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LABORATORY STUDIES ON SOME PLANT EXTRACTS AS MOSQUITO LARVICIDES

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Preliminary screening work on the biological activity of crude extracts of 24 local plants has been undertaken to study their larvicidal effects and growth inhibition properties against the 4th instar larvae of *Aedes aegypti* L. Alcoholic extracts of 12 plants resulted in the mortality of larvae to varying degree at 1000, 500 and 100 ppm doses. The extracts of *Ervatamia coronaria* proved highly toxic, whereas the petroleum ether extracts of this plant showed no larvicidal activity. The alcoholic extracts of *Cassia holosericea* also produced no lethal effects on larvae. The larvae exposed to these extracts could not pupate at 1000 ppm dose. These two plants seem to have juvenomimetic activity. There is need for further work to study these extracts for active ingredients to confirm the juvenile hormone (JH) like compounds in them.

INTRODUCTION

Since Williams *et al.* [1,2] discovered that certain plant products inhibited metamorphosis in Pyrrhocorid bugs, juvenile hormone (JH) activity has been discovered in few other plants so far, and the substances responsible for this activity have been isolated and their structure elucidated in certain cases. Most of the attempts to detect JH activity in plants gave negative results. Bowers [3] reported that of 52 plants he had investigated, extracts from only six showed JH activity. Staal [4-5] and Mansingh *et. al.* [6] also obtained mostly negative results. All these workers usually used coniferous trees. Tropical plants have been studied rarely. Prabhu and his colleagues [7-9] on the other hand have reported that JH activity is rather widespread in Indian plants. Grainge *et al* [10] have recently reviewed the literature pertaining to plant species possessing pest control properties including JH-like activities in some.

The present paper summarises the preliminary results of crude extracts of 24 indigenous plants. Out of these two plants seem to have JH-like activities. Further work has been undertaken to isolate active substances from them.

MATERIALS AND METHODS

one hundred g. air dried plant material was crushed and then extracted three times with 95 % alcohol at room temperature. The combined filtrates were concentrated under vaccum at 50° or less to remove the solvent completely.

Laboratory-reared fourth instar larvae of *Aedes aegypti* L. served as test insects. The viscous residues of the extracts after the removal of the solvent were further diluted to obtain 1000, 500, and 100 ppm concentrations. All plant extracts were first tried at the maximum conc. of 1000 ppm. If high kill was observed the insects were further tested at lower concentrations of 500 and 100 ppm. The larvae were kept in the treated medium till their emergence time at 28 ± 2° and 70 % RH.

The data collected were statistically analyzed corresponding to test designs. Abott's formula was applied to obtain the corrected percent mortality. Duncan's [11] new multiple range test was employed to evaluate the significance of the treatments.

RESULTS

The lethal effects of the plant extracts to fourth instar larvae of Aedes aegypti *L.* The scientific and common names of the plants, the parts used for extraction work, the known chemical composition of these plants are presented in Table 1.

Eighteen of the 24 plant extracts tested at 1000 ppm resulted in more than 64 % mortality at day-7, (Table 2). Twelve extracts, "tobacco, akri, chandni, sewar, kanghi, neem, babchi, bakain, kiramar, mushkbala, castorbean and yellow acacia", killed all larvae up to day-7. The first seven extracts mentioned above killed 100 % larvae in 24 hr. while the last three resulted in the mortality of all the larvae in three days.

Table 1. Plants investigated for biological activity against *Aedes aegypti* (L.) *Larvae*

S.No.	Family	Scientific name	Common name	Parts used	Known chemical composition of the plant
1.	2.	3.	4.	5.	6.
1.	Apocynaceae	*Rhazya stricta* (Dcne.)	"Sewar"	Leaves	Sewarine, rhazine, rhazidine,
		R. stricta	"Sewar"	Roots	vincadifformine, urosolic acid, triterpenoid, tetrahydroalstonine, vincadine
		R. stricta (partially purified)	"Sewar"		Steroids
		R. Stricta (partially purified)	"Sewar"		Asewerine
		Nerium indicum (Mill.)	"Kaner"	Flowers	Plunericin, karabin
		Ervatamia coronaria (Stapf.)	"Chandni"	Leaves	Ervatamine, lupeol
		Vinca rosea (L.)	"Sada bahar"	Shoots	Vinblastine, vincristine, leurocristine, leurosidine
2.	Aristolochiaceae	*Aristolochia bracteata* (Retz.)	"Kiramar"	Leaves Fruits	Ceryl alcohol, β-sitosterol, aristolochic acid
3.	Asclepiadaceae	*Calotropis procera* (Willd)	"AK"	Leaves	Asclepin, calatropagenin, coronadine, voacangine, ibogamine, isovoacangine, calotropin
4.	Crassulaceae	*Bryophyllum calycinum* (Salisb.)	Zakhmhaiyat	Leaves	Alkain and alkanol, α-amyrin, β-amyrin
5.	Euphorbiaceae	*Euphorbia pulcherrima* (Willd.)	Christmas flowers	Fruits Leaves	
		Ricinus communis (L.)	Castorbean	Leaves	Recinine, antigenin, allergenin
6.	Gentianaceae	*Erythraea roxburghii* (G. Don.)	Barik charayatah	Leaves	
7.	Leguminoseae	*Cassia holosericae* (Fresen.)	Jangli Senna	Leaves shoots	Sennosides and β-amyrin
		Pongamia glabra (Vent.)	Karanja	Fruits	Karanginin, glabrosponin, furanoflavole
		Glycyrrhiza glabra (L.)	Licorice	Roots	Glabrin
8.	Malvaceae	*Abutilon indicum* (Harv.)	Kanghi	Leaves	Glucosylbenzoic acid, farnesol caryophyllene, geraniol
9.	Meliaceae	*Azadirachta indica* (L.)	"Neem"	Shoots	Kaempterol, quercetin, myricetin, azadirachtin, deacetylazadiractinol
		Melia azedarach (L.)	"Bakain"	Shoots	Azaridine, meliotannic acid benzoic acid
10.	Mimosoideae	*Albizia lebbeck* (L.)	"Yellow acacia"	Leaves	Lebbekanin, friedelin, sitosterol, glycosides
11.	Oxalidaceae	*Averrhoa carambola* (L.)	"Kamrak"	Fruits	Acid potassium oxalate vitamin A

(continued.....)

Table 1, continue

12.	Papilionoideae	*Psoralae corylifolia* (L.)	"Babchi"	Seeds	Psoralen, isopsoralen, corylifolinin
13.	Rhamnaceae	*Ziziphus sativa* (Gaertn.)	"Beri"	Leaves	Ziziphic acid, ziziphotanic acid
14.	Solanaceae	*Datura metal* (L.)	"Datura"	Leaves	Hyocyamine,atropine,hyoscine
		Nicotiana tabacum (L.)	"Tobacco"	Leaves	nicotine, choline, anabasine, nornicotine
		Withania coagulans (Dunal.)	"Akri"	Leaves	Vegetable rennet, 4 enolides glycoproteins, withanolide, ergostadienediol
15.	Valerianaceae	*Valeriana wallichii* (DC.)	"Mushkbala"	Rhizomes Roots	Chatinine, velpotriate, chatinine and glucoside

Table 2. Larvicidal activity of various extracts of local plants

1 Name of plant	2 Common name	3 Solvent used	4 Mean percent corrected mortality* at 1000 PPM Day-1	 1000 PPM Day-3	 1000 PPM Day-7	 500 PPM Day-1	 500 PPM Day-3	 500 PPM Day-7	 100 PPM Day-1	 100 PPM Day-3	 100 PPM Day-7
Ervatamia coronaria	"Chandni"	Ethyl alcohol	100^{a}	–	–	80^{a}	100^{a}	–	65.2^{a}	80^{a}	100^{a}
Rhazya stricta (Partially purified)	"Sewar"	–	100^{a}	–	–	100^{a}	–	–	20^{c}	60^{bc}	75.87^{abc}
" "	"	–	100^{a}	–	–	34.28^{b}	100^{a}	–	0^{f}	15^{de}	46.58^{d}
" " (leaves)	"	Ethyl alcohol	100^{a}	–	–	10^{def}	75^{a}	83.48^{ab}	0^{f}	0^{g}	11.53^{fg}
Abutilon indicum	"Kanghi"	Chloroform	100^{a}	–	–	95^{a}	100^{a}	–	64^{a}	72^{a}	95.71^{a}
Azadirachta indica	"Neem"	Ethyl alcohol	100^{a}	–	–	32.86^{b}	65.14^{b}	100^{a}	10^{cd}	44.55^{cd}	81.48^{ab}
Psoralea corylifolia	"Babchi"	"	100^{a}	–	–	18.33^{bcd}	56.66^{b}	100^{a}	0^{f}	1.66^{efg}	41.01^{d}
Nicotiana tabacum	"Tobacco"	Ethyl alcohol	100^{a}	–	–	100^{a}	–	–	2^{ef}	20^{cde}	48.53^{d}
Withania coagulans	"Akri"	"	100^{a}	–	–	100^{a}	–	–	44.33^{b}	51.66^{bc}	71.40^{abc}
Rhazya stricta (Roots)	"Sewar"	"	97.5^{a}	100^{a}	–	96.25^{a}	100^{a}	–	62.5^{a}	68.75^{ab}	93.29^{a}
Valeriana wallichii	"Mushkbala"	"	95^{a}	100^{a}	–	88.66^{a}	100^{a}	–	14^{cd}	50^{bc}	84.98^{a}
Pongamia glabra	"Karanja"	Chloroform	55^{b}	62.5^{b}	89.99^{a}	0^{g}	20^{bcd}	66.96^{bcd}	0^{f}	2.5^{efg}	24.93^{ef}
Ricinus communis	"Castorbean"	Ethyl alcohol	40^{bc}	100^{a}	–	15^{cde}	65^{b}	94.44^{a}	0^{f}	1.66^{efg}	33.87^{de}
Euphorbia pulcherrima	"Christmas Flower"	Ethyl alcohol	23.33^{cd}	33.33^{cde}	89.99^{a}	0^{g}	16.66^{bcd}	39.99^{def}	0^{f}	10^{def}	14.21^{fg}
Aristolochia bracheata (Leaves)	"Kiramar"	"	20^{cd}	77.5^{ab}	100^{a}	30^{bc}	56^{b}	100^{a}	0^{f}	40^{cd}	77.5^{abc}
Albizzia lebbeck	"Yellow acacia"	"	13.33^{de}	100^{a}	–	8.57^{def}	44.28^{bc}	71.23^{bc}	8.33^{de}	14.99^{de}	56.56^{d}
Melia azedarach	"Bakain"	"	5^{def}	30^{cde}	100^{a}	5^{efg}	5^{def}	100^{a}	3.75^{de}	3.75^{efg}	41.02^{d}
Datura metal	"Datura"	"	4^{def}	14^{ef}	73.57^{bc}	4^{efg}	16.66^{bcd}	39.99^{def}	0^{f}	10^{def}	19.57^{efg}
Ziziphus sativa	"Beri"	"	2.5^{efg}	15^{ef}	72.5^{bc}	0^{g}	0^{f}	31.17^{def}	0^{f}	0^{g}	14.21^{fg}
Vinca reosea	"Sada bahar"	"	2.5^{efg}	32.5^{cde}	64.21^{cd}	0^{g}	27.5^{bcd}	59.79^{cde}	0^{f}	20^{cde}	51.74^{d}
Erythraea roxburgii	"Barik charayatah"	"	0^{g}	57.5^{bc}	80.73^{b}	0^{g}	0^{f}	75.22^{bc}	2.5^{ef}	2.5^{efg}	46.38^{d}
Aristolochia brachteata (Fruit)	"Kiramar"	"	0^{g}	45^{bcd}	100^{a}	0^{g}	0^{f}	77.97^{bc}	2.86^{ef}	10^{def}	29.53^{def}
Alochol check	–	–	0^{g}	0^{g}	9.63^{e}	0^{g}	0^{f}	9.63^{g}	0^{f}	0^{g}	10.8^{fg}
Acetone check	–	–	0^{g}	0^{g}	16^{e}	0^{g}	0^{f}	16^{g}	0^{f}	0^{g}	$10.8f^{g}$
Control	–	–	0^{g}	0^{g}	9.2^{e}	0^{g}	0^{f}	9.2^{g}	0^{f}	1.08^{efg}	6.75^{fg}

***Mean percent followed by the same letters above the figures are not significantly different at 5% level.**

At 500 ppm, the first ten extracts mentioned above caused 100% mortality up to seven days. The extracts of castorbean and yellow acacia resulted in significant mortality during this time. The toxicity induced by chandni significantly exceeded that of other plants at 100 ppm, (Table 2).

The effect of plant extracts on the inhibition of mosquito development. The extracts of kaner, licorice and

Table 3. Effect of plant extracts on development of *Aedes aegypti* (L) at day-7

Name of plant	Common name	Percent remaining alive at indicated stages		
		Larvae	Pupae	Adults
Test No. 1 (1000 ppm conc.)				
Cassia holosericea	"Jangli senna"	93.33 ± 3.33	0.0	3.33 ± 3.33
Ervatamia coronaria (Pet. ether ext.)	"Chandni"	73.33 ± 3.33	0.0	0.0
Datura metal	"Datura"	18.00 ± 11.16	0.0	6.00 ± 4.99
Erythraea roxburghii	"Barik charayatah"	12.50 ± 7.5	5.00 ± 3.80	0.0
Euphorbia pulcherrima (leaves)	"Christmas flowers"	6.66 ± 3.33	3.33 ± 3.33	0.0
Test No. 2 (500 ppm conc.)				
Albizzia lebbeck (leaves)	"Yellow acacia"	18.57 ± 6.83	2.85 ± 2.5	4.28 ± 3.33
Rhazya stricta (leaves)	"Sewar"	2.50 ± 1.92	5.00 ± 3.85	7.5 ± 5.76
Test No. 3 (100 ppm conc.)				
Rhazya stricta (partially purified steroids.)	"Sewar"	32.50 ± 3.33	12.50 ± 7.5	10.0 ± 2.88
Aristolochia bracteata (leaves)	"Kiramar"	15.00 ± 1.44	2.50 ± 1.94	5.0 ± 2.88
Valeriana walichii	"Mushkbala"	10.00 ± 8.66	2.00 ± 1.93	2.0 ± 1.92
Rhazya stricta (partially purified asewerine)	"Sewar"	7.50 ± 2.09	12.50 ± 7.5	2.5 ± 1.92
Melia azedarach	"Bakain"	7.50 ± 2.09	11.25 ± 4.2	36.25 ± 9.35
Psoralea corylifolia	"Babchi"	6.66 ± 3.33	5.00 ± 4.72	43.33 ± 5.77
Acetone check	–	0.94 ± 0.0	15.11 ± 3.79	69.09 ± 2.38
Alcohol check	–	6.01 ± 2.03	15.77 ± 2.82	66.68 ± 3.07
Control	–	3.86 ± 2.25	13.47 ± 2.22	72.65 ± 5.37

zakhmhyat were neither toxic nor delayed the larval development at 1000 ppm dose. Alcoholic extract of jangli senna and petroleum ether extract of chandni on the other hand significantly inhibited the larval development at this dose. The treated larvae could not pupate up to day-7, (Table 3). "Barik charayatah", "datura" and leave extract of "christmas flowers" significantly reduced the adult emergence at day-7. The extract of "yellow acacia" reduced the adult emergence at 500 ppm.

Partially purified extracts of "sewar" and crude extracts of "kiramar" and "mushkbala" reduced the adult emergence at 100 ppm, (Table 3).

DISCUSSION

The data presented in Table 2 show that out of the 24 plant extracts tested, 12 resulted in mortality of larvae to varying degrees at three doses during a period of 7 days. The toxicity induced is probably because of the cytotoxic compounds contained in them. Many plant extracts are known to have potent antimitotic activity and recently some effective compounds of plant origin are being tried against tumors in various organisms [12]. It is now well known from various reviews on this subject that there is hardly a chemical class of natural product which does not have a compound showing cytotoxic activity *in vivo* or *in vitro* [12].

In our experiments the extract of *Aristolochia bracteata* proved extremely toxic to the larvae of *Aedes aegypti* L. at 500 ppm dose. At 100 ppm, the toxicity induced was 77.5 percent, (Table 2). The crude extract of this plant is being used from old days to foul and neglected ulcers to destory insect larvae. The vernacular name "Kiramar" (insect killer) is expressive of this fact. Saxena *et al.* [13] topically treated adults of *Dysdercus koenigii, Aedes aegypti* and confused flour beetle *Tribolium castaneum* (Herbst.) with active principle of *Aristolochia bracteata* to observe sterility in these insects. Adults obtained from treated larvae of *Aedes aegypti* were reported to contain undeveloped eggs. Fagoonee *et al.* [14] reported the noxious effects of the methanolic extracts of neem on *Crocidolomia binotalis.* According to them these extracts not only caused antifeedant effects against cabbage webworm, but also caused high larval mortality, poor emergence, disruption of normal development, delay in larval moults and abnormal pupation. They discussed possible interference with the hormonal activity of the test insects. Many other workers [15-20] have studied the antifertility effects of crude extracts of plants, while some have noted juvenomimetic, anti-JH activity and growth inhibition action of plant extracts on insects.

The extracts of *Cassia holosericea* and *Ervatamia coronaria* produced no lethal effects even at highest doses. However, they resulted in delaying the development of the treated larvae, (Table 3). The treated larvae could not pupate and died in juvenile stages. These plants warrant further study as regards their active ingredients and minimum required amount to induce such effects. The juvenomimetic activity observed may be due to JH-like substances present in the extracts or they may be synergistic rather than intrinsically hormonal. Prabhu *et al.* [7-9] tested the extracts of various Indian plants against *Dysdercus cingulatus* and confirmed that the external and internal effects of the extracts was due to JH analogue present in the extracts rather than due to other substances.

The toxic effects of various plant extracts to mosquito larvae may play an important future role in mosquito control provided the cytotoxic compounds contained in them are removed or they get biodegraded into harmless chemicals. Juvenile hormone analogues of plant origin in purified form have no practical significance because of prohibitive cost factors. However, their synthetic preparation can be used as mosquito larvicides on large scale.

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