang 2012a.
2. Barnes C, Maxwell D, Reuman DC, Jennings S. Global patterns in predator–prey size relationships reveal size dependency of trophic transfer efficiency. Ecology 2010;91:222-232.
3. Barnes C, Maxwell D, Reuman DC, Jennings S. Global patterns in predator–prey size relationships reveal size dependency of trophic transfer efficiency. Ecology 2010;91:222-232.
4. Ceron J, Covarrubias L, Quintero R, Ortiz A, Ortiz M, Aranda E *et al*. PCR analysis of the Cry I insecticidal crystal family genes from *Bacillus thuringiensis*. Appl. Environ. Microbial 1994,
5. Dhanker R, Kumar R, Hwang JS. Predation by *Pseudodiaptomus annandalei* (Copepoda: Calanoida) on rotifer prey: size selection, egg predation and effect of algal diet. Journal of Experimental Marine Biology and Ecology 2012;414-415:44-53.
6. Diving as an anti-predator behavior in mosquito pupae. Zoological Studies Awasthi, AK, CH, Wu, KH, Tsai CC, King, Hwang JS. 2012b;51(8):1225-1234.
7. El-Badry AA, Al-Ali KH. Prevalence and Seasonal Distribution of Dengue Mosquito, *Aedes aegypti* (Diptera: Culicidae) in Al-Madinah Al Munawwarah, Saudi Arabia. Journal of Entomology 2010;7:80-880:353356.
8. How does the ambush predatory copepod *Megacyclopsformosanus* (Harada 1931) capture mosquito larvae of *Aedes aegypti*? Zoological Studies Barnes, C 2008;51(7):927-936.
9. Jude PJ, Dharshini S, Vinobaba M, Surendran SN, Ramasamy R. *Anopheles culicifacies* breeding in brackish waters in Sri Lanka and implications for malaria control. Malar J 2010;9:106.
10. Juliano SA, Reminger L. The relationship between vulnerability to predation and behavior of larval tree-hole mosquitoes: geographic and ontogenetic differences. Oikos 1992;63:465-467.
11. Maria A, Diuk-Wasser, Heidi E Brown, Theodore G Andreadis, Durland Fish, "Modeling the Spatial Distribution of Mosquito Vectors for West Nile Virus in Connecticut, USA", Vector Borne And Zoonotic Diseases 2006;6(3):283-295.
12. Marlylene de Brio. Toxity effect of *Delonixelata* and predatory efficiency of copepod, *Mesocyclops aspericornis* for control of dengue vector, Asian pacific journal of tropical disease 2015.
13. Marten GG, Borjas G, Cush M, Fernandez E, Reid JW. Control of larval *Aedes aegypti* (Diptera: Culicidae) by cyclopoid copepods in peridomestic breeding containers. J Med Entomol 1994;31:36-44.
14. Odalo J, Omolo M, Malebo H, Angira J, Njeru P, Ndiege I. Repellency of Essential Oils of Some Plants from the Kenyan Coast against *Anopheles gambiae*. Acta Tropica, 2005, 95.
15. Predator and prey body sizes in marine food webs. Ecology 89: 881. Barnes, C., D. Maxwell, D. C. Reuman & S. Jennings, Global patterns in predator–prey size relationships reveal size dependency of trophic transfer efficiency. Ecology 2010;91:222-232.
16. Sarwar M. Proposals for the Control of Principal Dengue Fever Virus Transmitter *Aedes aegypti* (Linnaeus) Mosquito (Diptera: Culicidae). Journal of Ecology and Environmental Sciences 2014c;2(2):24-28.
17. Tietze NS, Hester PG, Shaffer KR, Prescott SJ, Schreiber ET. Integrated management of waste tire mosquitoes utilizing *Mesocyclops longisetus* (Copepoda: Cyclopidae), *Bacillus thuringensis var Israelensis*, *Bacillus sphaericus*, and methoprene. J. Am. Mosq. Contr. Assoc 1994, 10.
18. Wiggelworth VB. The Effect of Salts on the Anal Gills of the Mosquito Larva 1993.
19. WHO, World Health Organization Manual on Practical Entomology in Malaria Part 1. WHO, Geneva 1975, 160.