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TOXIC EFFECT OF *UVARIA NARUM* (DUNAL) WALL AND ITS SYNERGISTIC PROPERTY WITH TEMEPHOS AGAINST THE FILARIAL VECTOR, *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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ABSTRACT

Larvicidal property of methanol extract of *Uvaria narum* leaf tested on III and IV instar larvae of the filarial vector, *Culex quinquefasciatus* Say and estimated 24 hr LC50 as 376.45 and 344.25 ppm for III and IV instar respectively. Temephos, the synthetic larvicide used for the present study was also subjected to find out the lethal concentration and estimated their 24 hr LC50 as 0.022 and 0.02 ppm against III and IV instar larvae, whereas in combination of the selected pesticides temephos and *U. narum* leaf extracts shows an LC_{50} value of 29.45 and 36.25 ppm respectively for III and IV instar larvae of *Cx. quinquefasciatus*. The data on the joint action of mixtures and the co-toxicity factor for III and IV instars (47.5% and 50.15%) pointed to its effects as potentiation, against the larvae of *Cx. quinquefasciatus*.

KEYWORDS: *Uvaria Narum*, Temephos, *Cx. Quinquefasciatus*, Synergistic Activity.

INTRODUCTION

Plants and plant derived substances have been used to kill insect pests and vectors for a long time before the advent of synthetic chemicals. Phytochemicals have broad range of activities against mosquitoes, such as ovicidal, larvicidal and adulticidal effects, oviposition-deterrence, developmental toxicity, hatching and emergence blocking effects etc..[1]. Azadirachtin and neem products exhibit various modes of action against insects such as anti-feedant, insect growth regulator, fecundity suppression and sterilization, oviposition repellency or attraction, affecting biological fitness and blocking development of vector-borne pathogens [2]. Phytochemicals are the simple and sustainable method of mosquito control which has comparatively low mammalian toxicity. Botanicals are basically secondary metabolites that serve as a means of defense mechanism of the plants to withstand the continuous selection pressure from herbivore predators and other environmental factors [3]. After facing several problems due to injudicious and over applications of synthetic insecticides like development of insecticide resistance in vector population and biomagnification of synthetic larvicides; refocus on phytochemicals that are easily bio degradable and causing no side effects were given emphasis while developing alternative methods in mosquito control as a part of the integrated mosquito management systems. Hence, the present study was undertaken to test the potency of plant *Uvaria narum* (Dunal) Wall. extracts, with commercial insecticides against larvae of *Culex quinquefasciatus* (Say) and analyze the joint action toxicity resulting from mixing botanical extracts with insecticides.

MATERIALS AND METHODS

Plant selected for the study: *Uvaria narum* (Dunal) Wall **(**Family: Annonaceae).

Uvaria narum is a scandent shurb, branchlet sparsely hairy commonly known as South Indian Uvaria. It is a medicinally important plant and its root and leaves used in treatment of intermittent fever, biliousness, jaundice, and also in rheumatic. Fresh mature leaves of *Uvaria narum* (Dunal) Wall, were collected from in an around Calicut University Campus, Kerala, India during the month of March and April and subjected for extraction.

Test Organism: *Culex quinquefasciatus* Say: The southern house mosquito *Culex quinquefasciatus* Say belonging to the order Diptera and family Culicidae, is the vector of Lymphatic Filariasis caused by the nematode *Wuchereria bancrofti*. In optimum temperature and humidity, the life cycle is about 7 days. Upon emergence the adult females prefer to feed blood and the species is highly anthropogenic.

Maintenance of *Culex* in the Laboratory: Mosquito larvae were collected from drainages and tanks from various places in Kozhikode District were brought to the laboratory and were maintained at 28 $\pm$ 1^{o}C and 80 $\pm$ 5%

relative humidity in plastic trays employing 0.08% saline [4]. The pupae were collected and kept for emergence in standard mosquito emergence cage. The adult males were fed with 10% sucrose solution and adult females were provided with blood meal from an immobilized quail on alternate nights. Bowls with water were provided in the cage to facilitate oviposition. The freshly laid egg raft were collected from the cage and transferred to a tray and allowed to hatch. The freshly hatched larvae were fed by fine powder of yeast and dog biscuit in the ratio 3:1. The water was changed in every alternate days. The freshly moulted III and IV instar larvae were used for bio assay.

Extraction of plant material: The leaves of the plant, *Uvaria narum* (Dunal) Wall collected from in and around the campus of the University of Calicut, were washed and dried under shade, powdered and extracted in analytical reagent methanol using soxhlet apparatus. The yield of the extract was calculated by weighing the dried extract and 1% stock solution of the extract were prepared and store in a refrigerator for the study.

Synthetic Larvicide – Temephos: The synthetic larvicide used for synergistic study is organophosphate insecticide Temephos. Its chemical name is 0, 0-(thiodi-4,1-phenylene) 0, 0, 0^1, 0^1 – tetra methyl phosphorothioate and It is soluble in common organic solvents and insoluble in hexane and methyl cyclohexane. Melting point of Temephos lies between 30-30.5^{o}C. Temephos is a non-systemic organophosphorus insecticide used to control mosquitoes, midge, black fly larvae and also fleas on dogs and cats etc.

Bioassay: The bioassay experiments were conducted according to standard WHO protocol [5] with slight modifications. III and IV instar larvae of C*x. quinquefasciatus* were used for bio assay. Each experiment was carried out in triplicate. The appropriate volumes of 1% stalk solutions were diluted in 100 ml 0.08% saline in disposable glass to obtain desired concentrations of test medium. 10 larvae were released to each glass containing a test medium and also in control set. Similarly, serial concentrations of Temephos were prepared and diluted in 100 ml with acetone. A control with 100 ml of 0.08% saline water and control with highest volume of acetone were also set. Percent mortality of treated larvae was observed after 24 hrs and calculated the lethal concentration LC_{50}. 24 hr LC_{50} and LC_{90} were calculated using a probit programme developed by Finney[6].

In another experiment five different concentrations of Temphos (0.1 ppm, 0.05 ppm, 0.02 ppm, 0.01 ppm and 0.005 ppm) and *Uvaria narum* leaf extract (200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm) were applied in to separate disposable glasses to find out the rate of treated larval mortalities. Synergistic effect of *U. narum* leaf extract with synthetic larvicide (Temephos) was also screened in yet another set of experiment keeping the plant extract/ temephos in constant concentration. After the assessment of toxicity of these groups, the plant extracts significantly potentiated the toxicities of Temephos were added to serial concentrations of larvicides to assess effect on the LC_{50}.

RESULT

Critical Lethal Concentrations: The yield of methanol extract of leaves of the tested plant, *U. narum* is calculated as the percentage in comparison with the initial weight of the powder taken for extraction. It is recorded as 14.75% and 1% stock solution was prepared and subjected for bioassay against III and IV larval instars of filarial vector *Cx. quinquefasciatus.* 24 hr LC_{50} and LC_{90} (ppm) of *U. narum*, Temephos and their synergistic effect was presented in table 1. The 24 hr LC_{50} of the methanol extract of the *U. narum* leaf is 376.45 ppm and 344.25 ppm for III and IV instars of *Cx. quinquefasciatus* respectively. Whereas 24 LC_{50} of the synthetic pesticides temephos is 0.022 and 0.026 ppm for III and IV instar *Cx. quinquefasciatus* larvae respectively (table 1). In combination of the selected pesticides and plant extracts shows an LC_{50} of 29.45 to 36.25 ppm respectively for III and IV instar larvae of *Cx. quinquefasciatus*.

Table. 1: 24 hr LC_{50} and LC_{90} (ppm) and associated statistics of Methanol extract of *U. narum* leaves, Temephos and their combinations against III and IV instar larvae of *Cx. quinquefasciatus*.

Extract/ Insecticides	Instar	LC50 (ppm)	Fiducial limit		LC90 (ppm)	Fiducial limit		Chi- square (χ^2)
			Lower	Upper		Lower	Upper	
Plant : *U. narum*	III	376.45	166.01	542.56	607.46	478.09	1681.19	25.957
	IV	344.25	105.07	469.75	593.25	468.75	1459.98	21.11
Synthetic Larvicide : Temephos	III	0.022	-0.50	-0.61	0.073	0.045	0.490	24.75
	IV	0.02	-0.107	0.099	0.080	0.047	1.411	30.40
Synergism	III	29.45	-71.25	68.57	158.45	105.91	443.68	11.18
	IV	36.25	26.01	45.29	128.25	111.65	152.91	4.25

Mortality percentage were determined after 24hr of exposure and the combined action of the different mixtures was expressed as cotoxicity factor according to Sun and Johnson, 1960[7] to differentiate between potentiation, Antagonism and additive using the following formula,

$$\text{Co-toxicity factor} = \frac{Observed\%\ of\ mortality - \exp ected\ \%\ moratality \times 100}{Expected\ \%\ mortality}$$

This factor differentiates the results into three categories. A positive factor of $\geq$ 20 indicates potentiation, a negative factor of $\leq$ 20 indicates antagonism, and the intermediate values of >-20 to < 20 indicate an additive effect. Table 2 provides the data on the joint action of mixtures of extracts with Temephos and *U. narum* against III and IV larval instars of *Cx. quinquefasciatus*. The co-toxicity factor for III and IV instars are 47.5 and 50.15 % points out its effects as potentiation, against the larvae of *Cx. quinquefasciatus*. The data shows 93.3% mortality against 63.3%, expected mortality when the third instar larvae treated with LC_0 and LC_{50} of temephos and *U. narum* respectively.

Table 2: Joint action of mixtures of U. narum leaf extract with insecticides against III and IV instar instar larvae of *Cx. Quinquefasciatus.*

Instar	Mixture		Expected mortality (%)	Observed Mortality (%)	Co-toxicity factor	Joint action
	Temephos (LC0)	*U. narum* (LC50)				
III	0.005	376.45	63.3%	93.3%	47.5%	*Po
IV	0.005	344.25	66.6%	100%	50.15%	Po

*Potentiation

Experiments in combination of LC_0 of Temephos and LC_{50} of *U. narum* tested against IV instar larvae of *Cx. quinquefasciatus* shows 100% mortality against an expected mortality of 66.6% (Table 2).

Synergistic/Antagonistic action

The insecticide (Temephos) at LC_0 (a concentration level causing no observed mortality) level mixed with the LC_{50} of *U. narum* showed synergistic effect against larvae of III and IV instar of *Cx. quinquefasciatus.* Synergistic factor (SF) of more than 1 indicates low synergism while SF approaching 2 meant high synergism. Data on synergistic factor for the selected instar of *Cx. quinquefasciatus* are provided in table 3. Synergistic factor calculated in with observed mortality of 93.3% against the expected mortality of 63.3% in combination experiment of LC_0 and LC_{50} of III instar *Cx. quinquefasciatus* larvae is 1.5 (table 3). Likewise experiments in combination of LC_0 temephos and LC_{50} *U. narum* tested against IV larvae of *Cx. quinquefasciatus* shows a synergistic factor of 1.5.

Table 3: Synergistic/Antagonistic effects of temephos with *U. narum* leaf Methanol extracts at LC_0 and LC_{50} concentration tested against III and IV instar larvae of C*x. Quinquefasciatus.*

Instar	Mixture		Test concentration		Sum of expected% of mortality values (A)	observed % mortality for the mixtures (B)	SF (C)	Effect
	Insecticides	Plant extracts	LC_0 ppm	LC_{50} ppm				
III	Temephos	*U. narum*	0.005	376.45	63.3%	93.3%	1.5	Synergism
IV	Temephos	*U. narum*	0.005	344.25	66.6%	100%	1.5	Synergism

(A) expected mortalities resulted from *Cx. quinquefasciatus* larvae exposed to LC_0 and LC_{50} of the tested toxicants in separate tests.

(B) Observed % of mortality refers to that of the mixture tested in the same experimental container at the LC_0 and LC_{50} levels.

(C) SF means synergistic/antagonistic factor which results from dividing the observed values by practical expected values; where S.F = 1.0 ± 0.05 (no effect); S.F.>1.05 (synergism); S.F.< 0.95 (antagonism)

DISCUSSION

Synergy has been reported for binary mixtures of various constituents of plant essential oils. Combinations of Thymol and linalool or 1,8-cineole or 1,8-cineole, or terpineol and linalool or 1,8-cineole, were synergistic in terms of toxicity to III stage *Chilopartellus* larvae (Lepidoptera: Pyralidae)[8]. Similarly, trans-anethole strongly synergized the toxicity of Thymol, citronellal and α-terpineol against *Spodoptera litura* (Lepidoptera: Noctuidae)[9]. Similar effects were also observed with the essential oils of *Ocimum kenyense* against stored product coleopterans, e.g. *Sitophilus zeamais* Mots and *Rhyzopertha dominica*[10]. Combinations of compounds may be more desirable due to increased benefits including broader insecticidal spectra, greater protection time and decreased residues and also reduced insect resistance and environmental effects[11].

In the present study, effects of *U. narum*, temephos and their synergistic activity against III and IV instars of *Cx. quinquefasciatus* shows that a combined effect speed up the mortality of the larvae, which could be helpful in the development of natural insecticides for the control of adult mosquito populations (Table 2). For III instar the mortality increases up to 12 folds and for 4th instar it increases up to 9 folds than their individual effects of plant extract.

The results of the present investigation reveal the broad-spectrum toxic properties of tested materials against III and IV instars of *Cx. quinquefasciatus*. Synergistic/

Antagonistic effects of insecticides (Temephos) with plant extracts (*U. narum*) at LC_O and LC_{50} concentration levels respectively against III and IV larval instars of *Cx. quinquefasciatus is* 1.5 (Table 3). Mortality of the larvae and adults of *Cx. quinquefasciatus* exposed to plant extracts or insecticides increased with time of exposure and concentration of toxicants, and their active ingredients. Joint action may well prolong the usefulness of synthetic insecticides that will eventually be unusable due to resistance[12]. Earlier studies with *Nigella sativa* seed extract[13], volatile oil of *Thymus capitatus*[14] and citrus peel oils[15] have shown potentiation of toxicity with different insecticides against *Culex pipiens* larvae. Present results on *Cx. quinquefasciatus* with plant extracts, insecticide and in combination also support these findings as the majority of the mixtures induced potentiation effects against tested larvae. For joint action, the co-toxicity factor for III and IV larval instars of *Cx. quinquefasciatus* are 47.5 and 50.15 respectively. These interesting results shows that synergistic/antagonistic action with conventional chemical pesticides determined in the present study could be exploited for integrated pest management.

CONCLUSION

The findings of the present investigation reveal that the plant extract potentiated the larvicidal activity of synthetic Larvicide against *Cx. quinquefasciatus*. The results of this study will contribute to a great reduction in the application of synthetic insecticides which in turn increase the opportunity for natural control of various medicinally important pests.

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