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Effect of *Murraya koenigii* extracts on feeding and ovipositional response of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae).

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ABSTRACT
The antifeedant and oviposition deterrent activity of hexane extract of *Murraya koenigii* leaves was evaluated at five different concentrations against the fourth instar larvae and gravid females of *Spodoptera litura*. Hexane extract showed feeding deterrence activity in a concentration dependent manner. The antifeedant index at all the five concentrations was significantly higher as compared to control being highest at 5% concentration (60.77) and lowest at 1% concentration (13.72). Similarly, leaf extract suppressed the egg laying of gravid females. The oviposition deterrence index was lowest at 1% (23.99) and highest at 5% (73.31) concentration of extract. The results clearly indicate the presence of some antifeedant and oviposition deterrent chemicals in *M. koenigii* leaves that can be exploited for the management of *S. litura*.

Keywords: Spodoptera litura, Murraya koenigii, Antifeedant, Oviposition deterrent.

1. Introduction
Spodoptera litura (Lepidoptera: Noctuidae) is a multivoltine, polyphagous insect pest of many agricultural crops such as cotton, groundnut, soybean tomato, sweet potato etc. [1]. High reproductive capacity, damaging potential and ability to migrate long distances has made it an economically important pest, that causes an estimated loss of 25.8 to 100% in crop production [2].

Infestation by *S. litura* has largely been controlled by the use of insecticides. This resulted in selective pressure on sprayed population and development of resistance against almost all the groups of insecticides [3, 4, 5]. In addition, over use and abuse of insecticides has resulted in ecological imbalance. Therefore, it is essential to search alternative sustainable methods for the management of this pest.

Plants have evolved rich sources of natural substances for their protection against herbivores. These botanicals are not only biodegradable and environment friendly but also there is less likelihood for insects to develop resistance against these natural substances [6]. Use of botanicals, particularly from neem, pyrethrum, tobacco etc., for controlling insect pests in the last few decades has given promising results [7, 8, 9].

M. koenigii, commonly known as curry leaf, is a small, deciduous-to-semi-evergreen tree found throughout India. It has been used as spice for its aroma, and as herb in Ayurvedic medicine. There are a few reports about the adverse effect of *M. koenigii* against some insect pests [10, 11, 12, 13]. The present study was initiated to evaluate the effect of non-polar leaf extract of *M. koenigii* on the feeding of larvae and oviposition of gravid females of *S. litura*.

2. Material and Methods
2.1 Insect culture
S. litura culture was maintained in BOD incubator at 27±2 ºC temperature, 65±5% relative humidity and 14L: 10D photoperiod. The adults were kept in clear plexiglass cage (20 X 20 X 20 cm) having side windows on the end walls (8 cm dia), and a fixed loose net cover on front door (10 cm X 10 cm) for handling the moths. The cage was provisioned with 10% sucrose solution on

cotton swab, which served as food for the adults. A castor leaf with its petiole dipped in water, filled in a reagent bottle, was provided in the cage as an oviposition substrate. Eggs laid in the cage were carefully collected, sterilized with sodium hypochlorite (0.2%) and formaldehyde (10%), rinsed thoroughly in water and kept in small jars. On hatching, larvae were reared on fresh castor leaves (*Ricinus communis* L.) in plastic jars, until pupation. Pupae were collected and washed with 0.05% sodium hypochlorite solution to prevent any infection [14]. Pupae were air dried, sexed and kept in separate jars for emergence of adult moths.

2.2 Plant extract

Fresh leaves of *M. koenigii* were collected from the field plots of Zoology Department, Delhi University that were grown under pesticide free condition. The leaves were washed and shade dried at room temperature to remove traces of water from surface. The air dried leaves (1000 gm) were submerged completely in hexane in a beaker for 24 h. The solvent was then decanted in another glass jar. The leaves were rinsed three times with solvent and collected in same jar. The pooled extract was then filtered through Whatman No. 1 filter paper. The filtrate was concentrated in a rotary evaporator at room temperature under reduced pressure. The concentrated extract was stored in deep freezer for use.

2.3 Preparation of test extract

Control solution was prepared by mixing 30% hexane and 70% distilled water (to avoid leaf damage due to organic solvent). 0.5% of Triton-X was added in control solution which served as an emulsifier. Five different concentrations of the crude extract, i.e. 1, 2, 3, 4, and 5% were prepared by serial dilution in control solution.

2.4 Antifeedant bioassay

No-choice leaf disc bioassays were carried out to determine the antifeedant activity of crude hexane extract of *M. koenigii*. For this overnight emerged fourth instar larvae were used as they have been reported to consume food with minimum fluctuation [15]. The late third instar larvae were kept individually in petridish, containing wet cotton wool in the evening at 8 pm. Those larvae which moulted successfully in night were used for the bioassay. Freshly excised tender castor leaves of same age were taken for the bioassay. Leaf discs of 5 cm diameter were cut and dipped two times for 0.5 sec each in desired concentration of extract. The control leaf disc was dipped in the control solution. The leaf discs were left for 20 minutes in the open to dry off the solvent. This was placed singly at the centre of a petridish (10 cm dia), having bottom lined with moist tissue paper. Single larva was released in each petridish, provisioned with leaf disc, and allowed to feed for 24 h. Thereafter, larvae were removed and remnants of leaf discs were kept between two transparent sheets. The leaf areas consumed by larvae were traced on the graph paper and consumption was calculated by the method [16]. Each experiment with individual concentration of extract was replicated 5 times, and each replicate consisted of responses of 10 larvae. The Antifeedant Index (AI) was calculated [17] by -

$$AI = 100 \{(C-T)/(C+T)\}$$

Where, C is control leaf area consumed and T is the treated leaf area consumed by larvae.

2.5 Oviposition bioassay

Oviposition bioassay was conducted in a plexiglass oviposition cage (40 x 20 x 20 cm), divided into three sections: treatment section (10 cm), middle section (20 cm) and control section (10 cm). The experiment was conducted in insectory, maintaining temperature 25±2 ºC, relative humidity of 60-65%, and dim diffused light.

No-choice bioassays were conducted for each concentration of extract. Two freshly excised tender castor leaves of approximately same size and age were taken. The petiole of leaf was dipped into a reagent bottle containing water, so as to avoid wilting. The open mouth of the bottle was plugged with cotton. Leaf surface was smeared on both sides with particular concentration of extract and control leaf was applied with control solvent. The leaves were left for about 20 minutes at room temperature to allow evaporation of water. The leaf bouquet treated with plant extract was placed at one end, while the control bouquet was placed at the opposite end. Two diet cups with cotton swabs, soaked in 10% sucrose solution, were placed in the middle section of cage which served as food for the adult moths. Five pairs of two days old mated adults were released in the middle section of the cage in the evening at the beginning of scotophase, i.e. 8 pm. The moths were removed next morning at the beginning of photophase, i.e. 7 am. The eggs deposited by females on the leaf surface and in the cage were collected and counted. The experiment was replicated 5 times for each concentration of extract, and each replicate consisted response of five pairs of adults from different batches. On the basis of eggs laid in the cage, oviposition Deterrent Index (ODI) was calculated [18] by the formula

$$ODI = 100\{(C_N-T_N)/(C_N+T_N)\}$$

Where, C_N is the number of eggs laid on control leaf, and T_N is the number of eggs laid on treated leaf.

2.6 Statistical analysis

The data for antifeedant index and oviposition index between different concentrations of extract were compared using one-way analysis of variance (ANOVA), followed by comparison of means by Tukey's test. The data for ovipositional and feeding response between control and treated leaves were subjected to student t-test. The statistical analysis was performed with the help of statistical software package 'Sigma stat 2.0 version' [19].

3. Results

3.1 Antifeedant activity

Leaf area consumption on treated leaf surface was significantly lower ($P<0.05$) at all the concentrations of extract as compared to control leaf surface (Table 1). Definite relationship was observed between the concentration of extracts and the area consumed by larva. Higher the concentration of extracts lower the area consumed by larva (Fig. 1). The antifeedant index was lowest at 1% concentration (13.7) and highest at 5% concentration of extract (60.8), the difference being highly significant ($P<0.001$). The AFI values at 3% and 4% of *M. koenigii* extract were 44.4 and 52.9 respectively, the difference being statistically not significant.

Table 1: Antifeedant activity of crude hexane extracts of *M. koenigii* foliage gainst fourth instar *S. litura* larvae [1]

	Treated leaf disc		Untreated leaf disc		
Extract conc.	**Leaf area consumed cm^2/larva**	**Percent feeding**	**Leaf area consumed cm^2/larva**	**Percent feeding**	**Antifeedant Index (AFI)[2]**
(%)					
	Mean ±SE		Mean ±SE		Mean[3] ±SE
1	5.63 ± 0.46	28.69	7.38 ± 0.45	37.61	13.72 ± 1.09[a]
2	3.65 ± 0.33	18.60	5.98 ± 0.24	30.47	24.70 ± 2.86[b]
3	2.58 ± 0.22	13.15	6.69 ± 0.36	34.09	44.38 ± 3.05[c]
4	2.05 ± 0.13	10.45	6.43 ± 0.29	32.76	52.88 ± 0.99[cd]
5	1.77 ± 0.15	09.02	7.21 ± 0.47	36.74	60.77 ± 0.94[d]

1. Freshly molted larvae were used for bioassay.
2. AFI was calculated as 100 {(C-T)/(C+T)}.
3. Mean AFI followed by different superscripts are significantly different (One-way ANOVA followed by tukey-test, P<0.05).

Table 2: Oviposition deterrent activity of crude hexane extracts of *M. koenigii* leaves against *S. litura* female [1]

	Treated leaf surface		Untreated leaf surface		
Extract conc.	**Number of eggs laid / Replicate**	**Percentage of eggs laid / Replicate**	**Number of eggs laid / Replicate**	**Percentage of eggs laid / Replicate**	**Oviposition Deterrent Index (ODI)[3]**
(%)					
	Mean ±SE		Mean ±SE		Mean ±SE
1	722.00 ± 40.34	28.55	1188.80 ± 97.32	47.01	23.99 ± 3.09[a]
2	492.20 ± 36.23	19.42	1257.00 ± 75.10	45.64	40.44 ± 3.03[b]
3	219.80 ± 11.02	09.46	1270.80 ± 67.67	52.96	69.18 ± 2.75[c]
4	197.40 ± 19.54	08.48	1194.20 ± 82.32	51.28	71.80 ± 1.21[c]
5	170.40 ± 8.10	06.49	1119.00 ± 65.70	42.62	73.31 ± 1.95[c]

1.Each replicate constituted the response of five pairs of males and females.
2.ODI was calculated as $100\{(C_N-T_N)/(C_N+T_N)\}$.
3.Mean ODI followed by different superscripts are significantly different (One-way ANOVA followed by tukey-test, P<0.05).

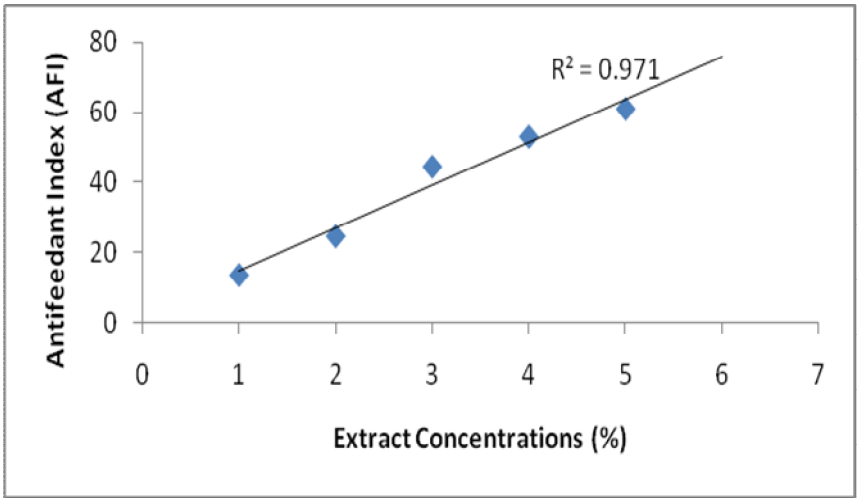

Fig 1: Correlation between antifeedant index values and different concentrations of *M. koenigii* hexane extract

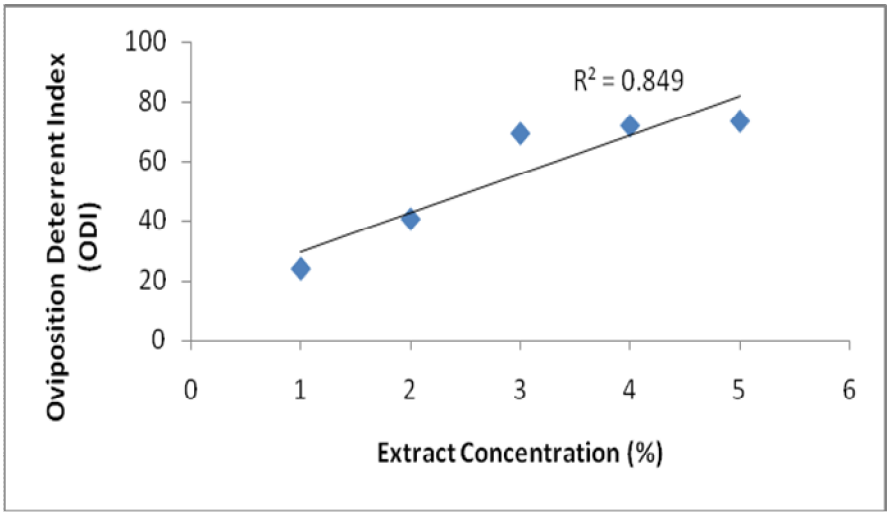

Fig 2: Correlation between oviposition deterrent index values and different concentrations of *M. koenigii* hexane extract

3.2 Oviposition activity

The hexane extract of *M. koenigii* leaves had significant effect on the ovipositional activity of *S. litura* females. The mean number of eggs laid by the females was significantly lower on leaves smeared with *M. koenigii* extracts in all the treatments as compared to control (Table 2). Mean number of eggs laid on the leaf smeared with 1% concentration of extract was 722 as compared to 1188 eggs in control. There was gradual decrease in egg deposition on the leaf surface by *S. litura* female with corresponding increase in concentration of *M. koenigii* extract (Fig. 2). The number of eggs deposited on the leaf surface treated with highest concentration of extract (5%) was almost ten times lower as compared to eggs on leaf surface smeared with lowest concentration of extract (1%). The mean oviposition deterrent index (ODI) of *S. litura* at 1, 2, 3, 4 and 5% concentrations of foliage extracts was 23.99, 40.44, 69.18, 71.80 and 73.31 respectively, the difference being statistically significant ($P<0.05$).

4. Discussion

Feeding and oviposition are the most important behavioural responses for establishment of insect population on a plant surface [20]. The results obtained in the present study indicate the presence of phytochemicals in non-polar foliage extract of *M. koenigii* that inhibit the feeding of *S. litura* larvae. This substantiates earlier finding about the antifeedant activity of acetone extract of foliage and hexane extract of plant of *M. koenigii* against *S. litura* [21, 22]. Such antifeedant activity in non-polar foliage extract of allied plant species, *M. exotica* has also been recorded against *S. litura* larvae [23]. These observations clearly indicate that *Murraya* foliage contains some phytochemicals that have antixenotic effect on insects. Non-polar foliage extract from *Porteresia coarctata* [24], *Clerodendron* spp. (*C. inerme and C. infortunatum*) [25], and pulp of *Momordica dioica* have also been observed to show antifeedant effect against *S. litura* larvae [26]. Phytotoxicity has been observed in another species of *Murraya, M. exotica* against maize weevil, *Sitophilus zeamais* and red flour beetle, *Tribolium castaneum* [27].

The bioassay results indicate the presence of non-polar chemicals in *M. koenigii* leaves that suppress oviposition of gravid *S. litura* females. This suppression in egg laying may be due to the presence of repellent or contact deterrent chemical or both in the foliage extract. Such oviposition deterrence effect against *S. Litura* was also observed in hexane extract of *Acorus calamus* [28]. Oviposition on cage walls and the blotting sheets corroborates the earlier finding that *S. litura* female may also oviposit on non-plant material despite of the presence of a host plant nearby [29].

5. Conclusion

This can be safely concluded from the present study that *M. koenigii* has the potential for use in the management of *S. litura*. The antifeedant and oviposition deterrent effects together would have synergistic effect in restricting population build-up of *S. litura* in the field [30]. Moreover, this method of management would be economical and sustainable, without any adverse effect on the environment. *M. koenigii* has been reported to contain steroids, saponins, alkaloids, flavonoides, glycolysis [31, 32]. Any of these phytochemicals or their combination may be responsible for antifeedant and oviposition deterrence activity against *S. litura*. However, further research is required for isolation and identification of phytochemicals, responsible for such activities so that these may be exploited for the management of *S. litura* population in the field.

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7. References

1. Noma T, Colunga-Garcia M, Brewer M, Landis J, Gooch A. Oriental leafworm *Spodoptera litura*, Michigan State University IPM Program and M. Philip of Michigan Department of Agriculture. Michigan State University's invasive species factsheets, 2010.
2. Dhir BC, Mohapatra HK, Senapati B. Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F.). Indian J Plant Protect 1992; 20(7-10):215-217.
3. Kranthi KR, Jadhav DR, Wanjari RR, Ali SS, Russell D. Carbamate and organophosphate resistance in cotton pests in India, 1995 to 1999. Bull Entomol Res 2001; 91(1):37–46.
4. Shad SA, Sayyed AH, Fazal S, Saleem MA, Zaka SM, Ali M. Field evolved resistance to carbamates, organophosphates, pyrethroids, and new chemistry insecticides in *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). J of Pest Sci 2012; 85:153–162.

5. Tong H, Su Q, Zhou X, Bai L. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. J Pest Sci 2013; 86:599–609.
6. Erdogan P, Yildirim A, Sever B. Investigations on the Effects of Five Different Plant Extracts on the Two-Spotted Mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae). Psyche 2012; Volume 2012, Article ID 125284, 5 pages.
7. Koul O, Singh G, Singh R, Singh J, Daniewski WM, Berlozecki S. Bioefficacy and mode of action of some limonoids of salanin group from *Azadirachta indica* A. Juss and their role in a multicomponent system against lepidopteran larvae. J Biosci 2004; 29(40):409-416.
8. Ulrichs C, Mewis J, Adhikary S, Bhattacharya A, Goswami A. Antifeedant activity and toxicity of leaf extracts from *Porteresia coarctata* Takeeka and their effects on the physiology of *Spodoptera litura* (F.). J Pest Sci 2008; 81:79-84.
9. Malarvannan S, Giridharan S, Sekar S, Prabavathy VR, Sudha N. Bioefficacy of crude and fraction of *Argemone mexicana* against Tobacco Caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). J Biopestic 2008; 55-62.
10. Devanand P, Rani PU. Biological potency of certain plant extracts in management of two lepidopteran pests of *Ricinus communis* L. Journal of Biopesticides 2008; 1:170-176.
11. Malwal M, Sarin R, Shakeet P, Bakshi S. Natural insect controlling agents from *Murraya koenigii* (L.) Spreng. J of Herbal Medicine and Toxicology 2009; 3(1):161-162.
12. Gandhi N, Pillai S, Patel P. Efficacy of Pulverized *Punica granatum* (Lythraceae) and *Murraya koenigii* (Rutaceae) leaves against Stored Grain Pest *Tribolium castaneum* (Coleoptera: Tenebrionidae). Int J Agric Boil 2010.
13. Li WQ, Jiang CH, Chu SS, Zuo MX, Liu ZL. Chemical Composition and Toxicity against *Sitophilus zeamais* and *Tribolium castaneum* of the Essential Oil of *Murraya exotica* Aerial Parts. Molecules 2010; 15:5831-5839.
14. Ignoffo CM, Dutky SR. The effect of sodium hypochlorite on the viability and infectivity of bacillus and beauveria spores and cabbage looper nuclear-polyhedrosis virus. J Invertebr Pathol 1963; 5:422-426.
15. Sadek MM. Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Leprdoptera: Noctuidae). Journal Applied Entomology 2003; 127:396-404.
16. Singh I, Singh G. Assessment of foliage loss caused by different larval instars of Bihar hairy caterpillar, *Spilosoma obliqua* (Walker) on sunflower. J Insect Sci 1993; 6(2):185-186.
17. Isman MB, Koul O, Luczynski A, Kaminski A. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J Agric Food Chem 1990; 38:1406-1411.
18. Huang XP, Renwick JAA. Cardenolides as oviposition deterrents to two Pieris species: structures-activity relationships. J Chem Ecol 1994; 20:1039-1051.
19. Jandel Scientific Software. Sigma Stat 2.0, Jandel Corporation, San Rafael, CA. 1995.
20. Saxena KN. Patterns of insect-plant relationships determining susceptibility or resistance of different plants to an insect. Entomol Exp Appl 1969; 12:751-766.
21. Devanand P, Rani PU. Biological potency of certain plant extracts in management of two lepidopteran pests of *Ricinus communis* L. Journal of Biopesticides 2008; 1:170-176.
22. Arivoli S, Samuel Tennyson. Antifeedant activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). Journal of Entomology and Zoology Studies 2013; 1 (4):87-96.
23. Wang LY, Lu YQ, Luo YP. Anti-feeding activities of 45 south herbs extracts against *Spodptera litura* Fabriciu. Hubei Agric Sci 2009; 48:628-630.
24. Ulrichs C, Mewis J, Adhikary S, Bhattacharya A, Goswami A. Antifeedant activity and toxicity of leaf extracts from *Porteresia coarctata* Takeeka and their effects on the physiology of *Spodoptera litura* (F.). J Pest Sci 2008; 81:79-84.
25. Krishna KGN, Balachandran J, Aravind S, Ganesh MR. Antifeedant and growth inhibitory effects of some neo-clerodane diterpenoids isolated from *Clerodendron species* (Verbenaceae) on *Earias vitella* and *Spodoptera litura*. Journal of Agricultural and Food Chemistry 2003; 51(6):1555-1559.
26. Narasimhan S, Kannan S, Hango K, Maharajan G. Antifeedant activity of *Momordica dioica* fruit pulp extracts on *S. litura*. Fitoterapia 2005; 76(7-8):715-717.
27. Li WQ, Jiang CH, Chu SS, Zuo MX, Liu ZL. Chemical Composition and Toxicity against *Sitophilus zeamais* and *Tribolium castaneum* of the Essential Oil of *Murraya exotica* aerial Parts. Molecules, 2010; 15:5831-5839.
28. Raja N, Elumalai MK, Jayakumar M, Jayasankar A, Muthu C, Ignacimuthu S. Biological activity of different plant extracts against armyworm, *Spodoptera litura* (Fab.) (Lepidoptera; Noctiudae). J Entomol Res 2003; 27(4):281-292.
29. Pitre HN, Mulrooney JE, Hogg DB. Fall armyworm (Lepidoptera: Noctuidae) oviposition: crop preferences and egg distribution on plants. J Econ Entomol 1983; 76:463-466.
30. Rao PJ, Mani C. Phytochemicals on the reproductive biology of insects. In: Chemical ecology of phytophagous insects. (Eds): T.N. Ananthakrishnan and A. Raman. International Science Publisher, New Delhi 1993, 105-114.
31. Pande M, Ingale S, Gupta S. The pharmacognostic and phytochemical studies on the leaves of *Murraya koenigii* (L) Spreng. Indian Journal of Science and Technology 2009; 2(3):53-54.
32. Mathur A, Dua VK, Prasad GBKS. Antimicrobial activity of leaf extracts of *Murraya koenigii* against aerobic bacteria associated with bovine mastitis. International journal of chemical, environmental and pharmaceutical research 2010; 1(1):12-16.