Testing the Insecticidal, Antifeedancy and Fungicidal Activity of Plant Extracts

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Abstract – Antifeedant, insecticidal and IGR potential of plant extract was tested against crop pests *Lipaphis erysimi Kaltenbach*, (Aphididae: Homoptera) and a lepidopteran insect pest, *Spodoptera litura*. The solvent extracts of four plants, viz. *Polyalthia longifolia*), *Paederia foetida* , *Limonia acidissima* and *Balanites aegyptiaca* from different families were screened for their bio-activity. It was observed that *Polyalthia longifolia* (Methanol extract) exhibited maximum potency with least concentration of 0.1% to give 50 percent antifeedancy, followed by *Polyalthia longifolia* (Pet ether) with a value of 0.2 and *Limonia acidissima* (Methanol) and *Limonia acidissima* (Pet ether). The least active compound for antifeedancy was found to be *Balanites aegyptica* (Water) among 16 extracts tested. Methanol showed potential antifeedancy than chloroform, water and petroleum ether extracts. Chloroform and petroleum ether extracts resulted promising insecticidal activity against aphids. *Limonia* (water), *Balanites* (methanol), *Polyalthia* (Methanol) and *Paederia* (water) didn't show insecticidal activity (LC_{50}) upto 0.1% as maximum concentration.

Key Word--- Antifeedant, insecticidal, IGR and plant Extracts.

I. Introduction

LIPAPHIS erysimi Kaltenbach, (Aphididae: Homoptera), commonly known as mustard aphid, is the most devastating pest and is distributed in many countries [9]. The yield loss due to aphid infestation in mustard ranged from 87.16 to 98.16%. Both the adults and nymphs of this aphid cause damage to mustard plants from seedling to maturity, but maximum damage is caused at flowering stage. The aphids suck sap from leaves, flowers, flower-buds, pod and twigs of the plants. They also secrete sticky honeydew which acts as a medium for sooty mold fungus development and reduce the photosynthetic efficiency of the plants. In case of severe infestation, leaves become curled, plant fails to develop pods, the young pods when developed fail to become mature and cannot produce healthy seeds. As a result, plants lose their vigour and growth becomes stunted [2].

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Synthetic chemicals may be used to plant protection programs to limit crop damage by pests. But because of growing concerns about health and environmental safety, the use of toxic, carcinogenic, and/or environmentally damaging chemicals is being discouraged. These chemicals leave toxic residues in consumable agricultural commodities. The survey of monitoring of farm-gate samples in different parts of the country recorded pesticide residues above Maximum Residue Limit (MRL) [8].

Due to the problems related with indiscriminate use of synthetic pesticides like resistance and effect on non-target organisms, there is diversion from these chemicals to plant based products. Many researchers have focused on the search for active naturally occurring essential plant products. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials [4]. Plant metabolites and plant based Pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides [7]. This led the authors to screen *in vitro* a large number of plants extracts for antifungal activity against important plant pathogenic fungi.

II. Materials And Methods

The antifeedant activity of all sixteen plant extracts were assessed by no-choice test methods, after 24 hours in each test, against 7 day old (3rd instar) larvae of *S. litura* on castor leaves

A. Plant Material

Fresh disease free plant parts (fruits and leaves) were collected from different regions of country. The plant parts used for extraction were, fruits of *Balanites aegytiaca*, arial parts (leaves with stems; without roots) of *Paederia foetida*; Leaves of *Limonia acididssima*; and seeds of *Polyanthia longifolia.*

B. Preparation of Extracts

Method1. Preparation of aqueous extract

For aqueous extractions, the leaf samples (100 gm) of plants were thoroughly washed, blot dried and powdered using blender. Ten gm dry powder was soaked in 200 ml distilled water and allowed to boil on water bath at 100ºC till the total volume of water was reduced to half. Filtered the solution using stainless steel sieve followed by Whatman filter paper No 1. The excess water was evaporated under

reduced pressure at 50 ^{0}C using rotary evaporator. The sample was sterilized at 120^{0}C for 30 min., which served as the mother extract. Dry extracts were stored in air tight glass vials [6].

Method 2. Preparation of organic solvent extract

Thoroughly washed mature leaves of all the test plants were shade dried and then powdered with the help of a blender. The powder (10g) was extracted with 100 ml of organic solvents of varied polarity methanol, chloroform and petroleum ether in 250 ml screw caped flask. The flasks were put on rotary shaker for 3-4 hours, at 120 rpm. After shaking the materials in solvents, the flasks were left overnight for cold maceration. The extracts were filtered through Watman filter paper no. 1, impregnated with same solvent. During filtrations, small amount (2g) of anhydrous sodium sulphate was put on filter paper to avoid any water contents in filtrate. The organic solvents were concentrated to near dryness under reduced pressure at temperature below 40°C. Dry extracts were stored in airtight brown bottle until further use. All the extracts were subjected to antifungal activity against the test fungi.

Method 3. Extraction Using Soxhlet Apparatus

Thoroughly washed mature leaves of all the test plants were shade dried and then powdered with the help of a blender. The plant powder (10g) was extracted with 200 ml organic solvent, in a flask of 500 ml capacity, using soxhlet apparatus. The temperature was maintained at 40ºC. Three repeat reflux were carried out for each plant sample. After extraction, the organic solvent was concentrated to near dryness under reduced pressure maintaining temperature below 40ºC.

The Method 1 was followed to prepare water extracts of all plants. The methanol and petroleum ether extracts were prepared following Method 2, while chloroform extracts were prepared using Method 3.

C. Rearing Of Test Insects

A nucleus culture of *S. litura* was maintained at 25±1^{0}C, 60±5% relative humidity and 16:8 hour photo: scotophase on artificial diet. An artificial diet was used in the study. Kidney bean (*Phaseolus vulgaris*) seeds procured from market were washed thoroughly and soaked overnight in water. Soaked seeds were ground in an electric grinder thoroughly with the addition of 400 ml double distilled water. Wheat bran, wheat germ, ascorbic acid, casein, yeast powder, methyl parahydroxybenzoate, sorbic acid, cholesterol, streptomycin sulphate, formaldehyde and multivitamin (ABDEC drops) were added to the ground material and mixed thoroughly. Agar was boiled in 200 ml double distilled water with constant stirring till it attained necessary consistency and then ground with the rest of the ingredients once again. The whole mixture was poured into plastic trays and covered with thin plastic film. After cooling, the diet was kept in refrigerator and used after 24 hours of ageing.

Neonates, upon hatching from egg, were transferred to glass jars containing fresh thoroughly washed castor leaves. Five-day old larvae were transferred to plastic boxes (30 cm long, 20 cm wide and 7 cm high) containing pieces of diet in groups of two larvae. Boxes were cleaned daily and larvae were fed with fresh diet. When the larvae exhibited gut purge and entered into non feeding wandering stage they were transferred to boxes containing saw dust for pupation. Pupae were collected after four to five days and disinfected with 0.02% sodium hypochlorite. Upon emergence of adults they were transferred to oviposition cages. Adults were fed with 20% honey solution containing vitamin C, E and streptomycin sulphate. Castor leaves with their petiole dipped in water were provided for oviposition inside the cages. All the containers used for rearing were periodically disinfected with Protasan DS® (Qualigens). This enabled to maintain a disease-free and healthy stock culture for further experiments. Larvae for experimental purposes were reared on washed and dried castor leaves in plastic boxes. Care was taken to avoid overcrowding and strict hygiene was maintained to prevent any infection.

D. Antifeedant Activity

The antifeedant activity of all sixteen plant extracts were assessed by no-choice test methods, after 24 hours in each test, against 7 day old (3rd instar) larvae of *S. litura* on castor leaves.

Stock solutions of the test extracts were prepared in the carrier solvents. Further dilution was done in emulsified water by maintaining emulsifier (Tween – 80) level at 0.5 % to yield various concentrations except for aqueous extracts. Concentrations were fixed after preliminary screening.

E. No-Choice Method

Leaf discs were punched out from the washed and dried castor leaves. They were dipped thoroughly in each of the concentration and air dried. Moist filter paper discs were placed in glass Petri plates (9 cm diameter) on which a single treated leaf disc was kept. Single pre-starved (3-4 hours), 7-day old larvae of *S. litura* was released into each Petri plate. Ten replicates were kept for each concentration. Leaf discs treated with solvent emulsified water served as control. The unfed area in each treatment was measured using a Licor-3100 leaf area meter after 24 hours (Table 1).

III. Results

The extracts viz, *Polyalthia longifolia* (methanol) and to the same extent *Polyalthia longifolia* (water) extracts are observed to give best results for fungal inhibition for in-vitro studies against all seven tested pests. *Limonia acidissima* (methanol) was found effective against *Pythium aphanidermatum*, *Rhizoctonia bataticola* and *Sclerotinia rolfsi*, but not against *R. solani*. *Balanites aegyptica* (water) was observed to be anfungal for *Rhizoctonia solani* but was least active for inhibiting *Pythium aphanidermatum* and *Sclerotinia sclerotiorum*. *Paederia foetida* (water) and *Paederia foetida* (methanol) did not show that much bio-activity against any of the tested fungi (Table 2).

Aqueous Extract

Among the 16 plant extracts screened, aqueous extract of *Acacia nilotica*, *Achras zapota*, *Datura stramonium*, *Emblica*

officinalis, *Eucalyptus globules*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Sygigium cumini* (Table 2) have recorded significant antifungal activity against one or the other *Aspergillus* species tested. The percent of inhibition of aqueous extract of the twelve plants were more than 50% against all the test fungi except *Manilkara zapota*, *Polyanthia longifolia* and *Eucalyptus globules* against *A. ochraceus* and *A. tamarii*. plants were extracted in four solvents, to give sixteen plant extracts for their bioactivity for crop pest management.

TABLE I

ANTIFEEDANCY OF PLANT EXTRACTS AGAINST *SPODOPTERA LITURA* LARVAE

Ser.	COMPOUND	Max concn used for test	df	χ^2	REGRESSION EQUATION	SLOPE B ± S.E.	AI 50%	AI 90%	FEDUCIAL LIMITS MIN	FEDUCIAL LIMITS MAX
i	*Balanites aegyptica* (Pet ether)	**1**	**4**	**7.608**	**5.1365+0.4307x**	**0.4307±0.066**	**0.4822**	**456.01**	**0.2244**	**1.0362**
ii	*Balanites aegyptica* (Water)	**1**	**4**	**1.06**	**5.0094+0.2452x**	**0.2452±0.063**	**0.9157**	**154709**	**0.1883**	**4.4525**
iii	*Balanites aegyptica* (Chloroform)	**5**	**6**	**3.137**	**4.9315+0.4009x**	**0.4009±0.047**	**1.4823**	**2333.9**	**0.761**	**2.8871**
iv	*Limonia acidissima* (Water)	**1**	**4**	**0.377**	**5.1098+0.4921x**	**0.4921±0.068**	**0.5983**	**240.67**	**0.2819**	**1.2696**
v	*Limonia acidissima* (Pet ether)	**1**	**4**	**6.98**	**5.2314+0.5026x**	**0.5026±0.067**	**0.3465**	**122.98**	**0.1895**	**0.6337**
vi	*Limonia acidissima* (Methanol)	**1**	**4**	**1.998**	**5.2035+0.4169x**	**0.4169±0.065**	**0.325**	**385.95**	**0.1592**	**0.6634**
vii	*Limonia acidissima* (Chloroform)	**5**	**5**	**9.4**	**4.8738+0.6288**	**0.6288±0.062**	**1.5873**	**173.5**	**1.0247**	**2.4588**
viii	*Polyalthia longifolia* (water)	**1**	**4**	**2.407**	**5.2824+0.7163x**	**0.7163±0.072**	**0.4034**	**24.84**	**0.2589**	**0.6286**
ix	*Polyalthia longifolia* (Methanol)	**1**	**4**	**2.169**	**5.3271+0.3390x**	**0.3390±0.063**	**0.1085**	**655.08**	**0.0535**	**0.22**
x	*Polyalthia longifolia* (Chloroform)	**1**	**4**	**4.923**	**5.0738+0.5619x**	**0.5619±0.070**	**0.7389**	**141.11**	**0.3811**	**1.4327**
xi	*Polyalthia longifolia* (Pet ether)	**1**	**4**	**1.278**	**5.4356+0.6950x**	**0.6950±0.070**	**0.2362**	**16.5**	**0.1578**	**0.3536**
xii	*Paederia foetida* (water)	**5**	**4**	**0.787**	**4.6760+0.4785x**	**0.4785±0.078**	**4.755**	**2269.5**	**2.1281**	**10.6246**
xiii	*Paederia foetida* (methanol)	**5**	**6**	**2.776**	**4.7522+0.3427x**	**0.3427±0.052**	**5.2845**	**29092**	**2.144**	**13.0254**
xiv	*Paederia foetida* (Pet ether)	**5**	**5**	**1.386**	**4.7870+0.4264x**	**0.4264±0.058**	**3.1585**	**3204.6**	**1.4768**	**6.7554**

TABLE II

INSECTICIDAL ACTIVITY (LC_{50} VALUES) OF PLANT EXTRACTS AGAINST APHIDS

Ser.	**Plant Extracts**	**LC50 Values (%)**		
		Estimate	95% Conf. intervals	
i	*Limonia acidissima* (Methanol)	**0.821**	**0.2498**	**2.6973**
ii	*Limonia acidissima* (Pet ether)	**0.777**	**0.1686**	**3.5830**
iii	*Limonia acidissima* (Chloroform)	**0.006**	**0.0013**	**0.0288**
iv	*Balanites aegyptica* (Water)	**3.013**	**0.1837**	**49.4168**
v	*Balanites aegyptica* (Pet ether)	**0.012**	**0.0016**	**0.0968**
vi	Balanites aegyptica (Chloroform)	**0.001**	**0.0001**	**0.0043**
vii	*Polyalthia longifolia* (water)	**0.682**	**0.1502**	**3.0961**
viii	*Polyalthia longifolia* (Pet ether)	**0.001**	**0.0002**	**0.0098**
ix	*Polyalthia longifolia* (Chloroform)	**0.005**	**0.0013**	**0.0160**
x	*Paederia foetida* (Pet ether)	**0.150**	**0.0571**	**0.3917**
xi	*Paederia foetida* (Chloroform)	**0.018**	**0.0022**	**0.1454**

Among fifty-two plants tested, aqueous extract of *Acacia nilotica*, *Achras zapota*, *Datura stramonium*, *Emblica officinalis*, *Eucalyptus globules*, *Lawsonia inermis*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum* and *Sygigium cumini* have recorded significant antifungal activity against one or the other *Aspergillus* species tested. *A. flavus* recorded high susceptibility and hence solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol extracts of all the twelve plants were tested.

Solvent Extracts

One gram of each of the dried evaporated solvent extract of all the test plants was dissolved in 10 ml of methanol. 500 µl of each of the solvent extract was amended with 15 ml of CDA medium before solidification of the medium. The medium amended only with 500 µl of methanol served as a control. *A. flavus* was inoculated and percent inhibition of the mycelial growth was determined as described earlier. As per the results of antifeedancy of various 16 plant extracts against *spodoptera litura*, it is observed that *Polyalthia longifolia* (Methanol extract) exhibited maximum potency with least concentration of 0.1% to give 50% antifeedancy, followed by *Polyalthia longifolia* (Pet ether) with a value of 0.2 and *Limonia acidissima* (Methanol) and *Limonia acidissima* (Pet ether). The least active compound for antifeedancy was found to be *Balanites aegyptica* (Water). So fractions of *Polyalthia longifolia* (Methanol extract), *Polyalthia longifolia* (Pet ether), *Limonia acidissima* (Methanol) and *Limonia acidissima* (Pet ether) could be bio-assayed for further studies. The results of extracts of chloroform and methanol from *Paederia foetida* and *Balanites aegyptica*, respectively are not included in the report as these could not give 50% inhibition in feeding, even up to concentration of 5 % level. Moreover *Balanites aegyptica* (chloroform and methanol), *Limonia acidissima* (chloroform), *Paederia foetida* (water, petroleum ether and methanol) were tested upto 5 % level of concentration for antifeedancy bio-assay.

The chloroform and petroleum ether extracts of all four plants gave very promising insecticidal activity against aphids, in comparison to other two extracts. *Limonia acidissima* (water), *Balanites aegyptica* (methanol), *Polyalthia longifolia* (Methanol) and *Paederia foetida* (water) did not show any insecticidal activity (LC_{50}) upto 0.1% of maximum concentration.

TABLE III
ANTIFUNGAL ACTIVITY (EC_{50} VALUES) OF PLANT EXTRACTS AGAINST VARIOUS FUNGI

	Pythium aphanidermatum	**EC_{50} values (%)**		
		Estimate	95% conf Intervals	
i.	*Polyalthia longifolia* (water)	**0.08**	**0.0676**	**0.1023**
ii.	*Balanites aegyptica* (water)	**6.38**	**1.7413**	**23.3955**
iii.	*Paederia foetida* (water)	**4.55**	**2.3549**	**8.8047**
iv.	*Polyalthia longifolia* (methanol)	**0.06**	**0.0480**	**0.0800**
v.	*Limonia acidissima* (methanol)	**0.34**	**0.2174**	**0.5436**
vi.	*Paederia foetida* (methanol)	**4.35**	**2.2856**	**8.2848**
vii.	***Rhizoctonia bataticola***	**4.82**	**2.2304**	**10.4163**
viii.	*Polyalthia longifolia* (water)	**0.37**	**0.2730**	**0.5129**
ix.	*Balanites aegyptica* (water)	**4.38**	**2.0319**	**9.4626**
x.	*Paederia foetida* (water)	**4.82**	**2.2304**	**10.4163**
xi.	*Polyalthia longifolia* (methanol)	**0.03**	**0.0275**	**0.0443**
xii.	*Limonia acidissima* (methanol)	**0.52**	**0.4028**	**0.6600**
xiii.	*Paederia foetida* (methanol)	**3.92**	**2.1390**	**7.1748**
xiv.	***Rhizoctonia solani***			
xv.	*Polyalthia longifolia* (water)	**0.16**	**0.1294**	**0.1886**
xvi.	*Balanites aegyptica* (water)	**0.55**	**0.3435**	**0.8752**
xvii.	*Paederia foetida* (water)	**2.09**	**1.2126**	**3.6153**

xviii.	*Polyalthia longifolia* (methanol)	**0.14**	**0.1240**	**0.1650**
xix.	*Limonia acidissima* (methanol)	**7.88**	**3.1345**	**19.8264**
xx.	*Paederia foetida* (methanol)	**3.57**	**2.1540**	**5.9110**
xxi.	***Sclerotinia sclerotiorum***			
xxii.	*Polyalthia longifolia* (water)	**0.27**	**0.1752**	**0.4127**
xxiii.	*Balanites aegyptica* (water)	**5.02**	**1.8237**	**13.8085**
xxiv.	*Paederia foetida* (water)	**3.11**	**1.5729**	**6.1563**
xxv.	*Polyalthia longifolia* (methanol)	**0.02**	**0.0140**	**0.0280**
xxvi.	*Limonia acidissima* (methanol)	**4.06**	**1.7588**	**9.3696**
xxvii.	*Paederia foetida* (methanol)	**4.42**	**2.1854**	**8.9353**
xxviii.	***Sclerotinia rolfsi***	**0.41**	**0.3000**	**0.5800**
xxix.	*Polyalthia longifolia* (water)	**0.15**	**0.1126**	**0.2018**
xxx.	*Balanites aegyptica* (water)	**2.17**	**1.1788**	**4.0055**
xxxi.	*Paederia foetida* (water)	**2.88**	**1.5662**	**5.2881**
xxxii.	*Polyalthia longifolia* (methanol)	**0.41**	**0.3000**	**0.5800**
xxxiii.	*Limonia acidissima* (methanol)	**0.63**	**0.4293**	**0.9177**
xxxiv.	*Paederia foetida* (methanol)	**3.22**	**1.8678**	**5.5448**
xxxv.	***Fusarium oxysporum***			
xxxvi.	*Polyalthia longifolia* (water)	**0.54**	**0.3252**	**0.8835**
xxxvii.	*Balanites aegyptica* (water)	**3.46**	**1.5126**	**7.9086**
xxxviii.	*Paederia foetida* (water)	**4.39**	**1.9785**	**9.7379**
xxxix.	*Polyalthia longifolia* (methanol)	**0.53**	**0.3110**	**0.9220**
xl.	*Limonia acidissima* (methanol)	**2.99**	**1.6979**	**5.2579**
xli.	*Paederia foetida* (methanol)	**5.16**	**2.9214**	**9.1244**
xlii.	***Alternaria alternata***			
	Polyalthia longifolia (water)	**0.24**	**0.1740**	**0.3416**
xliii.	*Balanites aegyptica* (water)	**4.39**	**2.1032**	**9.1821**
xliv.	*Paederia foetida* (water)	**4.34**	**2.2649**	**8.3020**
xlv.	*Polyalthia longifolia* (methanol)	**0.23**	**0.1380**	**0.3910**
xlvi.	*Limonia acidissima* (methanol)	**3.24**	**1.2772**	**8.2410**
xlvii.	*Paederia foetida* (methanol)	**3.60**	**2.0689**	**6.2557**

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