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IMPACT OF CERTAIN PLANT EXTRACTS AGAINST MULBERRY LEAF ROLLER *DIAPHANIA PULVERULENTALIS*

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The present investigation is intended to explore the possibility of using the medicinal plants such as *Lantana camara* Linn. (lantana), *Allium sativum* Linn. (garlic), *Zingiber officinale* Rose. (ginger), *Azadirachta indica* Juss.(neem) and *Vitex negundo* Linn. (vitex) having insecticidal properties against the mulberry leaf roller *Diaphania pulverulentalis*. The outcome of the findings indicated that the mortality of the second instar larvae of leaf roller recorded at each and every concentrations of the plant extracts found to be significant compared to untreated check at 24, 48, 72, 96, 120 and 144 h intervals in both methanol and aqueous extracts. Similarly, the per cent mortality of pupae and moths with deformation recorded maximum at lower concentrations of methanol and aqueous extract of plants compared at higher concentrations. While in case of third instar, the mortality was less compared to second instar. Higher mortality of the larvae was noticed with increase in concentration. After 144 h, the mortality range recorded by the methanol extract of the plants was maximum (85.00 to 95.00%) compared to aqueous extracts (58.00 to 76.00%). Based on the screening, one concentration from each plant extract which was more effective and statistically significant was selected and used to study its residual toxicity on silkworm *Bombyx mori* L.

Key words : Plant extracts, *Diaphania pulverulentalis, Bombyx mori*

INTRODUCTION

Sericulture is an agro based cottage industry mainly practiced by the small and marginal farmers of our country, which offers more employment opportunities and generating higher income. Though, Indian sericulture is showing a steady growth in domestic consumption as well as export of silk, the production of raw silk is still insufficient to meet the demand of domestic industries due to low productivity coupled with inferior silk quality. Under these circumstances there is an urgent need for strategic approach to improve the production of raw silk both in terms of quality and quantity. In mulberry cultivation, there are four important factors viz. climate, variety, agronomical inputs and plant protection measures which determine the leaf yield and quality (Rajanna *et al.*, 2005). However, some of the major constraints for the successful cultivation and production of quality mulberry leaf are insufficient rainfall, non availability of agronomical inputs and outbreak of pests and diseases (Sengupta *et al.*, 1990). Occurrence of different pests and diseases is also one of the major constraints of mulberry production.

The major pests of mulberry are Leaf roller, Bihar hairy caterpillar, Cut worm (Lepidoptera), Jassid, Spiralling white fly, Mealy bug and Scale insects (Homoptera) (Govindaiah *et al.*, 2005). Though the number of pests attacking mulberry is too high, mulberry leaf roller *Diaphania pulverulentalis* Hampson inflict crop losses 25-30% respectively. Among the various pests, the leaf roller *Diaphania* (= *Margoronia*)

pulverulentalis Hampson (Lepidoptera : Pyralidae) is causing serious damage with an average incidence of 27.85% in Karnataka, 20.98% in Andhra Pradesh and 16.48% in Tamil Nadu (Rajadurai *et al.*, 1999) and the incidence is severe during winter months (October-February). The incidence was also noticed on V1 with 61.22% and 41.20% on S-36 during the month of November (Manjunath Gowda *et al.*, 2004).

Hence, the present investigation is intended to explore the possibility of using different species of medicinal plants which have insecticidal properties against the leaf roller *Diaphania pulverulentalis.*

MATERIALS AND METHODS

The five selected medicinal plant extracts such as *Lantana camara* Linn.(lantana), *Allium sativum* Linn. (garlic), *Zingiber officinale* Rose. (ginger), *Azadirachta indica* Juss.(neem) and *Vitex negundo* Linn.(vitex) were evaluated against leaf roller both under *in vitro* and *in vivo* conditions.

Extraction of plant extract (using water) : The selected plant material were collected from the open field and thoroughly washed with tap water 3-4 times and finally washed with distilled water. The extract was prepared by taking the known quantity of plant material and crushed in mixer by adding required quantity of distilled water (100gm of plant material was crushed by adding 200 ml of distilled water). The extract was filtered through muslin cloth and the concentration of the filtrate was prepared to 100 per cent and stored in clean reagent bottle and maintained as stock solution. From this stock solution, 1, 3, 5, 7, 9, 11, 13, 15, 20 and 25 per cent following procedure outlined by Patil *et al.*(1997) and Mane (1998). Always fresh extracts were prepared and used in the experiments.

Extraction of plant extracts (Soxhlet method) : The selected plant material was collected from the field and thoroughly washed with tap water 3-4 times and finally with distilled water. These materials were wiped out with blotting paper, shade dried for 8-10 days and powdered using a mixer. 100 gm of plant material was used for the extraction in organic solvent methanol (200ml) by Soxhlet extraction method (Vogel, 1994). After complete extraction, the methanol was distilled off by placing on water bath at 30-35 °C to give viscous, semisolid, sticky greenish (from leaves), brownish (from zinger), creamish (from garlic) product. The final extract was preserved and stored in dark coloured glass bottle at 8-10 °C to avoid photo degradation and used as stock solution for further studies within three days to evaluate their pest management potency individually. Serial dilutions were prepared from the stock solution by adding distilled water to arrive 1, 3, 5, 7, 9, 11, 13, 15, 20 and 25 per cent.

In vitro evaluation of plant extracts against leaf rollers : Preliminary studies conducted on toxicity of plant extracts against second and third instar larvae of leaf roller using 1, 3, 5, 7, 9, 11, 13, 15, 20 and 25% in different intervals viz. 24, 48 72, 96, 120 and 144 h. In each concentration, five replications were maintained and the twenty leaf numbers of leaf rollers were kept per replication. Observations on the mortality of the leaf roller larvae were recorded once in 24 h. Based on the preliminary screening, one concentration per plant extract which was effective and statistically significant was selected as follows: 11% Lantana, 15% Garlic, 15% Ginger, 20% Neem and 25% Vitex extract. Further these concentrations were used to study the residual toxicity on silkworm.

RESULTS AND DISCUSSION

Mortality of second instar larvae of leaf rollers

The mortality of the second instar larvae of leaf roller recorded at each and every concentrations of the plant extracts found to be significant compared to untreated check at 24, 48, 72, 96, 120 and 144 h intervals in both methanol and aqueous extracts. The methanol extract exhibited maximum mortality of second instar leaf roller compared to aqueous extract. After 144 h maximum mortality was recorded by methanol extract of vitex (95.00%) followed by 20% neem (93.00%). In case of 11% lantana, 15% garlic and 15% ginger, it ranged from 85.00 to 88.00 per cent. In aqueous extracts, it was ranged from 68.00 to 82.00 per cent (Table I). The per cent mortality of pupae and moths with deformation recorded maximum at lower concentrations of methanol and aqueous extract of plants compared at higher concentrations. It was observed that there was no mortality of pupae and moths at higher concentrations of methanol extracts. This has revealed that at higher concentrations of methanol extract, maximum mortality was recorded in the larval instar of the leaf roller.

Mortality of third instar larvae of leaf rollers

The mortality was less in third instar compared to second instar. Higher mortality of the larvae was noticed with increase in concentration. After 144 h the mortality range recorded by the methanol extract of the plants was maximum (85.00 to 95.00%) compared to aqueous extracts (58.00 to 76.00%) (Table II). The mortality of pupae and moths at different concentrations of methanol and aqueous extract of the plants was found to be inversely proportional. The per cent mortality of pupae and moths with deformation was maximum at lower concentrations of aqueous extracts compared to higher concentrations. No mortality of pupae and moths was noticed at higher concentrations with methanol extracts revealing that, maximum mortality was recorded in the larval instar of leaf roller. The analysis of the results depicted better performance of lantana at lower concentration compared to other plant extracts.

Lethal time taken to kill fifty per cent second / third instar larvae of leaf roller (LT_{50})

The LT_{50} taken to kill second instar larvae of leaf roller was lower (69.05-102.29) compared to third instar (80.02-111.33). The percentage of mortality was increased with increase in the concentration. Based on the laboratory trial, the lethal time required by the methanol and aqueous extracts of the plants to kill the fifty per cent of the second instar larvae and third instar larvae (LT_{50}) was calculated and are depicted in Tables III and IV. At 11% concentration of methanol and aqueous extract of lantana, the LT_{50} values recorded against second instar leaf roller larvae were 84.32 and 102.29 h and the corresponding regression equation $Y= 4.255 + 0.019X$. At 25% concentration of vitex extract, the LT_{50} values recorded were 69.05 and 87.05 h and the corresponding regression equation value was $4.355 + 0.018X$. In case of the third instar larvae, the LT_{50} values recorded at 11% concentration of methanol and aqueous extract of lantana were 86.09 and 104.04 h and the corresponding regression equation value was $4.367 + 0.018X$. At 25% concentration of vitex extract, the LT_{50} values were 83.93 and 98.37 h and the corresponding regression equation value $Y = 4.359 + 0.019X$.

Table I : Effect of selected concentrations of the plant extract on second instar larvae of leaf roller *Diaphania pulverulentalis*

Plant extract/ Conc.	Per cent mortality in methanol extract								Per cent mortality in aqueous extract							
	24 h	48 h	72 h	96 h	120 h	144 h	Pupae	Adults	24 h	48 h	72 h	96 h	120 h	144 h	Pupae	Adults
11 % Lantana	0.00	26.00	50.0	64.0	74.0	87.0	7.00	2.00	0.00	18.0	43.0	50.0	59.0	69.0	13.00	5.00
15% Garlic	10.0	31.00	53.0	66.0	84.0	88.0	2.00	0.00	5.00	27.0	43.0	55.0	63.0	74.0	5.00	3.00
15% Ginger	10.0	30.00	50.0	67.0	80.0	85.0	2.00	2.00	3.00	21.0	39.0	54.0	61.0	68.0	5.00	4.00
20% Neem	19.0	31.00	50.0	67.0	80.0	93.0	2.00	0.00	14.0	30.0	47.0	62.0	71.0	81.0	4.00	2.00
25% Vitex	15.0	36.00	58.0	63.0	86.0	95.0	0.00	0.00	6.00	29.0	47.0	61.0	66.0	82.0	2.00	2.00
Control	0.00	0.00	1.00	2.00	3.00	3.00	0.00	0.00	0.00	0.00	1.00	2.00	3.00	3.00	0.00	0.00
CD at 5%	4.65	8.39	11.8	8.30	8.90	8.10	5.06	2.96	5.44	10.8	12.0	12.2	11.6	8.49	7.38	5.73

Table II : Effect of selected concentrations of the plant extract on third instar larvae of leaf roller *Diaphania pulverulentalis*

Plant extract/ Conc.	Per cent mortality in methanol extract								Per cent mortality in aqueous extract							
	24 h	48 h	72 h	96 h	120 h	144 h	Pupae	Adults	24 h	48 h	72 h	96 h	120 h	144 h	Pupae	Adults
11% Lantana	0.00	24.0	50.0	63.0	72.00	86.00	6.00	2.00	0.00	18.00	40.00	49.00	58.00	68.00	16.00	6.00
15% Garlic	4.00	27.0	47.0	66.0	76.00	89.00	0.00	0.00	0.00	23.00	38.00	50.00	58.00	67.00	4.00	2.00
15% Ginger	4.00	23.0	44.0	61.0	72.00	85.00	2.00	0.00	7.00	18.00	28.00	52.00	51.00	58.00	2.00	2.00
20% Neem	13.0	21.0	38.0	60.0	78.00	89.00	0.00	0.00	5.00	26.00	34.00	48.00	63.00	72.00	2.00	0.00
25% Vitex	8.00	24.0	43.0	64.0	85.00	95.00	0.00	0.00	5.00	29.00	34.00	46.00	60.00	76.00	0.00	0.00
Control	0.00	0.00	1.00	1.00	2.00	3.00	0.00	0.00	0.00	0.00	1.00	1.00	2.00	3.00	0.00	0.00
CD at 5%	6.64	9.88	10.9	12.4	11.46	7.16	4.00	2.53	6.82	10.51	10.85	12.07	9.12	7.33	7.21	4.74

Lethal concentration of plant extracts against second /third instar larvae of leaf roller (LC_{50})

The LC_{50} values of the methanol and aqueous extracts (µl) of five plant species were examined on second and third instar larvae of leaf roller. These values are presented in Tables V and VI along with the corresponding regression equation values. Among the plant extracts tested against second instar leaf roller, the methanol and aqueous extracts of lantana had the lowest LC_{50} values of 6783.18 and 8016.85 µl with the corresponding regression equation value Y = 0.861 + 0.0001X. However, the LC_{50} value of vitex extract was maximum i.e. 9120.31 and 11535.75 µl with the corresponding regression equation value was Y = 1.327 + 0.0001X. In case of the plant extracts tested against third instar leaf roller, the methanol and aqueous extract of lantana had the lowest LC_{50} value of 7216.25 and 9430.21 µl with the corresponding regression equation value Y = 1.044 + 0.0001X. The vitex extract recorded maximum LC_{50} values of 13196.12 and 14209.20 µ with the corresponding regression equation value Y = 1.422 + 0.0001X.

Table III : Lethal time taken to kill the second instar larvae of leaf roller *Diaphania pulverulentalis*

Plant material	Heterogeneity	Regression equation	LT_{50}	Fiducial limit
11% Lantana (meth.)	9.790	4.255+0.019x	84.32	78.38-90.25
11% Lantana (aqu.)			102.29	96.38-108.45
15% Garlic (meth.)	8.415	4.091+0.017x	76.33	69.91-82.60
15% Garlic (aqu.)			94.30	88.11-100.65
15% Ginger (meth.)	5.284	4.032+0.017x	77.60	71.07-84.00
15% Ginger (aqu.)			101.05	94.64-107.76
20% Neem (meth.)	2.526	4.057+0.016x	73.32	76.46-79.96
20% Neem (aqu.)			82.68	76.04-89.29
25%Vitex(meth.)	6.360	4.355+0.018x	69.05	62.83-75.05
25%Vitex (aqu.)			87.05	81.16-92.96

Table IV : Lethal time taken to kill the third instar larvae of leaf roller *Diaphania pulverulentalis*

Plant material	Heterogeneity	Regression equation	LT_{50}	Fiducial limit
11% Lantana (meth.)	7.702	4.367+0.018x	86.09	80.14-92.04
11% Lantana (aqu.)			104.04	98.09-110.28
15% Garlic (meth.)	8.851	4.295+0.018x	80.02	73.85-86.10
15% Garlic (aqu.)			103.39	97.31-109.78
15% Ginger (meth.)	13.248	4.067+0.016x	87.83	81.18-94.55
15% Ginger (aqu.)			111.33	104.50-118.73
20% Neem (meth.)	10.742	4.312+0.018x	82.30	76.21-88.37
20% Neem (aqu.)			99.40	93.41-105.64
25%Vitex(meth.)	15.341	4.359+0.019x	83.93	78.00-89.87
25%Vitex (aqu.)			98.37	92.56-104.41

Table V : Lethal concentration of plant extracts on the second instar larvae of leaf roller *Diaphania pulverulentalis.*

Plant material	Heterogeneity	Regression equation	LC50 (µl)	Fiducial limit
11% Lantana (meth.)	0.359	0.861+0.0001x	6783.18	6034.51-7589.51
11% Lantana (aqu.)			8016.85	7227.87-8914.32
15% Garlic (meth.)	0.267	1.048+0.0001x	8085.66	7226.22-9082.43
15% Garlic (aqu.)			10685.2	9635..-12004.50
15% Ginger (meth.)	0.757	1.149+0.0001x	8626.04	7793.34-9602.43
15% Ginger (aqu.)			10874.2	9871.08-12119.83
20% Neem (meth.)	0.414	1.217+0.0001x	9076.69	8218.46-10099.60
20% Neem (aqu.)			11454	10400.09-12777.97
25%Vitex(meth.)	0.0001	1.327+0.0001x	9120.31	8316.72-10067.97
25%Vitex (aqu.)			11535.8	10542.17-12766.48

Table VI : Lethal concentration of plant extracts on the third instar larvae of leaf roller *Diaphania pulverulentalis.*

Plant material	Heterogeneity	Regression equation	LC50 (µl)	Fiducial limit
11% Lantana (meth.)	0.649	1.044+0.0001x	7216.25	6479.40-8025.04
11% Lantana (aqu.)			9430.21	8582.65-10435.99
15% Garlic (meth.)	0.136	1.167+0.0001x	8917.81	8034.27-9973.04
15% Garlic (aqu.)			11507.1	10406.12-12904.97
15% Ginger (meth.)	0.009	1.351+0.0001x	9906.11	8996.63-11016.88
15% Ginger (aqu.)			12490	11344.06-13950.67
20% Neem (meth.)	0.900	1.378+0.0001x	11968.2	10750.39-13574.59
20% Neem (aqu.)			14213.7	12731.99-16214.01
5%Vitex(meth.)	0.028	1.422+0.0001x	13196.1	11860.11-14981.66
25%Vitex (aqu.)			14209.20	12752.22-16172.81

In the present study, the mortality range of second instar larvae of leaf roller at 11% concentration of methanol and aqueous extract of lantana from 24 to 144 h. was found to be 0.00 to 87.00% and 0.00 to 69.00% respectively whereas in third instar larvae, it was 0.00 to 86.00% and 0.00 to 68.00%. The present findings are in conformity with the findings of Pandey *et al.* (1983) who have reported more than 50% mortality of *A. gossypii* with lantana extract. Banerjee *et al.* (1982) who have reported that the alcoholic extract of garlic (2.5%) treated leaves provided 100% protection over control for the

larvae of *A.proxima*. This may be due to the pesticidal properties of garlic, a repellent, antifeedant, bactericide, fungicide and nematicide (Oparaeke & Dike, 1996). The active constituent in the garlic is allicin, which may inhibited the banana weevil. The garlic juice and its extracts form the basis of several commercially available pest control products (Prowse *et al.*, 2006). Devasingh *et al.* (2005) who have reported the mortality of the larvae of spotted boll worm *Earias vitella* in methanol extract of ginger, ranged from 12.7 - 78.9%. There was significant difference among treatments viz. extract of ginger in solvents like hexane, benzene, acetone and chloroform. The mortality of the larvae may be due to phenyl butanoids from rhizome of ginger.

This coincides with the results of Jaglan *et al.* (1997) who have reported that the mortality of larvae of gram pod borer *Helicoverpa armigera* in different treatments namely extraction at room temperature, hot extraction method and soxhlet extraction method of neem and lantana (0.5, 2.5, 5.0 and 7.0%) ranged from 8.0 to 88.0%. The exudates from reddish terminal leaves of neem exhibited higher per cent of antifeedency,

Table VII : Residual effect of plant extracts on bivoltine silkworm growth and cocoon characters (CSR2 CSR4)

T1 – 8th day after treatment

Name of the plant extracts	Total larval duration (days:hrs)	Vth instar larval wt. (g)	Single cocoon wt. (g)	Single shell wt.(g)	Single cocoon shell ratio (%)	Single cocoon filament length (mtr.)	Mortality (%)
11% Lantana	22.19	36.07	1.56	0.32	20.78	1152.58	5.33
15% Garlic	22.16	36.37	1.72	0.36	21.02	1190.75	6.00
15% Zinger	22.15	37.03	1.76	0.37	21.06	1201.00	6.67
20% Neem	22.15	37.27	1.76	0.37	21.23	1202.08	8.33
25% Vitex	22.14	38.80	1.76	0.38	21.30	1229.17	3.67
Control	22.10	40.00	1.82	0.40	21.94	1196.00	3.00
CD at 5%	0.06	1.53	0.06	0.00	1.07	62.17	2.92

T2 – 15th day after treatment

	Total larval duration (days:hrs)	Vth instar larval wt. (g)	Single cocoon wt. (g)	Single shell wt. (g)	Single cocoon shell ratio (%)	Single cocoon filament length (mtr.)	Mortality (%)
11% Lantana	22.19	36.80	1.70	0.35	20.65	1152.67	2.33
15% Garlic	22.16	37.53	1.73	0.36	20.17	1196.08	4.00
15% Zinger	22.15	37.57	1.76	0.37	21.37	1208.75	4.33
20% Neem	22.15	38.37	1.80	0.37	20.55	1227.17	5.67
25% Vitex	22.14	38.93	1.82	0.38	21.60	1235.33	2.33
Control	22.11	40.50	1.84	0.40	21.70	1197.67	3.67
CD at 5%	0.06	1.63	0.12	0.02	1.68	113.93	2.22

ovipositional repellency and insecticidal activity against fifth instar larvae of *Spodoptera litura*. The present findings are also in confirmity with Raghuraman & Singh (2000) who have reported that the neem extracts at 5% concentration recorded 50% mortality against Polyphagous parasitoid - an egg parasitoid *Trichogramma chilonis* and a larval parasitoid *Bracon hebetor*. The larval and pre-pupal stages are susceptible to insect growth regulatory effects of the extracts. The mortality of the larvae may be due to insecticidal action, feeding inhibition, gustatory repellency or impairment in the food assimilation. Butterworth & Morgan (1971) reported that the major constituent of neem namely azadirachtin exhibited inhibition in feeding of *Schistocerca gregaria*.

It was also in agreement with the observations of Perumal *et al.* (2004) who have reported that the larvicidal activities of vitex extract against *Spodoptera litura* at different concentrations were significant. Several treated larvae displayed morphological deformities which arrested their development.

Residual toxicity of the plant extracts against the silkworm

Bioassay was conducted to test the residual effect of aqueous extracts of selected plants viz. lantana (11%), garlic (15%), ginger (15%), neem (20%) and vitex (25%) against the bivoltine silkworm hybrid CSR2 x CSR4 by feeding the treated mulberry leaves on eighth (T1) and fifteenth day (T2) after treatment. After the treatment, the pre and post cocoon parameters were assessed viz. total larval duration, weight of ten larvae, single cocoon weight, single shell weight, shell ratio, single cocoon filament length and mortality and the data compiled are given in Table VII. The silkworm larvae fed with the mulberry leaves on fifteenth day after treating with the above extracts were showed better performance compared to the larvae fed with the mulberry leaves on eighth day after treament. However the untreated check has not shown any effect on the parameters assessed. The present results are in comparable with the reports of Santosh Kumar (1997) who have reported the improvement in economic traits of *Bombyx mori* without extension in larval duration due to dusting of 5% of *L.camara* extract on 48 h. old fifth instar larvae of silkworm. In contrary to the results, Mamadapur (1994) reported that, dusting of 5% *L.camara* to 48 h. old fifth instar larvae of silkworm prolonged the larval period and improved the economic traits.

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