Asian J. Exp. Sci., Vol. 16, No. 1&2, 2002, 9-19

Spilanthes acmella Murr. : Study on Its Extract Spilanthol as Larvicidal Compound

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Spilanthol, a major constituent of ethanolic extract of flower heads of *Spilanthes acmella Murr.* is having potent ovicidal, larvicidal and pupicidal activity. Maximum 7.5 ppm concentration causes 100% motility of eggs, larvae and pupae of Anopheles, Culex and Aedes mosquito. Spilanthol is more effective even at low doses against eggs and pupae. In pupae, it seems to work on nervous system which was evident by abnormal movement like jerks, spinning and uncoordinated muscular activity suggesting thereby that it disturbs nerve conduction.

Key Words : Spilanthol, Spilanthes acmella, Larvicidal, Ovicidal, Culex quifaciatus, Say, Anopheles culicifacies, Giles and Aedes aegypti, Linn.

Introduction :

In recent years use of eco-friendly and easily biodegradable plant products having natural insecticidal activity is increased. The first extensively used compound *pyrethrum* against mosquito was obtained from the flower extract of Chrysanthemum cinerariaefolium. Although the extract is effective, yet it is photodegenerative and requires high quantity to control the insect population. Hence, the investigations for such phyto-compounds are renewed which have selectively higher toxicity at low concentration to various or all developmental stages of mosquito. The said compound may diffuse from plant's parts in effective concentration if immersed in water body, longer stability in aqueous medium and occurs in plants in desired amount. But the said compounds have no or negligible effects on aquatic vertebrates and mammals. The present investigation is a step in this direction.

Mehra and Hiradhar (1998), Venkatachalam and Jebanesan (2001), Pendse *et. al*. (1946), Mehra *et. al*. (2000), Kalyansundaram and Das (1985) and many others tried the efficacy of many natural products from plants as larvicides. Pendse *et. al*. (1946) found the ethanolic extract of Spilanthes acmella Murr. one tenth active as compared to DDT against Anopheles larva.

Belonging to family *Asteraceae*, *Spilanthes acmella* (L.) Murray is also known as Toothache Plant and its various synonyms are *Bidens acmella, Bidens ocymifolia, Pyrethrum acmella, Spilanthes ocymifolia, Verbesina acmella, Blainvillea acmella*. It has the following place in plant kingdom.

Kingdom Plantae	–	Plants
Subkingdom Tracheobionta	–	Vascular plants
Superdivision Spermatophyta	–	Seed plants
Division Magnoliophyta	–	Flowering plants
Class Magnoliopsida	–	Dicotyledons
Subclass	–	Asteridae
Order	–	Asterales
Family Asteraceae	–	Aster family
Genus Spilanthes Jacq.	–	spilanthes

The present study deals with the laboratory investigations to ascertain the larvicidal properties of S. acmella Murr. in three species of mosquito viz. *Anopheles culicifacies* Giles, a vector of malaria, *Culex quinquefaciatus, Say*, a vector of filariasis and *Aedes aegypti Linn*, a vector of dengue.

The plant belongs to family compositeae, an annual herb upto 30-60 cm. in height that grows throughout India. Villagers use flower heads, which give burning taste, as a remedy for stammering and toothache. Other aerial parts and roots are used for curing of inflammation and diarrhoea.

Gokhle and Bhide (1945) extracted Spilanthol from the aerial parts of the plants . Later on Krishnaswami *et. al*. (1975), Bohalman *et. al*. (1980), Barges-Del-Castillo *et. al*. (1984), Lemos *et. al*. (1991) and Baruah (1993) reported â- cryophyllene, â-sitosterol, limonene, myrecene and other compounds from the plant *S. acmella* and its two other species. The flower tops and aerial parts have been found to be toxic to mosquito larvae and Periplanata. The compound Spilanthol has been identified as having larvicidal activity (Kadir *et. al*., 1989). In the present study one of the major constituents, Spilanthol, was use to confirm its activity against the eggs, various instar larvae and pupae of the above- mentioned mosquito species.

Material and Methods :

Collection : Flower heads of *Spilanthes acmella* (Murr) were collected locally from Gour Nagar hills. A herbarium specimen carting a number 105 IR (2000) has been deposited in the Herbarium of the Botany Department, Dr. Hari Singh Gour University, Sagar, after identification in the last week of June. Paracress is used which are leaves and particularly the flowers, which may better be termed flower heads. The herb is, in any case, best used fresh. The weird-looking flowerheads are set atop elongated stems, sometimes measuring more than 4 inches. The flowerheads begin as squat red affairs; they gradually elongate and turn yellow, still retaining the bloody red portion at the top, as they become chaffy seedheads that contain numerous slender black seeds. The red and yellow floral *contrast* makes for an effective and endearing sight.

Extraction : 1 kg. of powdered flower heads was extracted with petroleum ether (b/p 60-80^0 C) for 12 hrs. After vacuum evaporation of petroleum ether the residue (16gm.) was treated with methanol. So obtained Spilanthol was purified with successive washings with acetone. The yield of purified Spilanthol crystal was 407 mg/kg 90% dry powder.

Experiment : 100 mg. crystal were dissolved in 100 ml. ethanol. Aqueous solutions of 2 to 12 ppm were prepared from the stock solution.

The identified eggs of Culex, Anopheles and Aedes were obtained from Zoology Department of the University and kept separately in the aqueous medium containing 0.1% albumin at 28^{0}C +1^{0}C. The laboratory reared Ist to IVth instar larvae of Culex, Anopheles and IIIrd and IVth instar larvae of Aedes were reared in water containing 0.1% albumin and dried liver at 28^{o}C+1-2 ^{o}C at 12: 12 dark light regime.

The sets of 25 eggs of each species in triplicate were treated with 1, 3, 5 and 10 ppm solution (aqueous) of Spilanthol. The mosquito larvae were treated with different concentrations of the drug following the WHO (1981) larval susceptibility test method. The laboratory reared Ist, IInd, IIIrd and IVth instar larvae of Anopheles, Culex and IIIrd and IVth instar larvae of Aedes were treated with 1 to 10 ppm of the drug in water containing 0.1 % albumin and dried liver powder. For each of the given concentrations and instar stages

two replicates of 25 larvae per set were exposed. The pupae of the three species were exposed in a similar manner.

The results were scored after 24 hrs. of continuous exposure to the test solution. During the first three hours of drug exposure, the insects were constantly observed.

Untreated developmental stages (eggs to pupae) were kept at identical conditions except without the drug. The percent hatching or mortality was recorded. The results were analysed statistically and level of significance was marked asterically where it was not found.

Result and Discussion :

Tables 3 and 4 show that 4-7.5 ppm concentration of the drug proved deleterious to the various larval stages of mosquitoes. 100% mortality of Ist, IInd instar larvae of Culex and Anopheles was recorded with in 24 hrs. at 6.5 and 7 ppm respectively. Between 6.5 to 7.5 ppm concentrations no IIIrd and IVth instar larvae of any test species of mosquito could survive. At 1 and 2 ppm concentration of drug 12 to 48% eggs hatched normally (Table-2). No hatching was observed at 3 ppm concentration and eggs became decolorized within 21 hrs. However, the eggs when dissected and visualized under microscope partially formed embryos were noticed. 4-5 ppm concentration of the drug killed the experimental pupae within 3 to 5 hrs. of exposure. It is observed that as the concentration and exposure time increase progressively mortality increases in severity.

Abnormal spiral movements, incapability to reach 3-4 inch height from the bottom of water column, strong jerking movements exhibited intermittently by experimental larvae are some characteristics which appeared within 2 to 3 hrs. of the drug exposure. Slow upside-down movements, feeble jerks with lesser frequency occurring during the last phase are indicative characteristics of acute toxicity, which appeared between 10 to 15 hrs. after drug treatment.

Main constituents of Flowering paracress :

The pungent flavour of paracress is due to an unsaturated alkamid, spilanthol, which reaches its highest concentration (1%) in the flowers;

additionally, other pungent alkamides (isobutylamides of hendeca-2E,7Z,9E-trienoic acid and hendeca-2E-en-8,10-diynoic acid). In other work there are reports of C9 polyunsaturated alkamides. These compounds are chemically and physiologically related to the sanshools found in sichuan pepper. Besides the alkamides, pungent nonvolatile sesquiterpenoids have been found, e.g., polygodial and eudesmanolide II. The former is the dominant constituent of two other pungent spices, water pepper and Tasmanian pepper. From the flowers of paracress, traces of an essential oil have been isolated, whose main constituents were limonene, â-caryophyllene, Z-â-cimene, ã-cadinen, thymol, germacrene D and myrcene. (*J. Essent. Oil Res.*, 3, 369, 1991), (*J. Essent. Oil Res.*, 5, 693, 1993)

Spilanthol is more effective even at low doses against eggs and pupae. In pupae, it seems to work on nervous system as evident by abnormal movement like jerks, spinning and uncoordinated muscular activity. This suggests that the drug disturbs nerve conduction somewhere. The mortality of pupae in short span of time upon exposure to the drug also indicates that Spilanthol greatly disturbs the ongoing processes of histolysis and histogenesis. Though Kadir *et. al*. (1989) and Pendse *et. al*. (1946) reported Spilanthol as larvicidal yet the concentrations these reported are quite higher than what we observed in our study. Ramsewak *et. al*. (1999) observed 100% mortality of Aedes larvae at 12.75 µg/ml of Spilanthol. The hexane extract of dried flower buds of *Spilanthes acmella* afforded three N-isobutyl amides: spilanthol, undeca-2E,7Z,9E-trienoic acid isobutylamide and undeca-2E-en-8,10-diynoic acid isobutylamide. Their structures were determined by 1H and 13C NMR, MS and GC-MS spectroscopic methods. All were active against Aedes aegyptii larvae and Helicoverpa zea neonates at 12.5 and 250 micrograms/mL concentrations, respectively. In our case LD_{50} values for IIIrd and IVth instars larvae of Aedes were obtained at 4.5 and 5 ppm respectively. Interestingly, IIIrdand IVth instar larvae and pupae are more susceptible to Spilanthol at low concentrations i. e. 2 to 4 ppm; on exposing to these concentrations, unexpected higher mortality was observed during first 12 hrs. Sharma and Goel (1994) reported that alpha-T and erythrosine-B,the naturally occurring plant secondary metabolites are toxic to Ist to IVth instar larvae of Anopheles and Culex. Intereatingly, LC_{50} for first two and last two instar larvae and susceptibility of the compounds to the larvae differ much. They found the drugs more effective against Culex larvae than of Anopheles.

Ramsewak *et. al*. (1999) observed Spilanthol as larvicidal on larvae of one species, the concentration they reported are quite higher than what we observed in our study. Our results confirm the larvicidal activity of Spilanthol. However, we observed the effects on all developmental stages of three species of mosquito. Work in respect of other constituents of the extract is being carried out with the view to studying their synergistic effect and the mechanism of the drug affect the insect.

The study reports the free amino acid composition of the pollen of nine members of the family Asteraceae, i.e. *Ageratum conyzoides* L., *Blumea oxyodonta* DC., *Eupatorium odoratum* L., *Gnaphalium indicum* L., *Mikania scandens* Willd., *Parthenium hysterophorus* L., *Spilanthes acmella* Murr., *Vernonia cinerea* (L.) Lees. and *Xanthium strumarium* L. by thin layer chromatography. The amino acid content was found to vary from 0.5-4.0% of the total dry weight. Fourteen amino acids were identified, among which amino-n-butyric acid, aspartic acid and proline were present in almost all pollen samples. The other major amino acids present in free form included arginine, cystine, glutamic acid, glycine, isoleucine, leucine, methionine, ornithine, tryptophan and tyrosine (Mondal *et. al*., 1998).

Conclusion :

After the failure of National Malaria Eradication Programme because of the resistance developed by the medically important disease vector-mosquito against the synthetic chemical insecticides like DDT, BHC etc. and later on, due to ban on the use of these compounds by WHO (1981) because of their harmful effects on human beings other flora, fauna and ecology etc. the situation became even more difficult. Spilanthol, being more effective even at low doses against eggs and pupae and seems to work on nervous and the ongoing processes of histolysis and histogenesis, may prove quite effective insecticidal agent.

Acknowledgement :

The authors are thankful to the Head, Department of Zoology and Department of Pharmaceutical Science for providing facilities.

Table-1 : Showing the LC_{50} and LC_{95} values to the different larval stages of Culex, Anopheles and Aedes.

Species	**Instar**	**LC_{50} ppm**	**LC_{95} ppm**
Culex	I,II	4.0	6.0
	III & IV	4.5	6.0
Anopheles	I, II	5.0	6.5
	III & IV	5.09	7.0
Aedes	III	4.5	6.5
	IV	5.0	7.0

Table-2 : Ovicidal activity on Culex, Aedes and Anopheles.

Concentration	% of eggs hatched out			Time in hours
	Aedes	Culex	Anopheles	
1	48	32	52	12
2	24	16	12	14
3	0	0	0	20
Control	95%	96%	92%	8

Table- 3 : Showing the effect of drug on the larval stages of Culex.

S. No.	Concentration (ppm)	% Mean mortality in hour			
		6	12	18	24
I and II instar larva of Culex					
1.	2	1+ 0.05	7+0.53	25+2.10	35+2.43
2.	4	2+0.06	13+0.62	35+2.51	52+3.81
3.	6	12+0.61	25+2.11	60+3.90	95+4.12
4.	6.5	27+2.13	60+3.88	92+3.01	100+1.21
III and IV instar larva of Culex					
5.	2	4+0.42	12.5+0.52	30+2.23	42+2.91
6.	4	8+0.61	18+0.73	40+2.52	58+3.72
7.	6	20+1.97	25+2.12	55+3.95	92+4.20
8.	6.5	40+2.52	68+1.10	95+2.97	100+1.31

Table-4 : Exhibiting the effect of drug on the larval stages of Anopheles.

S.No.	Concentration (ppm)	%Mean mortality in hour			
		6	12	18	24
I and II instar larva of Anopheles					
1.	2	0	10+0.59	16+0.55	20+0.79
2.	4	2+0.04	11+0.60	30+2.46	48+3.77
3.	6	10+0.58	23+2.90	55+3.78	95+1.30
4.	6.5	25+2.02	55+5.82	93+2.42	100+1.89
III and IV instar larva of Anopheles					
5.	2	3+0.05	11+0.61	28+2.44	40+2.52
6.	4	9+0.58	15+0.64	38+3.66	53+3.76
7.	6	22+2.87	31+2.75	52+3.75	95+5.78
8.	6.5	35+3.50	59+3.75	97+2.68	100+5.12

Table-5 : Exhibiting the effect of drug on the III and IV larval stage of Aedes.

S.No.	Concentration (ppm)	%Mean mortality in hour			
		6	12	18	24
1.	2	0	5+0.50	20+2.85	30+2.46
2.	4	1+0.03	9+0.56	25+2.10	45+3.75
3.	6	10+0.57	30+2.46	70+4.62	96+5.01
4.	7.5	31+2.46	55+5.03	92+2.56	100+3.78

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