

## Effect of plant extracts and green synthesized silver nano-particles on mosquito predation of *Culex (lutiza) fuscanus*

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### Abstract

Chemical based insecticides and bio-pesticides are used to control mosquito larvae and in turn these larvicides pose problems to non-target co-inhabitants. In this regard the present study was carried out to find the influence of *Aegle marmelos*, *Coleus aromaticus*, *Colocasia esculenta* and *Wrightia tinctoria* plant extracts and green synthesized silver nanoparticles on the predation of mosquito predator, *Cx (L) fuscanus* which is also a mosquito larva. The study indicates that the plant extracts and green synthesized silver nano-particles have no effect ( $P < 0.05$ ) on the predation of *Cx (L) fuscanus*. Hence use of these plant extract and green synthesized silver nano-particles are recommended to control mosquito larvae along with this predator.

**Keywords:** predation, *Cx (L) fuscanus*, plant extracts, nanoparticles

### 1. Introduction

Synthetic insecticides such as DDT, Malathion and temephos used for vector control are non-degradable, non-selective and have harmful effect on non-target organisms the alternative for synthetic insecticide, plant derived natural products have many advantages. Being harmless to non-target organisms they do not cause pollution. They are also biodegradable [1]. The use of these natural chemicals in mosquito control program have no adverse effect on biocontrol agents, since biological control is one of the important management strategies of mosquito control where a variety of predators are in use since, different types of predators are used to control of mosquito larvae. Mosquito predator lists start from mosquito larvae of *Toxorhynchites* to some families of aquatic bugs and beetles, tadpoles, flatworms, nematodes, copepods, fishes, dragonfly nymph etc. Wide spectrums of biological agents are in use as biological agents in mosquito abatement programme [2]. Protozoa, fungi, bacteria and viruses also have been considered as biological control agents [3]. Larvivorous fishes such as *Gambusia holbrooki*, *Pseudomugil signifier* [4] *Gambusia affinis* [5] and *Poecilia reticulata* [6] are also used in mosquito control programme. Use of these larvivorous fishes show encouraging results [7] and *Gambusia affinis* is known as 'mosquito fish' due to its feed preference.

Pesticide/ larvicide applied to control mosquito larvae also have negative consequences on non-target organisms [8]. Pesticides both synthetic chemicals and plant derived chemicals are much in use as mosquito larvicides, have direct or indirect effect on the aquatic inhabitant including predator of mosquito [9]. These larvicides may have an indirect effect on larval predation which is reported earlier by Mariappan [10]. A comprehensive review on the effect of different type of chemical agents and its action on non-target organism are reviewed by Talebi [11].

Before advocating a larvicide in mosquito control programme it should be tested for its activity on non-target organism also. In this regard the present study is aimed to

find out the effect of plant extracts (*A. marmelos*, *C. aromaticus*, *C. esculenta*, and *W. tinctoria*) and green synthesized silver nano-particles on the predation of mosquito predator, *Cx (L) fuscanus* which is also a mosquito larvae.

### 2. Materials and methods

#### 2.1 Larval predation

The prey *Cx. quinquefasciatus* and the predator *Cx (L) fuscanus* were cultured in laboratory following the method adapted by WHO.2005 [12] and the IV instars of predator *Cx (L) fuscanus* were kept in speared containers without feed for a period of 12 hours before performing the experiment. In a series plastic aquarium of 300ml capacity with 250 ml of test concentrations (Sub lethal concentration of  $LC_{50}$  values, 1/4, 1/5, 1/7 ppm of  $LC_{50}$  values of methanol extract of *A. marmelos*, *C. aromaticus*, *C. esculenta*, and *W. tinctoria*) (1/2, 1/3, 1/6 concentration synthesized silver nanoparticles using *A. marmelos*, *C. aromaticus*, *C. esculenta*, and *W. tinctoria*) were taken. Sub-lethal concentrations were selected on basis of  $LC_{50}$  value obtained based on a series of experiments conducted for plant extracts and green synthesized silver nanoparticles ( $LC_{50}$  values of plant extracts *Aegle marmelos* 151.43 ppm, *Coleus aromatics* 188.36 ppm, *Colocasia esculenta* 165.69 ppm and *Wrightia tinctoria* 210.29 ppm and green synthesized silver nano particles of *Aegle marmelos* 33.40 ppm, *Coleus aromatics* 36.07ppm, *Colocasia esculenta* 32.69 ppm and *Wrightia tinctoria* 42.76 ppm for IV instar larvae). In each aquarium one *Cx (L) fuscanus* (predator) and 10 *Cx. quinquefasciatus* IV larvae (prey) were introduced simultaneously. Number of larvae consumed / killed by the predator was recorded for a period of one hour. A control aquarium was also maintained separately by one predator and 10 *Cx. quinquefasciatus* larvae. These experiments were repeated three times for each test concentration.

### 3. Results and Discussion

This experiment was conducted to study the compatibility of

the plant extracts and green synthesized silver nanoparticles as larvicides to use in integrated bio-control programme along with mosquito predator *Cx (L) fuscus*. *Cx (L) fuscus* is also mosquito species which is a co-inhabitant of other mosquito larvae.

Experiments were conducted in three types of chosen sub-lethal concentrations for plant extracts and green synthesized silver nanoparticles. The chosen concentrations for plant extracts *A. marmelos* are 23.3, 26.6 and 30, *C. aromaticus* are 23.3, 30, and 30, *C. esculenta* are 26.6, 30 and 30, and *W. tinctoria* are 23.6, 26.3 30 respectively (Table.1). Likewise green synthesized silver nanoparticles *A. marmelos* are 26.6, 26.6 and 30, *C. aromaticus* are 23.3, 26.6 and 30, *C. esculenta* are 26.6, 26.6 and 30 and *W. tinctoria* are 26.6, 30 and 30 respectively (Table.3)

There is no mortality (predator and prey) occurred during the period of experiment in the selected sub-lethal concentration of plant extracts and green synthesized silver nano-particles. An average of 30 % larvae was consumed by *Cx (L) fuscus* in the category of control and 27.45 % for plant extracts 27.74% for green synthesized silver nanoparticles.

A two way ANOVA was performed to study the effect of plant extracts and green synthesized silver nanoparticles and their concentrations on the predator organism. The analysis indicates that there is no significant difference in the predation of *Cx (L) fuscus* by the plant extracts. Likewise green synthesized silver nanoparticles also have such effect on the predation of *Cx (L) fuscus* (Table.2 and 4).

Insecticides of chemicals origin create several environmental problems in addition to killing non-target organisms such as beneficial natural predators and pollinators. In this regards one should be very careful in integration of biological control and chemical control agents in mosquito control programme. Both agents are must be complement to each other [13]. The effective larvicide and biological control agents are two important components in an integrated pest management system. Since most of the insecticides have a broad spectrum of action, they affect both prey and predator. Selective pesticides are available in few in numbers and these should be identified and integrated in to pest management. A selective pesticide is one that is toxic to pest (target), but has little or no effect on non-target organisms [14]. A list of non-target organism to *Bti* which is used to control mosquitoes is given by Glare and O'Callaghan [15]. Toxicological studies indicate that allethrin group of compounds are toxic to aquatic organisms like fish and stone fly and are less toxic to other aquatic insect larvae and *Daphnia*. For other species of arthropods the allethrin toxicity ranges from 20 to 2000 ppm [16]. Toxicity of pyrethrum against various fishes was reported and for *Salmo gairdneri* the toxicity is 0.056 ppm, for *Ictalurus punctatus* it is 0.096 ppm, for *Lepomis macrochirus* it is 0.080 ppm and for stone flies *Pteronarcys californica* it is 0.010 ppm [17]. Further synergistic effect of pyrethroids had been reported 5 times more toxic to rein trout [18].

Brown *et al.* [19] reported the application of organophosphorous has hazardous or unknown effect on associated non-target species. Effects of methanolic extract of *A. monophylla* on non-target organisms have revealed that this extracts is safer to predatory fishes *G. affinis* and *P. reticulata* and aquatic bugs *D. indicus*. Hence it is

Recommended to use the plants extracts along with these predatory fishes in Integrated Vector Control (IVM) [20].

Three medicinal plants such as *Mammea siamensis*, *Anethum graveolens* and *Annona muricata* were tested for larvicidal and pupal activity against *Ae. aegypti* and their effect on non-target organisms. The results show that these plants were toxic to *Ae. aegypti* larvae and pupae but had no adverse effect on guppy fish (*Poecilia reticulata*) [21].

Chloroform-methanol extract of *Solanum villosum* was tested for its larvicidal activity against *An. subpictus* larvae and its effect on larvae of *Chironomus circumdatus*. The results indicate that there is no effect of *S. villosum* extract on non-target organism [12]. Crude extract of Jasmine, *Cestrum diurnum* was tested for larvicidal activity against *Cx. quinquefasciatus*. *C. diurnum* extract have a toxic effect on target organisms and no mortality was noticed for non-target organisms, such as *Oreochromis niloticus* and Chironomid larvae under the laboratory condition [22].

Subarani *et al* [23] studied the larvicidal activity of green synthesized silver nanoparticles of *Vinca rosea* (L) leaves against the larvae of malaria vector *A. stephensi* and the filarial vector, *Cx. quinquefasciatus*. They have compared the toxicity of silver nanoparticles on the target (*Cx. quinquefasciatus*) as well as non-target organism, (*Poecilia reticulata* a predatory fish).

Comparative effect of *Alternanthera sessilis*, *Trema orientalis*, *Gardenia carinata* and *Ruellia tuberosa* leaves was evaluated against target species (*Cx. quinquefasciatus*) and non-target organisms *Diplonychus annulatum* and *Chironomus circumdatus*. There was no significant change in the physiological and behavior of non-target organisms [24].

Singha and Chandra [25] investigated the effect of crude and chloroform: methanol extracts of *Cuminum cyminum*, *Allium sativum*, *Zingiber officinale*, *Curcuma longa* and germinated tuber of *Solanum tuberosum* on *Toxorhynchites splendens*, *Gambusia affinis*, *Poecilia reticulata*, *Diplonychus indicus*, *Diplonychus annulatum*, *Anispos bouvieri* and *Chironomus circumdatus*. The studied concentrations had no influence on the survival of non-target organism indicating its safety in field applications on controlling mosquito larvae. Impact of pesticides/ larvicides on non-target arthropods organisms is given in table 5. The present study shows there is no influence of four plants extracts *A. marmelos*, *C. aromaticus* *C. esculenta* and *W. tinctoria* and green synthesized silver nanoparticles on the predation of *Cx (L) fuscus* a non-target organism. The results of this study indicates that both plant extract and synthesized silver nanoparticles have an effect on target organisms and have no adverse effect on non-target organisms. It is assumed that the plant extracts are safe to the environment and may be included in the IPM.

**Table 1:** Influence of *A. marmelos*, *C. aromaticus*, *C. esculenta* and *W. tinctoria* methanolic leaf extracts on the predation of IV instar of *Cx. quinquefasciatus* by *Cx. (L) fuscus*

Plant species	Control	Concentration (ppm) (% of consumption)		
		1/4	1/5	1/7
<i>A. marmelos</i>	30.0 ± 3.3	23.3 ± 3.3	26.6 ± 3.3	30 ± 0.0
<i>C. aromaticus</i>	30.0 ± 0.0	23.3 ± 3.3	30 ± 0.0	30 ± 0.0
<i>C. esculenta</i>	30.0 ± 0.0	26.6 ± 3.3	30 ± 0.0	30 ± 0.0
<i>W. tinctoria</i>	30.0 ± 0.0	23.3 ± 0.0	26.6 ± 6.6	30 ± 0.0

**Table 2:** Influence of *A. marmelos*, *C. aromaticus*, *C. esculenta* and *W. tinctoria* green synthesized silver nanoparticles on predation of IV instar of *Cx. quinquefasciatus* by *Cx. (L) fuscanus*.

Plant species	Control	Concentration (ppm) (% of consumption)		
		1/2	1/3	1/6
<i>A. marmelos</i>	30.0 ± 0.0	26.6 ± 3.3	26.6 ± 3.3	30 ± 0.0
<i>C. aromaticus</i>	30.0 ± 0.0	23.3 ± 3.3	26.6 ± 3.3	30 ± 0.0
<i>C. esculenta</i>	30.0 ± 0.0	26.6 ± 3.3	26.6 ± 3.3	30 ± 0.0
<i>W. tinctoria</i>	30.0 ± 0.0	26.6 ± 0.0	30 ± 0.0	30 ± 0.0

**Table 3:** Analysis of Variance (ANOVA) to test the influence of plant extracts and their concentrations on the predatory efficacy of *Cx. (L) fuscanus* on *Cx. Quinquefasciatus*

Source	SS	df	MS	F	P
Main effect Concentration	149.99	3	49.99	2.66	0.0644*
Plant extract	66.66	3	2.22	1.88	0.3309*
Interaction Concentration × Plant extract	83.33	9	9.25	1.49	0.8677*
Error	600	32	18.75		
Total	900	47			

\*Statistically not significant at 0.05% level

**Table 4:** Analysis of variance (ANOVA) to test the influence of green synthesized silver nano-particles and their concentrations on the predatory efficacy of *Cx. (L) fuscanus* on *Cx. Quinquefasciatus*

Source	SS	df	MS	F	P
Main effect Concentration	106.25	3	35.41	2.42	0.0834*
Silver nano-particles	6.24	3	2.08	0.14	0.9335*
Interaction Concentration × Silver nano-particles	18.75	9	2.08	.14	0.9979*
Error	466.66	32	14.58		
Total	597	47			

\*Statistically not significant at 0.05% level

**Table 5:** Impact of pesticides/ larvicides on non-target arthropods organisms

Pesticides /Larvicides	Bio-control agent	Impact	Reference
Extract of <i>Azadirachta indica</i> seed	<i>Encarsia sp; Aleurodiphilus sp</i>	Reduce the parasitoids population	Price and Schuster 1991 <sup>[26]</sup>
d-allethrin	<i>Culex (Lutzia) fuscanus</i>	Harmless	Mariappan <i>et al.</i> , 1997 <sup>[27]</sup>
Acetonic fraction of <i>Trichilia havanensis</i>	<i>Chrysoperla carnea</i>	Harmless	Huerta <i>et al.</i> , 2003 <sup>[28]</sup>
Natural pyrethrins	<i>Chrysoperla caenea</i>	Harmful	
Azadirachtin	<i>Trichogramma cacoeciae</i>	Reduced life table parameter	Saber <i>et al.</i> , 2004 <sup>[29]</sup>
	<i>Amblyseius cucumeris</i>	Harmless	Thoeming and Poehling, 2006 <sup>[30]</sup>
	<i>Hypoaspis aculeifer</i>	Moderately harmful	
<i>Azadirachta indica</i> azal	<i>Opius chromatomyiae</i>	Slightly harmful	Hossain and Poehling, 2006 <sup>[31]</sup>
<i>Melia volkensii</i> seed extract	<i>Chilocorus bipustulatus and Phoroscyrnus anchorago</i>	Slightly harmful	Peveling and Ely, 2006 <sup>[32]</sup>
Natural oil of jajoba plant	<i>Diaerilla rapae</i>	Harmless	Farag and Gesreha, 2007 <sup>[33]</sup>
<i>Azadirachta indica</i>	<i>Campoletic chloridae</i>	Slightly harmful	Rao <i>et al.</i> , 2007 <sup>[34]</sup>
Pyrethrin	<i>Harmonia axyridis</i>	Highly toxic to first instar, harmless to third instar, pupa and adult	Kraiss and Cullen, 2008 <sup>[35]</sup>
<i>Aegle marmelos</i> leaf extract	<i>Culex (L) fuscanus</i>	Harmless	Present study
<i>Coleus aromaticus</i> leaf extract	<i>Culex (L) fuscanus</i>	Harmless	Present study
<i>Colocasia esculenta</i> leaf extract	<i>Culex (L) fuscanus</i>	Harmless	Present study
<i>Wrightia tinctoria</i> leaf extract	<i>Culex (L) fuscanus</i>	Harmless	Present study
<i>Aegle marmelos</i> silver nano particles	<i>Culex (L) fuscanus</i>	Harmless	Present study
<i>Coleus aromaticus</i> silver nano-particles	<i>Culex (L) fuscanus</i>	Harmless	Present study
<i>Colocasia esculenta</i> silver nano-particles	<i>Culex (L) fuscanus</i>	Harmless	Present study
<i>Wrightia tinctoria</i> silver nano-particles	<i>Culex (L) fuscanus</i>	Harmless	Present study

Adopted from Talebi *et al.*, 2008

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