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REPELLENT AND REPRODUCTIVE INHIBITORY EFFECTS OF *STRYCHNOS NUXVOMICA* L. AND *LEPIDIUM SATIVUM* L. AGAINST *SITOPHILUS ORYZAE* (L.) (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

In order to use eco friendly alternatives to chemical insecticides for the management of rice weevil *Sitophilus oryzae* L., hexane, ethyl acetate and methanol plant extracts from the leaves of *Strychnos nuxvomica* L., *Lepidium sativum* L. and *Azadirachta indica* A. Juss. were evaluated for repellent (area preference method) and reproductive inhibitory activities. Results showed that all the plant extracts were repellent at doses between 78.4 and 235.8 µg/cm² after 1, 6, 24, 48 and 72 h exposure. In repellency tests, hexane and ethyl acetate extracts of *A. indica* (>90.0%) followed by *L. sativum* (87.3%) were found to be the most effective compared to the extracts of *S. nuxvomica* at 235.8 µg/cm². In majority of the plant extracts, the per cent repellency increases from 6 to 24 h and then decreases gradually whereas in neem extracts the repellent activity exists even after 72 h. All the plant extracts significantly reduced F_1 progeny emergence compared to untreated control. At 7.5% w/w, ethyl acetate extract of *S. nuxvomica* (96.3%), and hexane extract of *L. sativum* (95.3%) effectively suppressed the F_1 progeny production followed by hexane and methanol extracts of *A. indica* (94.2%, 90.5%), respectively. The present work indicated that the plant extracts from Indian origin were potential grain protectants against *S. oryzae*.

Farmers store maize from short term to long term duration for food purpose, higher prices and to supply for industrial purposes. During this storage period, maize suffers with post harvest losses mainly due to insect pests. *Sitophilus oryzae* (L.) is the most destructive internal feeder of stored maize causing quantitative and qualitative losses. It feeds on grain kernels voraciously and was reported that grub consumes more grain (14 mg) than adult (0.4 mg)/ day (Giolebiowska , 1969). The infestation causes 53.30% damage resulting in 14% loss in weight over four months storage period (Lakshmi Soujanya et al., 2013). In India, chemical insecticides are commonly used to manage storage pests. However, due to ban of effective fumigant methyl bromide (Butler and Rodriguez, 1996); and development of resistance to phosphine, (Daglish et al., 2014; Lee et al., 2001) alternate control measures are essential. Plant derived products serve as best option and can be used as stored grain protectants for small scale storage (Utono and Gibson, 2015; Santos et al., 2015; Hossain et al., 2014). India has rich source of flora of indigenous medicinal plants which are traditionally being used for health purposes. *Strychnos nux-vomica* L. (Loganiaceae) contains caffeic acid- cinnamic acid derivative active against snake bite (Mors et al., 2000) and leaves possess antioxidative properties particularly used for treatment of diabetes and tumour diseases in humans. Phytochemical analysis of *S. nux-vomica* indicated the presence of flavonoids, phenols, terpenoids, tannins and saponins (Mathivanan et al., 2014). In south Asia, *Lepidium sativum* L. (Cruciferae) is used to treat asthama, bronchitis and cough (Duke et al., 2002).The chief phytochemical constitutents mainly contains alkaloids, saponins, anthracene glycosides, flavonoids and sterols. Five new dimeric imidazole alkaloids lepidine B, C, D, E and F were found in seeds of *L. sativum.* (Raval, 2016). *Azadirachta indica* A. Juss. (Meliaceae) is an ever green tree cultivated in various parts of India possessing antifeedant, antimicrobial, antibacterial and antifungal properties and contains phytochemical constitutents such as diterpenoids, triterpenoids, flavonoids, glycosides, tannins etc. The test plants were chosen based on medicinal importance and their ease of availability to the farmers. Although extensive work has been done on neem, but no research has been done so far on the insecticidal efficacy of plant extracts of *L. sativum*, *S. nux vomica* against *S .oryzae*.. Hence, in this study, hexane, ethyl acetate and methanol plant extracts from the leaves of *L.*

sativum, *S. nux vomica* were tested and *A. indica* in comparison for repellent and reproductive inhibitory activities against *S. oryzae.*

MATERIALS AND METHODS

Adults of *S. oryzae* were reared on whole maize at 26 ±1°C and 60± 5% relative humidity (RH). Leaves of *S. nux vomica, L. sativum* were collected in June 2015 from Herbal garden, Sri Konda Laxman Horticultural University, Hyderabad and leaves of *A. indica* were collected from Winter Nursery Centre fields, Hyderabad, Telangana. The leaf material was air dried at room temperature (25-28°C) for one week and then powdered. Leaf powder was extracted with three different solvents of analytical grade based on their increasing polarity- hexane, ethyl acetate and methanol by using Soxhlet apparatus for 10-14 hr. The extracts were concentrated and stored at 4°C for further use.

The repellent tests were carried out according to the method described by Jilani and Saxena (1990). Whatman # No 1 filter paper of 9 cm dia was divided into two equal parts. Each solution was applied to half a filter paper disc with micropipette. The other half of filter paper was applied with acetone as control. After drying of solvent, treated and untreated halves were attached with adhesive tape and placed in petri plates. Thirty adults (5-10 days old) of mixed sex were released at the centre of filter papers. The dishes were then covered and sealed with parafilm. Three replications were used for each concentration. Observations on the number of insects present in treated and untreated portion were recorded after 1, 6, 24, 48 and 72 h.Per centage repellency was calculated according to Nerio et al. (2009): PR = [(NC-NT) /(NC+NT)] × 100.

Three concentrations of 2.5, 5.0 and 7.5% of plant extracts were prepared and mixed with 25g of maize grain. After drying of grain, twenty adults of five day old were released into each plant extracts treated maize jar which was covered with perforated lid. Five replications were made for each concenteration. A control was set up with the same volume of acetone treated grain. After seven days of oviposition, weevils were removed from jars and the entire setup was left for 45 days for F_1 progeny emergence. The % reduction in adult emergence was calculated according to Talukder and Howse (1994) as follows: % reduction = $[C_N-T_N/C_N] \times 100$, where C_N= Number of newly emerged adult weevils in the untreated control; and T_N = Number of newly emerged adults in the treated dish.

RESULTS AND DISCUSSION

The % repellency of *S. oryzae* with the plant extracts are given in Table 1, which reveal that the plant extracts exhibited significant repellency. The repellent action was directly proportional to the concenteration; repellency was observed even at low concenterations and differed significantly ($F = 7.27$; $df = 44$; $p< 0.0001$). At 78.4 µg/cm^2, hexane and ethyl acetate extracts of *A. indica* and *L. sativum* repelled 76.6, 77.9 and 74.6, 66.6% respectively. The methanolic extract of neem gave 55.9% repellency while the remaining extracts exhibited <50% repellency. At 125.8, 157.2 and 188.6 µg/cm^2,all the extracts exhibited moderate repellent effect. ANOVA revealed that all the plant extracts differed significantly at highest concentration ($F= 3.74$; $d.f = 44$; $p< 0.0001$).

Hexane and ethyl acetate extracts of *Azadirachta indica* at 235.8 µg/cm^2 showed the highest mean repellent activity (>90.0%) followed by hexane and ethyl acetate extracts of *L. sativum* (87.3%). The % repellent action was 64.4%, 81.9% from hexane and ethyl acetate extracts of *S. nuxvomica,* respectively. The methanolic extracts of *S. nuxvomica* and *A. indica* exhibited 76.4% and 80.0% repellency, but it was only 65.3% with *L. sativum.* Extracts of *A. indica* and *L. sativum* exhibited highest repellent activity followed by *S. nuxvomica.* Plants of Meliaceae and Cruciferae are known for producing large quantities of terpenoids which might be responsible for repellent action. In majority of the plant extracts, the repellency increases from 6 to 24 hr and then decreases gradually whereas in neem extracts the repellent activity exists even after 72 hr.

Several workers had reported repellent activity of plant extracts towards *Sitophilus* sp (Akhtar et al., 2013; Khani et al., 2011; Tavares et al., 2014), but none available on the efficacy of *S. nux vomica* and *L. sativum* against *S. oryzae*. Wei et al. (2014) reported that ethyl acetate extract of dried fruits of *Illicium verum* Hook.f.exhibited maximum repellency of 76.9% with *S. zeamais* followed by petroleum ether and methyl alcohol extract. Pretheep Kumar et al. (2004) found that an extract of protein enriched bean flour had a high level of repellency on *S. oryzae*; 76.3% and 91.2% of repellency with a concentration of 0.1% and 1% of the extract, respectively, was observed after 48 hr of the assay was initiated. Nattu Durai et al. (2015)

Table 1. Repellency due to plant extracts in adults of *S. oryzae*

Plant extract	Exposure time (h)	Dose (µg/cm^2)					*Concentration*	Progeny emergence (Number)	F_1 reduction (%)
Strychnos nuxvomica		78.4	125.8	157.2	188.6	235.8	2.5%	13 ± 0.3	65.8
Hexane	1	40.0 ± 0.0	42.2 ± 4.4	48.8 ± 2.2	75.5 ± 2.2	80.0 ± 3.8	5%	14.4 ± 1.0	62.1
	6	29.8 ± 8.9	34.7 ± 8.7	46.8 ± 13.7	59.3 ± 14.5	62.1 ± 14.6	7.5%	8.8 ± 0.73	76.8
	24	40.0 ± 0.0	42.2 ± 8.0	51.1 ± 2.2	68.8 ± 2.2	75.5 ± 2.2			
	48	22.2 ± 5.8	17.7 ± 8.0	51.1 ± 5.8	60.0 ± 3.8	71.1 ± 2.2			
	72	33.3 ± 13.3	42.2 ± 13.5	42.2 ± 11.1	48.8 ± 4.4	64.4 ± 4.4			
	Mean	33.3	42.2	42.2	48.8	64.4			
Ethyl acetate	1	62.2 ± 5.8	66.6 ± 6.6	71.1 ± 2.2	79.9 ± 3.8	82.2 ± 2.2	2.5%	8.2 ± 0.8	78.4
	6	66.6 ± 3.8	71.1 ± 2.2	73.3 ± 3.8	75.5 ± 2.2	83.4 ± 2.0	5%	2.2 ± 0.6	94.2
	24	71.0 ± 2.2	73.3 ± 3.8	75.5 ± 8.0	77.7 ± 4.4	84.4 ± 2.2	7.5%	1.4 ± 0.2	96.3
	48	44.5 ± 2.2	64.4 ± 2.2	68.8 ± 9.6	75.5 ± 11.7	77.7 ± 7.7			
	72	33.4 ± 7.8	45.5 ± 9.1	57.6 ± 2.3	69.0 ± 5.8	71.1 ±5.8			
	Mean	55.5	68.8	69.26	75.52	81.925			
Methanol	1	53.3 ± 3.3	60.0 ± 3.8	64.4 ± 5.8	66.6 ± 6.6	80.0 ± 3.8	2.5%	11.0 ± 1.4	71.1
	6	40.0 ± 10.1	60.0 ± 3.8	64.4 ± 5.8	70.0 ± 15.2	82.2 ± 4.4	5%	9.2 ± 0.3	76.8
	24	40.0 ± 10.1	57.7 ± 4.4	60.0 ± 4.4	80.0 ± 3.8	84.4 ± 2.2	7.5%	3.6 ± 0.5	90.5
	48	51.1 ± 2.2	55.5 ± 8.0	44.4 ± 8.0	77.7 ± 4.4	73.3 ± 7.7			
	72	42.2 ± 11.1	51.1 ± 5.8	46.6 ± 3.8	53.3 ± 6.6	62.2 ± 5.8			
	Mean	45.3	56.8	55.96	69.52	76.42			
Lepidium sativum									
Hexane	1	70.0 ± 11.5	73.3 ± 6.6	76.6 ± 3.3	80.0 ± 5.7	83.3 ± 12	2.5%	18.8 ± 0.4	50.5
	6	73.3 ± 3.3	80.0 ± 5.7	83.3 ± 3.3	83.3 ± 3.3	86.6 ± 3.3	5%	15.4 ± 0.7	59.5
	24	73.3 ± 8.8	76.6 ± 8.8	76.6 ± 3.3	76.6 ± 6.6	83.3 ± 3.3	7.5%	1.8 ± 0.8	95.3
	48	73.3 ± 6.6	80.0 ± 5.7	80.0 ± 5.7	90.0 ± 5.7	90.0 ± 5.7			
	72	83.3 ± 3.3	86.6 ± 6.6	90.0 ± 3.3	90.0 ± 5.7	93.3 ± 3.3			
	Mean	74.6	79.3	81.3	83.98	87.3			
Ethyl acetate	1	60.0 ± 11.5	66.6 ± 12	70.0 ± 5.7	73.3 ± 8.8	80.0 ± 3.8	2.5%	24.2 ± 0.4	36.3
	6	50.0 ± 5.7	53.3 ± 8.8	66.6 ± 6.6	73.3 ± 8.8	80.0 ± 5.7	5%	21 ± 2.4	44.7
	24	80.0 ± 5.7	83.3 ± 6.6	83.0 ± 3.3	83.3 ± 3.3	93.3 ± 6.6	7.5%	10 ± 1.6	73.7
	48	63.3 ± 3.3	80.0 ± 5.7	86.6 ± 3.3	86.6 ± 6.6	96.6 ± 6.6			
	72	80.0 ± 5.7	83.3 ± 3.3	83.3 ± 3.3	86.6 ± 3.3	86.6 ± 3.3			
	Mean	66.6	81.6	77.9	80.62	87.3			
Methanol	1	26.6 ± 8.8	36.6 ± 3.3	50.0 ± 10.0	56.6 ± 3.3	70.0 ± 5.7	2.5%	26 ± 0.7	31.6
	6	36.6 ± 6.6	43.3 ± 8.8	46.6 ± 3.3	60.0 ± 0.0	63.3 ± 3.3	5%	23.6 ± 0.8	37.9
	24	30.0 ± 5.7	36.6 ± 6.6	40.0 ± 5.7	40.0 ± 15.2	53.3 ± 15.2	7.5%	16.6 ± 1.4	56.3
	48	36.6 ± 3.3	46.6 ± 3.3	46.6 ± 6.6	66.6 ± 3.3	73.3 ± 3.3			
	72	20.0 ± 5.7	33.3 ± 3.3	46.6 ± 3.3	60.0 ±5.7	66.6 ± 5.7			
	Mean	29.9	39.2	45.9	56.6	65.3			

contd...

Table contd...

Azadirachta indica									
Hexane	1	76.6 ± 3.3	83.3 ± 3.3	86.6 ± 3.3	86.6 ± 3.3	93.3 ± 6.6	2.5%	17.8 ± 0.8	53.2
	6	76.6 ± 6.6	86.6 ± 3.3	86.6 ± 3.3	83.3 ± 3.3	96.6 ± 3.3	5%	11.6 ± 0.6	69.5
	24	83.3 ± 3.3	80.0 ± 5.7	93.3 ± 3.3	93.3 ± 3.3	96.6 ± 3.3	7.5%	2.2 ± 0.9	94.2
	48	73.3 ± 3.3	83.3 ± 3.3	86.6 ± 3.3	93.3 ± 3.3	93.3 ± 3.3			
	72	73.3 ± 3.3	86.6 ± 3.3	83.3 ± 3.3	93.3 ± 6.6	93.3 ± 3.3			
	Mean	76.6	83.9	87.2	89.9	94.6			
Ethyl acetate	1	73.3 ± 8.8	73.3 ± 3.3	80.0 ± 0.0	83.3 ± 3.3	93.3 ± 3.3	2.5%	21.4 ± 0.8	43.7
	6	76.6 ± 6.6	76.6 ± 6.6	83.3 ± 3.3	86.6 ± 3.3	93.3 ± 3.3	5%	16 ± 0.6	57.9
	24	83.3 ± 3.3	86.6 ± 3.3	90 ± 5.7	93.3 ± 3.3	100 ±0.0	7.5%	13.4 ± 0.8	64.7
	48	83.3 ± 3.3	86.6 ± 6.6	90 ± 5.7	93.3 ± 3.3	96.6 ± 3.3			
	72	73.3 ± 3.3	83.3 ± 3.3	83.3 ± 3.3	93.3 ± 3.3	93.3 ± 3.3			
	Mean	77.9	81.2	85.3	89.9	95.3			
Methanol	1	46.6 ± 14.5	53.3 ± 12	53.3 ± 6.6	60.0 ± 5.7	60.0 ± 11.5	2.5%	25.2 ± 0.9	33.7
	6	53.3 ± 12.0	60.0 ± 5.78	60.0 ± 5.7	70.0 ± 5.7	70.0 ± 5.7	5%	16.6 ± 1.8	56.3
	24	60.0 ± 5.78	66.6 ± 6.6	73.3 ± 6.6	73.3 ± 3.3	76.6 ± 12.0	7.5%	15.4 ± 0.6	59.5
	48	66.6 ± 3.3	70.0 ± 5.7	80.0 ± 5.7	80.0 ± 10.0	86.6 ± 3.3			
	72	53.3 ± 3.3	70 ± 10.0	76.6 ± 10.0	86.6 ± 6.6	90.0 ± 10.0			
	Mean	55.9	63.9	68.6	77.47	80.8			

reported that diethyl ether fruit extract of *Toddalia asiatica* (L.) Lam. exhibited 100% repellent activity against *S. oryzae* adults at 20 ìL concentration.

As regards F_1 progeny emergence, significantly lower number of progeny (1.4 to 26) (F = 50.28; df =112; p<0.0001) was produced in most of the treatments in comparison to the untreated control (38). The F_1 progeny emergence was minimum in ethyl acetate extract of *S. nuxvomica* (1.4) followed by hexane extracts of *L. sativum* (1.8) and *A. indica* (2.2) at 7.5% w/w. Even at lower dose of 2.5%w/w, few progeny emerged (8.2) from maize treated with ethyl acetate extract of *S. nuxvomica.* Similarly, the % reduction in F_1 progeny was also higher with ethyl acetate extract of *S. nuxvomica* (96.3%), hexane extract of *L. sativum* (95.3%) and hexane and methanol extracts of *A. indica* (94.2%, 90.5%) at 7.5% w/w. There was 31.6 to 78.4% reduction in F_1 progeny emergence when maize treated with the remaining plant extracts at 2.5 and 5.0%w/w concentrations. The F_1 progeny reduction in maize treated with plant extracts might be due to increased adult mortality and inhibition of oviposition by *S. oryzae*.

The results revealed that at all the concentrations, ethyl acetate extract of *S. nux-vomica* reduced the F_1 progeny emergence to maximum extent followed by hexane and methanol extracts. These agree with those of other authors who reported such reduction. Yankanche and Gadache (2010) reported that ethanol extracts of *Clerodendron inerme* (L.) and *Withania somnifera* (Dunal) completely suppressed progeny emergence of *S. oryzae* at 2.5 and 5% concentration. Similarly, Udo (2005) reported significant reduction of F_1 progeny emergence in maize treated with *Dennetia tripetala* Bak. f. leaf powder at 10% concentration. Recently, Akter and Akter (2016) reported reduced adult emergence of *S. oryzae* with neem leaf powder @ 1g/100g grain.

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