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Residual toxicity of essential oils from *Murraya koenigii* and *Ocimum tenuiflorum* against *Spodoptera litura*

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Abstract
Murraya koenigii (curry leaf) and *Ocimum tenuiflorum* (lemon tulsi) oils at 2.5, 2.0, 1.5, 1.0 and 0.5% showed toxicity to 7days old larvae of *Spodoptera litura* at 24, 48 and 72 hours after exposure (HAE). *M. koenigii* oil was more toxic compared to *O. tenuiflorum* oil with LC_{50} of 1.78 and 2.40, respectively at 24 HAE and LC_{50} of 1.26 and 1.38, respectively at 48HAE. A comparison of LT_{50} values between *M. koenigii* and *O. tenuiflorum* oils at 2.5 and 2.0% indicated almost similar response 18.8 and 28.4; and 18.6 and 29.1hours, respectively. It revealed that *O. tenuiflorum* oil was quicker in action than *M. koenigii* oil. *M. koenigii* at 1.5 and 2 and 2.5% was most toxic to tobacco caterpillar.

Keywords: Essential oils, *Murraya koenigii*, *Ocimum tenuiflorum, Spodoptera litura,* tobacco caterpillar

Introduction
Spodoptera litura is a highly polyphagous insect which feeds on various hosts plants and at least 180 plant families are attacked by this pest [1]. It affects the yield of cotton, flax, groundnut, vegetables, castor, tomato, sweet potato, colocasia spp., ornamentals, wild plants and shade trees [2, 3, 4] by feeding gregariously on leaves, and causes large economic losses up to 30-50% in major crops [5]. The pest is widely distributed in temperate and tropical regions of the world [6].
Chemical control has been the most common method for the control of *S. litura* which has the potential to develop resistance to various classes of insecticides [7, 8, 9, 10]. From time immemorial there has been a continuous need for the production of safe pesticides and in this context essential oils derived from plants have shown promising results for the quick control of various notorious insect pests. Plants are producers of many compounds that are insect repellents, growth and development inhibitors and oviposition deterrents. Plant essential oils are safe to use and do not pollute the environment and for this reason most of the research work is being focused on plant oils or horticultural oils in developing safe insecticides due to their unique mode of action [11]. Insects which are acutely poisoned by essential oils show symptoms similar to chemicals that are nerve poisons including agitation, hyperactivity, paralysis and quick knockdown [12, 13]. Many plant oils have been tested against *S. litura* and they have shown insecticidal action, to list a few are karanja, clove and calamus oil [14], tulsi [15] and *Jatropha* oil [16] fennel and pepper oil [17]. The present study was undertaken to test the insecticidal activity of two plant essential oils, *M. koenigii* and *O. tenuiflorum* against *S. litura.*

Materials and Methods
The studies on the bioefficacy of oils against tobacco caterpillar were conducted in the bioactive plant natural products laboratory at the department of entomology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The population of *S. litura* was maintained on castor leaves in the laboratory. *Murraya koenigii* and *Ocimum tenuiflorum* plants were obtained from medicinal plants research and development centre (MRDC), Pantnagar and their leaves were shade dried, extracted by hydrodistillation method [18] using clevenger apparatus. The distilled oils were separated from water by a separating funnel and stored in refrigerator. Five concentrations 0.5, 1.0, 1.5, 2.0 and 2.5% of each of the oils were prepared in acetone and tested against 7d old larvae of *S. litura* under laboratory conditions (avg. temp. 28°C and RH 88%) by residue contact bioassay method [19]. One ml of each of the concentration was coated as a thin film in the lower and upper lids of petriplate (dia. 9cm).The

solvent was allowed to dry at room temperature. After evaporation of the solvent, freshly moulted 7d old larvae of *S. litura* (n=8) were released in each petridish and given contact exposure for 30 minutes. In the control, the larvae were exposed to acetone alone [20]. Each concentration was regarded as a treatment and replicated thrice. Thereafter, the larvae were transferred to respective rearing boxes containing fresh castor leaves. The data on mortality was recorded at 12, 24, 48 and 72 HAE. Moribund larvae were counted as dead.

Statistical analysis

The observed mortality was corrected using Abbott's formula [21]. The LC50 and LT50 values were determined using Probit analysis [22] based computer programme STPR718 at the computer centre, college of basic sciences and humanities of the University.

Results and Discussion

It is evident from the table No. 1 and 2 that *M. koenigii* and *O. tenuiflorum* oils caused significant mortality only at 48 and 72 HAE enabling to go for probit analysis as the lower concentrations caused mortality ranging from 12.5 to 37.5 at 12 HAE and 20.8 to 58.3% at 24 HAE. The essential oil from *M. koenigii* was more toxic than *O. tenuiflorum* oil with LC_{50} of 1.78 and 2.40, respectively; and LC_{90} values of 12.4 and 14.0, respectively at 24 HAE. This trend was maintained at LC_{50} 48 HAE (1.26 and 1.38, respectively), but was reversed at LC_{90} 48 HAE (10.0 and 4.22, respectively). The RT at LC_{90} level at 48 HAE were 1.0 and 2.37 for the two oils indicating that *O. tenuiflorum* was 2.37 times more toxic than the essential oil from *M. koenigii* (Table No.1 and 2). A further reduction in LC values were observed at 72 HAE, the LC_{30}, LC_{50} and LC_{90} values of *M. koenigii* being 0.51, 0.98 and 4.78; and 0.67, 1.04 and 3.02% for *O. tenuiflorum*, respectively. The *O. tenuiflorum* oil in all the three observations recorded at 24, 48 and 72 HAE at LC_{50} level proved to be less toxic than curry leaf oil to the larvae of *S. litura*, but at LC_{90} level, 48 and 72 HAE *M. koenigii* oil was less toxic than *O. tenuiflorum* oil (Table 3). A comparison of LT_{50} values (Table 4) between *M. koenigii* and *O. tenuiflorum* oils at 2.5 and 2.0% concentrations indicated almost similar response (18.8 and 28.4h ; and 18.6 and 29.1h, respectively). *O. tenuiflorum* oil at 2.5% concentration caused 90% mortality in 58.5h whereas *M. koenigii* oil required 124.9h. It proved that *O. tenuiflorum* oil was quicker in action than *M. koenigii* oil. At the next lower concentration of 2.0 and 1.5% reverse trend was observed. The LT_{90} values for *M. koenigii* were 355.8 and 725.1h and for *O. tenuiflorum* 190.5 and 602.6h, respectively. It was interesting to note that at LT_{90} *O. tenuiflorum* oil was quicker in expressing its toxicity against *S. litura* than *M. koenigii* oil whereas at LT_{30} and LT_{50} levels *M. Koenigii* oil was quicker in toxic action as evident from their LT values. This oil has also been reported to possess toxicity by topical application to *S. litura* larvae with LD50 = 116.3 µg/ larva [23]. 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. The antifeedant effect was more than 50% at the concentration of 250 µg/ ml. Amongst crude extracts, benzene extract was more effective than hexane extract; the ED 50 being 710 and 4261 µg/ ml, respectively and curry leaves showed antifeedant activity against fifth instar larvae of *S. litura* with ED_{50} value of 130 µg/ ml. [24]. Toxicity of *M. koengii* (LC50= 6.58µl) has also been reported against black ant [25]. The insecticidal propeties may be due to the presence of various phytochemicals for example mahanine, koenine, koenigine, koenidine, isomahanine, murrayamine, D-alpha -pinene, caryophyllene, dipentene, D-sabinene etc. [26, 27, 28]. A very little work has been done on curry leaf and lemon tulsi oils and we could not find direct references on the toxicity of oils against *S. litura*, however many other researchers have found toxicity against *S. litura* in various other plant oils such as *Ocimum basilicum*, *Satoreja hortensis*, *Thymus serpyllum* and *Origanum creticum*. Further *O. basilicum* has shown moderate contact toxicity (LC 50 = 59.8 and LC 95 = 125.3 ppm) against *S. litura* 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* [29, 30, 31] .Linalool is a terpenoid present in *O. basilicum* and has shown potential in controlling *S. frugiperda* by causing a repellent effect, acute toxicity, nonpreference and knockdown effect [32, 33].The bioactivity reflected by essential oils is indicative of the fact these essential oils may find place under the category of biopesticides in the integrated pest management programmes.

Table 1: Dosage-mortality response of two plant essential oils against 7d old larvae of tobacco caterpillar, *Spodoptera litura* at 24 hours after exposure

Plant oils	LC values (%)						Chi square	Regression equation Y= a+bx	Fiducial limit (LC_{50})	
	LC_{30}	*RT at LC_{30}	LC_{50}	RT at LC_{50}	LC_{90}	RTat LC_{90}			Lower	Upper
Murraya koenigii	0.80	1.46	1.78	1.34	12.42	1.12	0.064	Y=4.01+0.25x	1.24	3.72
Ocimum tenuiflorum	1.17	1.00	2.40	1.00	14.02	1.00	1.40	Y=3.66+0.29x	1.69	6.60

*Relative toxicity (RT) = LC value of least toxic insecticide/LC value of candidate insecticide

Table 2: Dosage-mortality response of two plant essential oils against 7d old larvae of tobacco caterpillar, *Spodoptera litura* at 48 hours after exposure

Plant oils	LC values (%)						Chi square	Regression equationY= a + bx	Fiducial limit (LC_{50})	
	LC_{30}	*RT at LC_{30}	LC_{50}	RT at LC_{50}	LC_{90}	RTat LC_{90}			Lower	Upper
Murraya koenigii	0.54	1.61	1.26	1.09	10.01	1.00	0.51	Y= 4.26+0.25x	0.7	
Ocimum tenuiflorum	0.87	1.00	1.38	1.00	4.22	2.37	1.99	Y=3.52+0.47x	1.10	1.74

*Relative toxicity (RT) = LC value of least toxic insecticide/LC value of candidate insecticide

Table 3: Dosage-mortality response of two plant essential oils against 7d old larvae of tobacco caterpillar, *Spodoptera litura* at 72 hours after exposure

Plant oils	LC values (%)						Chi square	Regression equation Y=a+bx	Fiducial limit (LC_{50})	
	LC_{30}	*RT at LC_{30}	LC_{50}	RT at LC_{50}	LC_{90}	RTat LC_{90}			Lower	Upper
Murraya koenigii	0.51	1.31	0.98	1.06	4.78	1.00	1.18	Y=4.21+0.33x	0.60	1.31
Ocimum tenuiflorum	0.67	1.00	1.04	1.00	3.02	1.58	4.59	Y=3.76+0.50x	0.78	1.28

*Relative toxicity (RT) = LC value of least toxic insecticide/LC value of candidate insecticide

Table 4: Duration-mortality response of two plant essential oils against 7d old larvae of tobacco caterpillar, *Spodoptera litura*

Plant oils	Conc. (%)	LT values in Hours			Chi square	Regression equation Y = a + bx	Fiducial limits (LT50)	
		LT30	LT50	LT90			Lower	Upper
Curry leaf, *Murrayakoenigii*	2.5	8.74	18.85	124.99	0.18	Y=4.3+0.41x	7.99	27.96
	2.0	10.19	28.44	355.83	0.80	Y=4.26+0.31x	11.39	54.56
	1.5	13.54	42.76	725.16	0.54	Y=4.16+0.27	22.41	310.08
Lemon tulsi (*Ocimum tenuiflorum*)	2.5	11.73	18.67	58.54	0.50	Y=3.97+0.61x	12.50	24.35
	2.0	13.59	29.15	190.56	0.18	Y=3.98+0.42x	17.51	44.94
	1.5	17.59	48.84	602.62	0.10	Y=4.0+0.30x	29.17	235.27

Conclusion

In the present investigation curry leaf and lemon tulsi oils showed larvicidal properties against 7d old larvae of tobacco caterpillar at 48 and 72HAE. Curry leaf oil when compared to lemon tulsi oil was more effective in causing larval mortalities, though both the oils were effective at concentration above 1% at 24, 48HAE, however less time was taken by lemon tulsi oil in killing the larvae as compared to curry leaf oil. *M. koengii* and *O. tenuiflorum* oils are promising natural control agents in plant protection programmes as they slow down the process of resistance development in insects due to their unique mode of action which is different from the conventional insecticides and may function as nerve toxins, respiratory poisons, ecdysis inhibitors and antifeedants

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