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Synergistic Activity of *Andrographis paniculata* Nees Extracts Against the Larvae of the Malarial Vector *Anopheles stephensi* Liston (Diptera: Culicidae)

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ABSTRACT

An investigation has been made on the Deltamethrin and different solvent extracts of *Andrographis paniculata* Nees were evaluated under laboratory conditions for larvicidal activity against the malarial vector *Anopheles stephensi* Liston.Ethanolic extract with lethal concentration LC_{50} and LC_{90} of 35.47and 47.43 after 24h and LC_{50}25.22 and LC_{90} 46.32 ppm after 48h, respectively was found to be the most effective, followed by Acetone, Methanol, Chloroform, Hexane, Petroleum ether and Benzene extracts. LC_{50} and LC_{90} for Deltamethrin were 0.0036 and 0.0097 ppm after 24h and 0.0055 and 0.0085 ppm respectively after 48h of exposure respectively. Combined formulations were evaluated for synergistic activity and a 1:4 ratio of Deltamethrin and Ethanolic extract was observed to be more effective than 1:2 and 1:1 ratios. Combinations of *Andrographis paniculata* extracts with Deltamethrin demonstrated higher larvicidal activity, indicating synergistic activity.

Key words: *Andrographis paniculata*, *Anopheles stephensi*, deltamethrin, synergistic, larvicide.

INTRODUCTION

Integrated vector control is an effective and essential part of any successful vector control program. Chemical control, although effective, is often used only as a temporary solution to disease outbreaks. The over use of chemical control often leads to resistance to these chemicals, resulting in a rebounding vector population and disease potential. Over the past decade, phytochemicals have received progressively more attention as insecticide alternatives. Selected botanicals have been shown to be effective larvicides and adulticides and in some cases are more eco-friendly against non-target animals (Prakash *et al*., 1997). Phytoextracts have been successfully tested for various biocontrol programs (Markouk *et al*., 2000; Jeyabalan *et al*., 2003; Choochote *et al*., 2004; Sharma *et al.,* 2006). Natural plant extracts provide potential for the development of botanical pesticides and synthetic analogs (e.g. pyrithrin). Identifying insecticides

that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Synergistic activity between current effective pesticides and phytochemicals is a powerful tool for developing insect control strategies (Bernard *et al*., 1993) Most studies on the synergistic, antagonistic, and additive toxic effects of binary mixtures involving phytochemicals have been conducted on agricultural pests rather than vectors of diseases. So an attempt has been made to evaluate the Synergistic action of an ethanopharmacologically importantant medicinal and insecticidal plant *Andrographis paniculata* Nees with Deltamethrin against *Anopheles stephensi* Liston. The malarial vector of India and other Asian regions are areas where control has not yet been established.

MATERIALS AND METHODS

Mosquito Culture

Anopheles stephensi Liston were maintained in the laboratory from the lines obtained from National Institute of Communicable Diseases-Field station Cunnoor. The colonies of mosquitoes were maintained at conditions 27± 2^0 C and 80% ± 5 relative humidity under 14L:10D cycles. The larvae were reared in enamel trays and fed finely ground dog biscuits and Yeast at 60:40 ratio. Water in rearing containers was refreshed every 2 days, pupae were transferred from the trays to a cup filled with dechlorinated tap water and placed in screened cages where adults emerged.The adult mosquitoes were maintained in a net cage (90×90×90 cms) and were continuously given 10% sucrose solution provided in a jar with a cotton wick. For continuous culture selected number of Mosquitoes were allowed to feed chicken blood and every third day, thereafter moist filter paper was kept in a beaker in the cages for mosquitoes to lay their eggs on, eggs laid on the filter paper were immersed in larval basins containing water for the maintenance of the colony.

Phytoextract Preparation

The leaves and roots of *Andrographis paniculata* Nees were collected locally from the foot hills of the Western Ghats area adjacent to Bharathiar University, Coimbatore. Tamil nadu, India. The whole plants of were washed with double distilled water and shade dried at room temperature. Dried parts were chopped into small pieces of approximately 1 cm size by a falcon stem cutter (Biocraft scientific India) and powdered with the help of an electric blender. The dried powder was subjected to Ethanol, Methonal, Benzene, Hexane, Chloroform and Acetone in a Soxhlet apparatus

(Borasil, Mumbai, India) for 72 hrs (Saxena *et al*., 1994), after removing the solvents from plant extracts in a Vacuum rotary evaporator viscous paste residues were obtained.The Volume of stock solution of 1% obtained by weighing residues of 200 mg in 20 ml ethanol and kept in a screw-cap vial with aluminum foil over the mouth of the vial.The stock solution was then serially diluted ten-fold in ethanol (2ml solution to 18 ml solvent).Test concentrations were obtained by adding 0.1-1.0 ml (100-1000ul) of the appropriate dilution to 100 ml distilled water (WHO,2005).

Larvacidal Bioassay(WHO 2005)

The larvicidal bioassay was assessed by using standared WHO Protocols (WHO, 2005). For experimental treatment, one ml of *A. paniculata* extract was added to 100 ml of distilled water in a 250 ml of enamel bowel which was shaken lightly to ensure a homogenous test solution, then 25 early fourth instar larvae of *Anopheles stephensi* were transfered by means of strainers of the bowel, each experiment was performed in 4 replicates with a final total of 100 larvae for each concentration and the equal number of controls were set up simultaneously with distilled water to which 1 ml of ethanol was added, experiments were conducted at 27+1^{o}C , 85% Relative humidity (RH) with photoperiod of 12L:12D. Symptoms of the treated larvae were observed and recorded immediately at timed intervals and no food was offered to the larvae. Mortality and survival was registered after 24 and 48 h of the exposure period.The moribund and dead larvae in four replicates were combined and expressed as a percentage of larval mortality of each concentration. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. The larvae showed discoloration, unnatural positions, tremors, unco-ordination or rigor were also counted.

Cypermethrin Bioassay

Deltamethrin, purchased from a local market, was diluted in dechlorinated tap water to obtain a stock solution (20 ppm). Different test concentrations ranging from 0.0015 to 0.05 ppm. were prepared by diluting this solution, and a bioassay against *Anopheles stephensi* larvae was conducted as for the extracts of *A.paniculata.*

Combined Bioassay of Phytoexract and Deltamethrin

For combination studies, 20 ppm, stock of deltamethrin together with the most efficient extract of *A.paniculata* was prepared. Keeping deltamethrin as the standard,

its stock was mixed with the extract in ratios of 1:1, 1:2 and 1:4. Test concentrations for each of the mixed formulation ratios were prepared by further diluting the combination mixture in water. Larval efficacy for each formulation was observed as above and lethal concentration LC_{50} as well as LC_{90} were determined.

Data Analysis

Mortality data produced for phytoextracts and Deltamethrin bioassays and for mixed formulations were analyzed by Probit Analysis (Finney, 1971). The corrected percent mortality was calculated by Abbot's formula (Abbot, 1925) on mortality if it ranged from 5 to 20% in controls:

Mortality (%) = [(T-C) / (100-C)] × 100

Where T is the percent mortality in the test concentration and C is the percent mortality in the control.

Regression equations, LC_{50} and LC_{90} were obtained along with standard error and fiducial limits at 95% confidence level. A co-toxicity coefficient (Sarup *et al* 1980) and a synergistic factor (Kalyanasundaram *et al*., 1985) for mixed formulation experiments were calculated after calculating LC_{50} and LC_{90} for each combination.

Co-toxicity coefficient (CTC) = [toxicity of insecticide (alone) /toxicity of insecticide with plant extract] × 100.

Synergistic factor (SF) = toxicity of insecticide (alone) / toxicity of insecticide with plant extract.

A value of SF > 1 indicates synergism and SF < 1 indicates antagonism.

The co-toxicity co-factor differentiates the results into three categories. A positive factor of +20 indicates potentiation, A negative factor of -20 indicates antagonism and the intermediate values of -20 to +20 indicate an additive effect.

RESULTS

Larvicidal Activity of *Andrographis Paniculata Nees*

The larvicidal activity of extracts of *A.paniculata* Nees against *Anopheles stephensi* larvae shown in Table 1.The LC_{50} and LC_{90} were 35.47, 47.43, 36.51, 50.70, 35.87, 48.39, 36.54, 49.27, 37.68, 51.88 ppm at 24h and 38.05, 51.36, 3.02, 52.35 ppm at 48 h exposure for ethanol, methanol, acetone, choloroform, hexane, petroleum ether, benzene respectively. All of the values fit well within 95% confidence limits, All fractions of

A.paniculata Nees demonstrated appreciable larvicidal activity after 24 and 48 hrs exposure. The ethanolic extract showed greater larvicidal activity than the other extracts.

Bioassay of Mixed Formulations

The results of different ratios of deltamethrin and ethanolic extract combined against *Anopheles stephensi* larvae are shown in Table 2. The ratio 1:1 had LC_{50} and LC_{90} of 0.0026 and 0.0057 ppm. and 0.0016 and 0.0055 ppm. after 24 and 48 h of exposure, respectively. The LC_{50} and LC_{90} values for the ratio 1:2 of deltamethrin and plant extract were 0.0025 and 0.0034 ppm. and 0.0012 and 0.0020 ppm. after 24 and 48 h of treatment, respectively; and for the ratio of 1:4 were 0.0022 and 0.0028 ppm. and 0.0012 and 0.0020 ppm. after 24 and 48 h of treatment, respectively.

For LC_{50} values, the co-toxicity co-efficient of the ratio 1:1 was 138.46 and 343.75 and the combined factor was 1.38 and 3.43 after 24 and 48 h of exposure, respectively, synergism was seen at both time points. In the case of LC_{90} values, the co-toxicity coefficient was 170 and 154 and the combined factor was 1.70 and 1.54 after 24 and 48 h of exposure, respectively, synergistic action against *Anopheles stephensi* larvae at both time points. For the ratio 1:2, the co-toxicity coefficient was 144 and 458 and the combined factor was 1.44 and 4.58 for LC_{50} values after 24 and 48 h of exposure, respectively, which showed synergism. For LC_{90} values, the co-toxicity coefficient was 285 and 425 and the combined factor was 2.85 and 4.25 showing synergism after 24 and 48 hrs of treatment. The ratio 1:4 had a co-toxicity coefficient of 163 and a combined factor of 1.63 (synergism) after 24 h and 458 and 4.58 (synergism) after 48 h for LC_{50} values, and for LC_{90} values the co-toxicity coefficient was 345 and the combined factor was 3.45 (synergism) after 24 h, and 425 and 4.25 (synergism) after 48 h.

DISCUSSION

Economical and efficient mixtures of toxicants have been used in pest control over the years. When certain pairs of toxicants are administered together, the resultant effect may be greater than, equal to or less than what might be expected from the sum of the activity of the toxicants when administered separately. In “synergism”, the joint toxic action of mixtures is significantly greater than that which can be predicted from the individual action of the components; conversely, toxic “antagonism” is the toxic action resulting from a mixture of two or more components in which the resulting response is significantly less than the minimum effect predictable from the separate action of each component (Sarup *et al*., 1980).

Table 1. Efficacy of different solvent whole plant extracts of *Andrographis paniculata* Nees against larvae of malarial vector *Anopheles stephensi* Liston.

Solvent Extraction	Exposure time (hrs)	Regression equation	X^2	LC_{50}+SE (Fiducial limits)(p.p.m)	Relative Toxicity (ppm)	LC_{90}+SE (Fiducial limits)(ppm)	Relative Toxicity (ppm)
Ethanol	24	Y=1.07×-3.80	4.06	35.47±4.5 (28.90-39.79)	6.42	47.43±5.3 (42.24-67.76)	6.11
	48	Y=0.06×-1.53	5.097	25.22±4.9 (20.52-28.79)	6.16	46.32±4.3 (40.32-58.37)	7.01
Acetone	24	Y=1.02×-3.67	3.32	35.87±6.2 (34.44-37.17)	4.85	48.39±3.7 (41.65-60.45)	4.29
	48	Y=2.10×-0.07	3.16	27.24±4.9 (22.88-29.85)	5.02	43.83±6.2 (41.36-47.90)	5.02
Methanol	24	Y=0.09×-3.34	4.06	36.51±5.9 (26.87-43.10)	4.46	50.70±4.7 (43.75-101.23)	4.29
	48	Y=0.03×-0.75	0.02	21.34±8.1 (61.44-28.27)	4.15	57.47±5.2 (46.44-197.91)	5.20
Chloroform	24	Y=0.10×-3.67	2.43	36.54±6.5 (35.13-37.85)	3.82	49.27±6.5 (46.84-52.85)	2.85
	48	Y=0.07×-2.13	2.36	28.68±5.9 (24.7-31.1)	4.20	45.89±4.9 (43.72-50.60)	3.11
Hexane	24	Y=0.09×-3.40	1.22	37.68±9.0 (36.19-39.16)	3.02	51.88±4.5 (48.90-50.28)	1.98
	48	Y=0.06×1.99	1.69	29.67±6.5 (25.60-32.13)	3.46	48.76±5.1 (45.35-54.93)	2.86
Petroleum ether	24	Y=0.09×-3.66	2.98	38.05±6.7 (37.99-39.47)	2.96	51.36±6.1 (48.60-55.54)	2.20
	48	Y=0.54×-1.51	3.45	27.68±7.2 (21.12-30.99)	3.00	51.15±,5.6 (46.64-60.72)	2.58
Benzene	24	Y=0.08×-3.39	0.41	38.02±6.7 (37.97-39.52)	3.05	52.35±7.6 (49.28-57.14)	2.19
	48	Y=0.05×-1.62	1.82	28.59±6.7 (22.95-31.61)	2.97	51.08±6.2 (46.75-59.91)	2.62

Table 2. Combined bioassay of Deltamethrin (D) with whole plant ethanolic extract of *Andrographis paniculata* Nees against the Malarial vector *Anopheles stephensi* Liston, Syn –Synergism, D-Deltamethrin, E-Ethanolic Extract.

Treatment	Ratio (D:E)	Exposure Time (hrs)	Regression Equation	X^2	LC50+SE (FiducialLimits) (ppm)	CTC	SF	Type of Action	LC90+SE (Fiducial limits) (ppm)	CTC	SF
Deltamethrin	-	24	Y=0.03×-3.60	2.45	0.0036±7.1 (0.0030-0.0048)	-	-	-	0.0097±7.9 (0.0058-0.0084)	-	-
	-	48	Y=0.95×-3.77	0.89	0.0055±6.1 (0.0040-0.0073)	-	-	-	0.0085±7.6 (0.0061-0.0129)	-	-
D with E	1:1	24	Y=0.28×-3.68	2.98	0.0026±7.3 (0.0022-0.0035)	138.46	1.38	Syn	0.0057±6.2 (0.0028-0.0066)	170	1.70
	1:1	48	Y=0.96×-5.77	2.63	0.0016±8.1 (0.0006-0.0028)	143.75	1.43	Syn	0.0055±,6.0 (0.0022-0.0092)	154	1.54
	1:2	24	Y=0.14×-4.36	1.30	0.0025±5.9 (0.0013-0.0038)	144	1.44	Syn	0.0034±5.2 (0.0025-0.0035)	285	2.85
	1:2	48	Y=0.82×-6.65	1.94	0.0012±6.4 (0.0004-0.0032)	458	4.58	Syn	0.0020±9.6 (0.0009-0.0035)	425	4.25
	1: 4	24	Y=0.11×-5.10	4.76	0.0022±5.6 (0.0010-0.0032)	163	1.63	Syn	0.0028±4.7 (0.0019-0.0037)	346	3.46
	1: 4	48	Y=0.82×-6.65	1.94	0.0012±5.2 (0.0004-0.0032)	458	4.58	Syn	0.0020±6.1 (0.0009-0.0035)	425	4.25

The synergistic effects observed in bioassays using a combination of botanical extracts and different synthetic insecticides have been observed in several previous studies (Kalyanasundaram & Babu, 1982; Kalyanasundaram & Das, 1985; Mulla & Su, 1999; Thangam & Kathiresan, 1990, 1991). Some extracts have also produced synergistic effects with insect growth regulators. Mulla & Su (1999) showed that neem seed kernel extract has synergistic effects when combined with the juvenile hormone analog methoprene. A few studies have mentioned synergism between different botanical extracts. Mwaiko (1992) reported that a mixture of the peel oils extract of three citrus species (lemon, orange, and bitter orange) was much more effective than for the peel oils extract for the individual species.

In the present study, extracts of *A. paniculata* Nees were found to act synergistically. The combination of deltamethrin and the plant extract was greatly enhanced at different ratios. However, the larvicidal activity was minimal when the mixed formulation contained an equal amount of both the constituents (i.e. a 1:1 ratio). The co-toxicity coefficient and synergistic factor for ratio 1:1 were lower than for the other ratios of the mixed formulations tested. In this ratio, the plant component decreases the toxicity of deltamethrin earlier but later increases its toxicity against *Anopheles stephensi* larvae by degrading the detoxifying enzymes in the mosquito, and thus it exhibits synergism as observed by Thangam & Kathireasan (1991) in *Aedes aegypti*. The present study is favorably supported by the finding of Bansal and Singh (2006), deltamethrin, an approved insectide, is effective for the control of mosquitoes. Furthermore, Thangam & Kathireasan (1991) noticed the synergistic properties of *Rhizophora apiculata*, *Caulerpa scalpelliformis* and *Dictyota dichotoma* individually and with DDT. Moreover, the larvicidal potential of *Vinca rosea*, *Leucus aspara*, *Pedalium murax*, *Clerodendron inerme*, *Turnera ulmifolia* and *Parthenium hysterophorus* in combination with phenthoate and fenthion against *Anopheles stephensi* has been observed by Kalyanasundarm & Das (1985) and significant synergism was noted with fenthion, the synergistic factor was 1.40, 1.31, 1.61, 1.48, 1.38 and 2.23, respectively. The inferences regarding the larvicidal potential of crude extracts of *A.paniculata* suggests its suitability as an eco-friendly, effective larvicide in the management of mosquito populations and in limiting the outbreak of various vector borne epidemics. In addition, the results concerning the mixed formulation of the extract and deltamethrin may prove helpful in finding an effective combination of natural and synthetic products against mosquito larvae, which may be an alternative to existing, conventional chemical insecticides and provide a solution to combat the ever increasing problem of the world's mosquito population.

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