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Toxicity of some bioactive medicinal plant extracts to Asian army worm, *Spodoptera litura*

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Abstract: Comparative toxicity of *Andrographis paniculata* (leaves), *Anethum graveolens* (leaves), *Azadirachta indica* (fruits), *Cassia fistula* (seeds), *Cuscuta reflexa* (stem), *Dendropthoe falcata* (leaves), *Lantana camara* (leaves and fruits), *Melia azedarach* (leaves) *and Vitex negundo* (stem) plant extracts against 2nd days (first instar) and 6th days(third instar) old larvae of *Spodoptera litura* was studied at 5 and 10mg/ml concentration. *D. falcata* leaves (98.58%) and *A. indica* fruits= *C. reflexa* (85.72%) were most effective in reducing weight gain in 2nd days old larvae at 5mg/ml and at 10mg/ml, *C. reflexa* (98.58) followed by *C. fistula* seeds and *L.camara* fruits(both 92.86%) were effective in retarding growth. *L.camara* fruit extract (99.43 and 99.93%) showed greater toxicity and reduced growth as compared to *A. graveolens* leaves (76.16%) at 5mg/ml. At 10mg/ml *L.camara* (99.94%) and *A. paniculata* (89.54%) leaves exhibited reduction in weight gain over control against 6d old larvae. All the medicinal plants showed varying degree of toxicity. *D.falcata*, *A.indica* and *L.camara* exhibited high larval mortality as compared to the other plant extracts.

Keywords: Botanicals, Medicinal plants, Mortality, *Spodoptera litura*, Toxicity

INTRODUCTION

Insect pest management is facing economic and ecological challenge worldwide due to the human and environmental hazards caused by majority of the synthetic pesticides (Rattan, 2010). The indiscriminate use of chemical pesticides has given rise to many well-known and serious problems, including genetic resistance of pest species, toxic residues in stored products, increasing costs of application, hazards from handling and environmental pollution (Rembold, 1994). Plants have evolved as a whole arsenal of defence strategies against herbivores, including the synthesis of a tremendous variety of chemical compounds. Chemical defence products may range from low molecular weight secondary metabolites, to peptides and proteins that are active against insects (Ibanez, 2012).The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment. These natural products generally inhibit insect's development, behaviour and provoke precocious moulting and alter the growth regulating hormones to cause malformations, sterility or death in insects (Celis *et al.*, 2008). Therefore the present investigation was conducted to compare the toxicity and growth regulatory properties of some plant extracts against 2nd and 6th day old larvae of Asian army worm *Spodoptera litura*.

MATERIALS AND METHODS

Preparation of methanol powders: The fresh plant parts *viz. Andrographis paniculata* (leaves), *Anethum graveolens* (leaves), *Azadirachta indica* (fruits), *Cassia fistula* (seeds), *Cuscuta reflexa* (stem), *Dendropthoe falcata* (leaves), *Lantana camara* (leaves and fruits), *Melia azedarach* (leaves) *and Vitex negundo* (stem) were washed in running tap water and dried in shade for a week. The dried plant samples were weighed and macerated in electric grinder into a fine paste, completely dipped in methanol in separate flasks, and kept at room temperature. After 3-4 days, the extract was filtered through Whatmann filter paper and centrifuged at 5000 rates per minute (rpm) for 10minutes. The supernatant was evaporated under vacuum (Kurucheve *et al.*, 1997).The crude powder obtained after evaporation of the supernatant was kept in sealed glass vials and stored in refrigerator. A test solution (on dry wt/volume basis) was prepared for each extract by dissolving appropriate amount of powder in definite volume of water (Vitthalrao, 2004).

Testing of toxicity: The comparative toxicity of nine medicinal plant extracts (methanol powders) viz. *A. paniculata* leaves; *A. graveolens* leaves; *A. indica* fruit; *C. fistula* seeds; *C. reflexa*; *D. falcata* leaves; *L. camara* fruits and leaves; *M. azedarach* leaves and *V. negundo* stem were evaluated against 2 and 6d old larvae of *S.litura*. Two concentrations, 5 and 10mg/ml

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were prepared in water. Sandwiches (size 3×3cm^2) were prepared from fresh leaf disc of *Ricinus communis*. Two leaf discs were used to make one sandwich. One ml of each of the concentrations of 5 and 10mg/ml was spread thoroughly on the leaf disc with the help of a glass rod and then the other untreated leaf disc was sandwiched onto it. Control (*R. communis*) leaf sandwiches received only water. Three replications were maintained. The sandwiches were fed to 2days and 6days old larvae for 3days and observations were recorded on larval weight, larval weight gain and mortality parameters.

Statistical analysis: The experiment was conducted in completely randomized design (CRD) (Gomez and Gomez, 1984) and the data was analyzed by one way Analysis of Variance (ANOVA) following Snedecor and Cochran (1967) and the means were separated using, Duncan Multiple Range Test (DMRT) (Duncan, 1955) based SPSS16 computer programme.

RESULTS AND DISCUSSION

Effect on larval weight gain: All the medicinal plant methanol powders at both of the tested concentrations were effective in significantly reducing the weight gain of 2days and 6days old larvae of *S. litura* in comparison to control. The maximum reduction at 5mg/ml concentration against 2days old larvae was seen in *D. falcata* leaves (98.58%) and *A. indica* fruit and *C. reflexa* stem (both 85.72%) over control (larval weight gain at 3days after feeding=0.014g) followed by *L. camara* fruits (58%), *L. camara* leaves (50%), *A. graveolens* leaves (42.86%), *M. azedarach* leaves (35.72%), *A. paniculata* stem (35.18%), and *C. fistula* seeds and *V. negundo* stem (both 28.58%) (Table1).

At 10mg/ml concentration against 2days old larvae of *S. litura*, the maximum reduction was caused by *C. reflexa* stem (98.58%), *C. fistula* seeds and *L. camara* leaves (both 92.86%) followed by *A. paniculata* stem and *V. negundo* stem (both 78.58%), *M. azedarach* leaves (71.43%) over control (larval weight gain at 3days after feeding=0.014g). *A. indica* fruit (57.15%), *D. falcata* leaves and *A. graveolens* leaves (both 50%) and *L.camara* fruit (21.43%) also lowered weight gain over control. *C. reflexa*, *A. indica*, *D. falcata* and *L. camara* caused a negative weight gain, indicating loss in weight of the larvae at 3days after feeding (Table2).

Maximum reduction in weight gain over control (0.239g) against 6days old larvae of *S.litura* at 5mg/ml conc. was exhibited by *L. camara* fruit (99.43%) followed by *A. graveolens* leaves (76.16%), *M. azedarach* leaves (55.65%), *C. fistula* seeds (53.56%), *A. indica* fruit and *D. falcata* leaves (both 48.96%), *L. camara* leaves (37.66%), *A. paniculata* stem (36.51%), *V. negundo* stem (35.15%) and *C. reflexa* stem (9.2%) (Table 3).

At 10mg/ml concentration the maximum reduction in weight gain over control (0.239g) in 6days old larvae of *S.litura* was observed with *L. camara* fruit (99.93%) and *A. paniculata* stem (89.54%) followed by *A. graveolens* leaves (76.99%), *D. falcata* leaves (74.99%), *C.reflexa* stem (71.13%), *A.indica* fruit (70.72%), *M. azedarach* leaves (69.88%), *C. fistula* (66.11%), *L. camara* leaves (61.09%) and *V. negundo* stem (59.42%) (Table 4).

Toxicity: Five of the nine plant extracts viz., *A. graveolens*, *A. indica*, *D. falcata*, *L. camara* (both leaves and fruits) and *M. azedarach* at both the concentrations (5 and 10mg/ml) reflected varying

Table 1. Comparative effect of nine medicinal plants (methanol powder) on growth of 2 days old larvae of tobacco caterpillar, *S. litura* (Fab.) at 5mg/ml concentration.

Plant species scientific name	Weight gain/larva 3DAF$^{\#}$ (g)	Reduction in weight gain over control (%)
Andrographis paniculata	0.009±0.0005^{a}	35.18
Anethum graveolens	0.008±0.0005^{bc}	42.86
Azadirachta indica	0.002±0.003^{c}	85.72
Cassia fistula	0.01±0.001^{a}	28.58
Cuscuta reflexa	0.002±0.0007^{a}	85.72
Dendropthoe falcata	0.0002±0.001a	98.58
Lantana camara (F)	0.0009±0.001^{a}	93.58
Lanana camara (L)	0.007±0.001^{a}	50.00
Melia azedarach	0.009±0.001^{bc}	35.72
Vitex negundo	0.01±0.0005^{ab}	28.58
Control	0.014±0.0005^{d}	-
SEM(±)	0.0007	-
CD at 1%	0.003	-
CD at 5%	0.002	-
F value	**	-

Means followed by common letter do not differ significantly by Duncan Multiple Range Test (p=0.05), Mean±SD of replicates; **=Highly significant; DAF= Days after feeding; (F)= Fruits; (L)= Leaves

Table 2. Comparative effect of nine medicinal plants (methanol powder) on growth of 2 days old larvae of tobacco caterpillar, *S. litura* (Fab.) at 10mg/ml concentration.

Plant species scientific name	Weight gain/larva 3DAF# (g)	Reduction in weight gain over control (%)
Andrographis paniculata	0.003±0.001^{bc}	78.58
Anethum graveolens	0.007±0.001^{bc}	50
Azadirachta indica	-0.006±0.007^{a}	57.15
Cassia fistula	0.001±0.001^{c}	92.86
Cuscuta reflexa	-0.0002±0.0002^{a}	98.58
Dendropthoe falcata	-0.007±0.006^{a}	50
Lantana camara (F)	-0.011±0.002^{a}	78.57
Lanana camara (L)	0.001±0.0002^{b}	92.86
Melia azedarach	0.004±0.003^{c}	71.43
Vitex negundo	0.003±0.001^{c}	78.58
Control	0.014±0.0005^{d}	-
SEM(±)	0.001	-
CD at 1%	0.007	-
CD at 5%	0.005	-
F value	**	-

Means followed by common letter do not differ significantly by Duncan Multiple Range Test (p=0.05); Mean±SD of replicates; **=Highly significant; DAF= Days after feeding; (F)= Fruits; (L)= Leaves

Table 3. Comparative effect of nine medicinal plants (methanol powder) on growth of 6 days old larvae of tobacco caterpillar, *S. litura* (Fab.) at 5mg/ml concentration.

Plant species scientific name	Weight gain/larva 3DAF# (g)	Reduction in weight gain over control (%)
Andrographis paniculata	0.512±0.01^{b}	36.41
Anethum graveolens	0.057±0.01^{a}	76.16
Azadirachta indica	0.122±0.01ab	48.96
Cassia fistula	0.111±0.01^{ab}	53.56
Cuscuta reflexa	0.261±0.03^{c}	9.2
Dendropthoe falcata	0.122±0.01^{ab}	48.96
Lantana camara (F)	0.137±0.04^{b}	99.43
Lanana camara (L)	0.149±0.002^{b}	37.66
Melia azedarach	0.106±0.01a$^{\b}$	55.65
Vitex negundo	0.155±0.0.02^{b}	35.15
Control	0.239±0.007^{c}	-
SEM(±)	0.021	-
CD at 1%	0.084	-
CD at 5%	0.062	-
F value	**	-

Means followed by common letter do not differ significantly by Duncan Multiple Range Test (p=0.05); Mean±SD of replicates; **=Highly significant; DAF= Days after feeding; (F)= Fruits; (L)= Leaves

degree of toxicity against 2d old larvae of *S. litura*, the values being 16.66 and 50.00%, 50.00 and 83.33%, 50.00 and 83.33% , 16.66 and 100%, 0.00 and 16.66%, 00.00 and 50.00% respectively. Only *L. camara* fruits proved toxic to 6 days old larvae of *S. litura* giving 100% mortality (Table 5).

It is evident from our observations that younger larvae are more susceptible to the effect of these medicinal plants whereas the older ones are quite resistant. They are able to withstand/tolerate the doses responsible for killing the younger larvae. *S. litura* is a generalist having a wide host range and tolerant/resistant to many insecticides (Srivastava and Joshi, 1965; Armes *et al*., 1997; Stanley *et al*., 2006; Bhargava *et al*., 2008; Muthusamy *et al*., 2011). It is known to metabolize secondary plant metabolites (Zhau *et al.*, 2011) and thereby it is capable of damaging even the toxic medicinal plants (Verma, 2006). Enough literature is available on the bioactivity (feeding deterrence, oviposition deterrence, repellence, toxicity, inhibition of growth and development) of neem tree, *Azadirachta* genus against various insect pests including *S. litura* (Schumutterer and Ascher, 1987; Ayyangar and Rao 1989; Kulkarni, 1999; Teik *et al.*, 2005) where methanolic extract of *A. indica* and *A. exelsa* showed repellent activities at 0.02%, inhibited feeding at 0.3% and decreased body length and weight and caused high mortality at a

Table 4. Comparative effect of nine medicinal plants (methanol powder) on growth of 6 days old larvae of tobacco caterpillar, *S. litura* (Fab.) at 10mg/ml concentration.

Plant species scientific name	Weight gain/larva 3DAF[#] (g)	Reduction in weight gain over control (%)
Andrographis paniculata	0.025±0.002[b]	89.54
Anethum graveolens	0.055±0.01[bc]	76.99
Azadirachta indica	0.07±0.02[cd]	70.72
Cassia fistula	0.081±0.006[cd]	66.11
Cuscuta reflexa	0.069±0.01[cd]	71.13
Dendropthoe falcata	0.06±0.01[cd]	74.99
Lantana camara (F)	-0.17±0.004[a]	99.93
Lanana camara (L)	0.093±0.01[cd]	61.09
Melia azedarach	0.072±0.009[cd]	69.88
Vitex negundo	0.097±0.01[d]	59.42
Control	0.239±0.007[e]	-
SEM(±)	0.012	-
CD at 1%	0.049	-
CD at 5%	0.036	-
F value	**	-

Means followed by common letter do not differ significantly by Duncan Multiple Range Test (p=0.05); Mean±SD of replicates; **=Highly significant; DAF= Days after feeding; (F)= Fruits; (L)= Leaves

Table 5. Comparative effect of nine medicinal plants (methanol powder) on toxicity of 2 and 6 days old larvae of tobacco caterpillar, *S. litura* (Fab).

Plant species scientific name	2days		6days	
	Abbotts corrected mortality (%)			
	5 mg/ml	10 mg/ml	5 mg/ml	10 mg/ml
Andrographis paniculata	-	-	-	-
Anethum graveolens	16.66	50.00	-	-
Azadirachta indica	50.00	83.33	-	-
Cassia fistula	-	-	-	-
Cuscuta reflexa	-	-	-	-
Dendropthoe falcata	50.00	83.33	-	-
Lantana camara (F)	16.66	100	-	100
Lanana camara (L)	-	16.66	-	-
Melia azedarach	-	-	-	-
Vitex negundo	-	50	-	-
Control	-	-	-	-
SEM(±)	-	-	-	-
CD at 1%	-	-		-
CD at 5%	-	-	-	-
F value	-	-	-	-

concentration of 0.5%.

Significant insecticidal activity in methanolic extract of *Artemisia nilagirica*, *Lantana wightiana* and *Synedrella nodiflora* have been reported against fourth instar of *S. litura* (Rathi and Gopalakrishnan, 2004, 2006 and 2010). In a recent study, the methanolic/ethanolic extracts of *Ocimum canum* and *Rhinacanthus nasutus* and *Clerodendron inerme* and *Cassia fistula* have been reported insecticidal against third and fourth instar larvae of *S. litura* (Chauhan *et al*., 2011). The LC/LD50 values have been reported as 36.46 and 68.08 ppm (*O. canum* and *R. nasutus*) against fourth instar and 3.846 and 1.703ppm (*C. inerme* and *C. fistula*) against third instar larvae of *S. litura*.

Our investigation is in conformity with the findings of Pathrose *et al.* (2007) and Deepthy *et al*. (2010) where in the growth inhibiting activity and toxicity of methanol extract of *Andrographis paniculata* and *Vitex negundo* have been reported against larvae of *S.litura*.

Conclusion

All the medicinal plants tested showed reduction in larval weight gain over control showing their deleterious effect on growth of 2 days and 6 days old larvae of *S.litura*. The most prominent were *D. falcata*, *A. indica*, *C. reflexa*, *L. camara* (fruits) and *A. graveolens*. Significant reduction in larval weight resulted in death of the larvae, with highest mortality

in *L. camara* (fruits), *D. falcata* and *A. indica* against smaller larvae while only *L. camara* (fruit) showed toxic effects to older larvae.

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