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Bioefficacy of *Murraya koenigii* oil against *Spilosoma obliqua* and *Spodoptera litura*

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Abstract
The insecticidal and biorational properties of *Murraya koenigii* oil were tested at five different doses against fifth instar larvae of *Spilosoma obliqua* and *Spodoptera litura*. *M. koenigii* oil significantly reduced the larval weight gain of *S. obliqua* at all the doses, ranging from 127.8 to 47.3% at 2.5 to 0.5 µl/larva, in comparison to control. At the doses of 2.5 and 2.0 µl/larva, a negative weight gain (0.053 and -0.016 g/larva at 2 days after feeding) was observed. The insecticidal activity was only 33%, pupation 66.6% and adult emergence 63.3% at 2.5 µl/larva. The same dose showed reduction in larval weight gain over control (-106.25%), resulting into 26.66% larval mortality against *S. litura*. The mean pupal weight at the higher doses was significantly reduced to 0.21 g. Pupation (50.0%) and adult emergence (43.3%) were also significantly reduced at 2.5 µl/larva. *M. koenigii* essential oil shows promising results as a biorational chemical for the control of these two insect pests of agricultural significance.

Key words: *Murraya koenigii*, Growth and Development Parameters, *Spilosoma obliqua*, *Spodoptera litura*, Essential oils

1. Introduction
Plant derived products namely azadrachtin from *Azadrachta indica*, pyrethrin from *Chrysanthemum cinerariaefolium*, carvone from *Carum carvi* and alkyl isothiocyanate from mustard and horseradish oil have received global attention due to their pesticidal properties and potential to protect several food commodities [3]. Essential oils produced by different plant genera have been reported to be biologically active and are endowed with insecticidal, antimicrobial and bio regulatory properties [8, 16]. Pest control by direct or indirect use of essential oils, is a promising approach [4] to repel insects or protect stored products [15]. Moreover, essential oils are easily biodegradable in the environment [5] and possess little or no toxicity against fishes, birds and mammals [28]. The presence of essential oils in members of family Rutaceae with diverse activities has valued their increasing demand as natural sources of insecticides and thus encouraged us to undertake a comprehensive study of the insecticidal and growth regulatory activity of *Murraya koenigii* Sprenge (L.) oil against two notorious insect pests, *Spodoptera litura* (Fabricius) and *Spilosoma obliqua* (Walker) which have consistently gained resistance to the chemical pesticides [2, 18].

2. Materials and methods
2.1 Insect Culture
Wild population of the test insects, *S. obliqua* and *S. litura* was collected from Norman E. Borlaug Crop Research Centre (NEBCRC), G. B. Pant University of Agriculture and Technology Pantnagar. A rolling culture of the test insects was maintained on castor, *Ricinus communis* leaves under laboratory conditions (Temp. 28 ^{0}C and RH 88%) in plastic tubs. The larvae of the required age group were taken from the culture, as and when required. All the experiments were conducted in the Bioactive Plant Natural Products Laboratory at the department of Entomology.

2.2 Extraction of oil
The leaves of plant *M. koenigii* were obtained from Medicinal Plants Research and Development Centre (MRDC), Pantnagar and the essential oil was extracted by hydrodistillation method using Clevenger apparatus [24]. The distilled oil was separated from water by a separating funnel and stored in refrigerator.

2.3 Insecticidal activity

Five doses of each of the oils viz., 2.5, 2.0, 1.5, 1.0 and 0.5µl were topically applied to the thoracic region of cold immobilised larvae (10days old larvae of *S.litura* and 16days old larvae of *S.obliqua* (both fifth instar)), individually using a repeating topical dispenser attached to 25 µl Hamilton microapplicator syringe[14, 23]. Each dose of the oil constituted a treatment which consisted of 30 freshly moulted larvae, divided in three replications. Control was untreated. The treated larvae were transferred to separate plastic boxes (size: l 24 x b 15 x ht 8 cm) containing untreated castor leaves as food and other observations were recorded on the following parameters:-larval weight (g), larval period (d), pupal period (d), pupal then covered with muslin cloth. The data on leaf area consumed was visually quantified in cm^2 with the help of graph paper. The weight (g), terminal larval mortality (%) and adult emergence (%).

2.4 Statistical analysis

The experiment was conducted in completely randomized design (CRD) and the data was analysed by one way Analysis of Variance (ANOVA).The means were separated using Duncan's Multiple Range Test (DMRT) based SPSS16 computer programme.

3. Results

3.1 Effect of *M. koenigii* oil on *S. obliqua*

It is evident from the Tables 1and 2 and Fig. 1 that *M. koenigii* oil had a significant effect on the weight gain of the larvae which was significantly reduced at all the doses, ranging from 127.8 to 47.3% at 2.5 to 0.5 µl/larva, in comparison to control, respectively. At the two higher doses *viz.*, 2.5 and 2.0 µl/larva in fact, a negative weight gain of (-0.053 and -0.016 g/larvae at 2 days after exposure (DAE) was observed. The oil, at the higher doses significantly increased the larval and pupal periods. At the doses of 2.5 and 2.0 µl/larva, the larval periods were 21.2 d and 21.6 d against 20.0 d in control; and the pupal periods were 10.3 and 10.4 d against 10.0d in control, respectively. The mean pupal weight at the three higher doses of 2.5, 2.0 and 1.5 µl/larva was 0.16, 0.22 and 0.26g respectively as against 0.38 g in control. A significant reduction in per cent pupation was observed only at higher doses of 2.5µl/larva and 2.0µl/larva. The per cent pupation at 2.5 µl/larva was 66.6 and adult emergence was 63.3%.

The *M. koenigii* oil was virtually non-insecticidal at the doses below 2.0 µl/larva. The highest terminal larval mortality was 33.3% at 2.5 µl/larva which was significantly higher than control at p=0.05%. The oil was effective in reducing the growth and development parameters, severely affecting mean larval and pupal weights and also pupation. Although, it was non insecticidal at the lower doses, its insecticidal property could be checked at doses above 3 µl/larva.

3.2 Effect of *M. koenigii* oil on *S. litura*

The curry leaf oil significantly reduced feeding in *S litura* at the doses ranging from 2.5 to 1.5 µl/larva (0.0 to 1.5 cm^2), as compared to control (2.16 cm^2) at p= 0.05%. The highest dose of 2.5 µl/larva caused 100 per cent reduction in feeding over control, whereas at the lowest dose of 0.5 µl/larva the reduction in feeding was 9.72%. Since the feeding was nil at highest dose, the larvae lost weight and there was -106.25% reduction in larval mean weight gain over control resulting into 26.66% larval mortality at 2 DAE and 46.6% terminal larval mortality. At the lowest dose of 0.5 µl/larva 12.5% reduction in larval weight gain was observed with a larval mortality of 3.3% at 2 DAE (Tables 3 and 4) and Fig.1.

The larval period was significantly increased at all the doses (12.66 to 12.07 d) except at 0.5 µl (11.7d) which was *at par* with control (11.3d). A significant increase in pupal period (8.26 to 7.73d) was observed at three highest doses as compared to the lowest dose and control (7.03 d). The mean pupal weight was significantly reduced at the higher doses of 2.5 and 2.0µl/larva (0.21 g) as compared to control (0.31 g); at the lower doses it was *at par* to control. Percent pupation and adult emergence was significantly reduced at the higher doses (50.0 and 43.3 at 2.5, and 60.0 and 53.3 at 2.0 µl/larva respectively) as compared to control (96.6%). The lower doses also caused a significant reduction in adult emergence as compared to control, the values ranged between (63.3 to 86.6%) at 1.5 to 0.5 µl/larva. With regard to percent pupation the lower doses of 1.0 and 1.5 µl/larva were *at par* to control.

Table 1: Effect of Curry leaf, *Murraya koenigii* (L.) Spreng, oil on growth of 16d old larvae of Bihar hairy caterpillar, *Spilosoma obliqua* (Walker) by topical application bioassay method

Plant species Common name, (Scientific name, Family)	Dose(µl/larva)	Larval Period(d)	Terminal larval mortality (%)	Pupal period(d)	Mean pupal weight(g)	Pupation (%)	^Adult emergence (%)
Curry leaf, (*Murraya koenigii* (L.) Spreng., Rutaceae)	2.5	21.23±0.34^{c}	33.33±3.33^{d}	10.33±0.33^{a}	0.16± 0.01^{a}	66.66±3.33^{a}	63.33±3.33^{a}
	2.0	20.67±0.13^{bc}	16.66±3.33^{c}	10.40±0.23^{a}	0.22±0.02^{b}	83.33±3.33^{b}	73.33±3.33^{b}
	1.5	20.70±0.08^{bc}	16.66±3.33^{c}	10.63±0.19^{a}	0.26±0.02^{c}	83.33±3.33^{b}	80.00±0.00^{bc}
	1.0	20.58±0.18^{ab}	13.33±3.33^{ab}	10.75±0.14^{a}	0.30±0.01^{cd}	86.66±3.33^{b}	83.66±3.33^{cd}
	0.5	20.34±0.22^{ab}	6.66±3.33^{ab}	10.23±0.23^{a}	0.31±0.01^{d}	93.33±3.33^{b}	86.66±3.33^{cd}
Control	-	20.00±0.00^{a}	3.33±3.33^{a}	10.06±0.03^{a}	0.38±0.00^{e}	96.66±3.33^{b}	93.33±3.33^{e}
Sem(±)	-	0.191	3.33	0.214	0.012	3.33	3.04
CD at 1%		0.828	14.39	0.924	0.055	14.39	13.13
CD at 5%	-	0.590	10.26	0.659	0.039	10.26	9.37
F Value	-	*	**	ns	**	**	**

Means (±SE) followed by common letters do not differ significantly by DMRT (p=0.05%)

^ Adult emergence was calculated on the basis of initial number of larvae, ns= non- significant, *= Significant **= Highly significant

Table 2: Effect of Curry leaf *Murraya koenigii* (L.) Spreng. oil on development of 16d old larvae of Bihar hairy caterpillar, *Spilosoma obliqua* (Walker)

Plant species Common name, (Scientific name, Family)	Dose(µl/larva)	Larval Period(d)	Terminal larval mortality (%)	Pupal period(d)	Mean pupal weight(g)	Pupation (%)	^Adult emergence (%)
Curry leaf, (*Murraya koenigii* (L.) Spreng., Rutaceae)	2.5	21.23±0.34^{c}	33.33±3.33^{d}	10.33±0.33^{a}	0.16± 0.01^{a}	66.66±3.33^{a}	63.33±3.33^{a}
	2.0	20.67±0.13^{bc}	16.66±3.33^{c}	10.40±0.23^{a}	0.22±0.02^{b}	83.33±3.33^{b}	73.33±3.33^{b}
	1.5	20.70±0.08^{bc}	16.66±3.33^{c}	10.63±0.19^{a}	0.26±0.02^{c}	83.33±3.33^{b}	80.00±0.00^{bc}
	1.0	20.58±0.18^{ab}	13.33±3.33^{ab}	10.75±0.14^{a}	0.30±0.01^{cd}	86.66±3.33^{b}	83.66±3.33^{cd}
	0.5	20.34±0.22^{ab}	6.66±3.33^{ab}	10.23±0.23^{a}	0.31±0.01^{d}	93.33±3.33^{b}	86.66±3.33^{cd}
Control	-	20.00±0.00^{a}	3.33±3.33^{a}	10.06±0.03^{a}	0.38±0.00^{e}	96.66±3.33^{b}	93.33±3.33^{e}
Sem(±)	-	0.191	3.33	0.214	0.012	3.33	3.04
CD at 1%		0.828	14.39	0.924	0.055	14.39	13.13
CD at 5%	-	0.590	10.26	0.659	0.039	10.26	9.37
F Value	-	*	**	ns	**	**	**

Means (±SE) followed by common letters do not differ significantly by DMRT (p=0.05%)
Adult emergence was calculated on the basis of initial number of larvae, ns= non- significant, *= Significant **= Highly significant

Table 3: Effect of Curry leaf, *Murraya koenigii* (L.) Spreng, oil on feeding and growth of 10d old larvae of tobacco caterpillar, *Spodoptera litura* (Fab.) by topical application bioassay method

Plant species Common name, (Scientific name, Family)	Dose (µl/larva)	#MLAC (cm^2)/larva 2DAE	Reduction in Feeding over control (%)	Mean weight /larva at 2^DAE (g)	Reduction in mean wt. gain over control (%)	Larval Mortality at 2DAE (%)
Curry leaf, (*Murraya koenigii* (L.) Spreng. Rutaceae)	2.5	0.00±0.00^{a}	100.00	0.48±0.01^{a}	-106.25	26.66±3.33^{d}
	2.0	1.59±0.12^{b}	26.38	0.53±0.02^{b}	75.00	13.33±3.33^{c}
	1.5	2.02±0.06^{c}	6.48	0.58±0.01^{c}	37.50	10.00±0.00^{bc}
	1.0	1.96±0.02^{bc}	22.22	0.61±0.01^{cd}	18.75	6.66±3.33^{abc}
	0.5	1.95±0.02^{bc}	9.72	0.62±0.00^{de}	12.50	3.33±3.33^{ab}
Control		2.16±0.10^{c}	-	0.64±0.01^{e}	-	0.00±0.00^{a}
Sem(±)	-	0.120	-	0.008	-	2.721
CD at 1%	-	0.519	-	0.038	-	11.75
CD at 5%	-	0.370	-	0.027	-	8.38
F Value	-	**	-	**	-	**

Means (±SE) followed by common letters do not differ significantly by DMRT (p=0.05%) #MLAC= Mean leaf area consumed;
^DAE = Days after exposure Mean initial weight (g/larva) = 0.480 g; **= Highly significant

Table 4: Effect of Curry leaf, *Murraya koenigii* (L.) Spreng, oil on development of 10d old larvae of tobacco caterpillar, *Spodoptera litura* (Fab.) by topical application bioassay method

Plant species Common name, (Scientific name, Family)	Dose (µl/larva)	Larval period (d)	Terminal larval mortality (%)	Pupal period (d)	Mean pupal weight (g)	Pupation (%)	^Adult emergence (%)
Curry leaf, (*Murraya koenigii* (L.) Spreng. Rutaceae)	2.5	12.66±0.26^{c}	46.66±3.33^{d}	8.26±0.13^{d}	0.21±0.01^{a}	50.00±5.77^{a}	43.33±6.67^{a}
	2.0	12.71±0.06^{c}	33.33±3.33^{c}	7.73±0.13^{c}	0.21±0.00^{a}	60.00±0.00^{a}	53.33±3.33^{ab}
	1.5	12.17±0.04^{bc}	26.66±3.33^{c}	7.73±0.03^{c}	0.27±0.01^{b}	73.33±3.33^{b}	63.33±6.67^{bc}
	1.0	12.07±0.07^{bc}	16.66±3.33^{b}	7.50±0.26^{bc}	0.27±0.01^{b}	83.33±3.33^{bc}	76.66±3.33^{cd}
	0.5	11.70±0.35^{ab}	10.00±0.00^{ab}	7.10±0.06^{ab}	0.28±0.01^{b}	90.00±0.00^{c}	86.66±3.33^{de}
Control	-	11.33±0.33^{a}	3.33±3.33^{a}	7.03±0.03^{a}	0.31±0.04^{b}	96.66±3.33^{cd}	96.66±3.33^{e}
Sem(±)	-	0.227	3.042	0.136	0.016	3.33	4.714
CD at 1%		0.981	13.13	0.587	0.070	14.39	20.35
CD at 5%	-	0.700	9.37	0.419	0.050	10.26	14.523
F Value	-	**	**	**	**	**	**

Means (±SE) followed by common letters do not differ significantly by DMRT (p=0.05%) **= Highly significant

4. Discussion

Plants from Rutaceae family are known for their toxicity towards insect's pests for example essential oil from the leaves of *Aegle marmelos* showed insecticidal activity against

Callosobruchus chinensis, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* [19]. This oil has also been reported to possess toxicity by topical application to *S. litura* larvae with LD50 = 116.3 µg/ larva [30]. The hydrodistilled essential oil from *M. koenigii* leaves has been reported to possess antifeedant activity against fifth instar larvae of *S. litura* with ED_{50} value of 130 µg ml^{-1}. The antifeedant effect was more than 50% at the concentration of 250 µg ml^{-1}. Amongst crude extracts, benzene extract was more effective than hexane extract; the ED_{50} being 710 and 4261 µg ml^{-1}, respectively [29]. A recent investigation also reports toxicity of *M.koengii* to black ant *Lasius niger*. *M. koengii* oil was toxic with LC50= 6.58µl followed by *M. paniculata* (LC50= 8.41µl) and *Skimmia laureola* (LC50= 10.15 µl) were found toxic to black ant [22]. Similar studies have been reported where essential oil of *Ageratum conyzoides* caused 43.0–68.75% mortality at 0.025–0.25 µl concentration [25]. Pulegone is shown to be effective against *Musca domestica* and, *S. litura* with the range of LD_{50} = 38–753.9 µg/insect [20, 13]. Pulegone containing diet at 0.1% retarded development and inhibited reproduction of last instar of southern armyworm, *Spodoptera eridania* [12]. Considerable feeding inhibition (70.21–80.21%) was recorded for 3rd in stars of *S. obliqua* when treated with 0.4% concentration of *Artemisia nilagirica* and *Juglans regia* var. *kumaonica* oils, while at 0.3% these oils induced feeding deterrence of 63.12–83.76% among 5th instars of *S. litura* [7]**.** Essential oils from *Elsholtzia densa*, *E. incise* and *E. piulosa* also showed significant antifeedant activity against 3rd instars of *S. litura* [27]. These oils are rich in 1, 8- cineole, linalool, eugenol, carvacrol and thymol, which are known compounds to show effects against various insect species, and fumigant activity in above cases could be attributed to them in the respective essential oils. The essential oil from *P. cablin* was found to possesses fumigant properties against *S. litoralis* with LD_{50} value of 14.8 ml/cm^3 of space [23]. Patchouli oil has been reported toxic to obliquely banded leaf roller, *Choristoneura rosaceana* larvae (LC50=2.8 µl/ml and LD50=8.0 µg/insect) [21]. The turmeric (*Curcuma longa*) leaves, the unutilized part of turmeric plant, on hydrodistillation yields oil rich in α-phellandrene (70%). This oil induces growth inhibition and larval mortality against *Spilosoma obliqua* and diamond back moth, *Plutella xylostella* (Linnaeus) at 1% concentration [1, 11, 31]. The leaf oil is also ovicidal and nymphicidal against *Dysdercus koenigii* and induces moderate knockdown effect against *T. castaneum*. Citronellal is toxic to *S. litura*, and *M. domestica* (LD_{50} = 66.0–111.2 µg/insect) [14], *d*-Limonene in the range of 50–273.7 µg/insect is toxic to *M. domestica and S. litura*. The essential oil from root of sweet flag, *Acorus calamus* is also known for its insecticidal and antigonadal actions associated with its most abundant constituent β-asarone [17]. *A. calamus* has been shown to induce mortality of 80.87% in 3rd instars of *Spilosoma obliqua* in laboratory and 74.26% under field conditions at 2.0% concentration [9].

Some essential oils and their components exhibited both a repellent and a larvicidal action for example *Ocimum* volatile oils including camphor, cineole, methyl eugenol, limonene, myrcene and thymol, strongly repelled mosquitoes and *O. basilicum* exerted a larvicidal activity evaluated at EC_{50}=81 ppm [6]. Further *O. basilicum* has shown moderate contact toxicity (LC_{50} = 59.8 and LC_{95} = 125.3 ppm) against *S. litura* [10] and the oil of *O. sanctum* and *O. basilicum* have been reported to cause 100% feeding difference at 10% concentrations, resulting into 20% mortality against *S. litura* [26].

5. Conclusion

In the present investigation *M. koenigii* oil significantly reduced the larval weight gain, pupation per cent and adult emergence; the larval and pupal periods of *S. obliqua* were significantly increased at the higher doses. It virtually proved non-insecticidal causing 33.3% mortality at a topical dose of 2.5 µl/larva. The curry leaf oil had almost same effect on 10d old larvae of *S. litura* being relatively more severe in comparison to *S. obliqua*. For example at the higher dose of 2.5 µl/larva feeding was nil which resulted into the loss of body weight (-106.25%) at 2DAE and 46.6% terminal larval mortality. The oil shows its potential for exploitation in insect pest management programmes.

6. Acknowledgement

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