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EFFICACY OF HEXANE EXTRACTS OF SOME PLANTS AGAINST RICE WEEVIL *SITOPHILUS ORYZAE* (L.) IN STORED MAIZE

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ABSTRACT

Hexane extracts of certain botanicals were evaluated in the laboratory for their oral, contact, fumigant toxicity and repellent activity at 5% concentration against rice weevil *Sitophilus oryzae* L. at the Natural Pesticide Laboratory, Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai, Tamil Nadu during 2019-2021. The results revealed that all the botanicals were effective. Considering the contact toxicity, 5% hexane extract of *Mentha spicata* 5% (80.00%) performed better at 72 hr after treatment. *M. spicata* and *Vitex negundo* 5% hexane extract exhibited maximum oral toxicity (90.00%). Fumigant effect was maximum in *V. negundo* (73.33%), *M. spicata, Ocimum sanctum* and *Tagetes erecta* flower extracts (70.00%). *Ocimum sanctum* (70.56%) and *M. spicata* (67.22%) exhibited maximum repellency. Thus, *M. spicata* at 5% was the most effective as contact, oral, fumigant toxicant and repellent against *S. oryzae* in stored maize.

Key words: *Sitophilus oryzae*, hexane extracts, botanicals, contact toxicity, oral toxicity, repellency, fumigant, *Mentha spicata*, *Vitex negundo*, *Ocimum sanctum*, *Curcuma longa*.

Maize is the major cereal crop in the world after wheat and rice. In developing countries, maize production and consumption often falls below demand as a result of post-harvest losses due to storage pests (Mohale et al., 2010). Post-harvest losses by stored insect pests amount to 9% in developed countries and 20% or more in developing countries (Philips and Throne, 2010). In stored maize, rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae), is the most destructive both in field and storage and caused losses of 30-80%. One pair of *S. oryzae* can reproduce about one million of its species within a period of 3 months under favourable conditions (Thomas et al., 2002). Adults are internal feeders and cause vertical infestation (Prakash et al., 1987). Already registered insecticides are few in number and fumigant chemicals are in restriction for use (Philips and throne, 2010). To combat these problems, botanical insecticides could be viable as component of IPM. Plant materials with insecticidal properties are locally readily available, affordable, relatively it is nonpoisonous to human health and less detrimental to the environment (Talukder and Howse, 1995). Plants contain a large number of secondary metabolites viz., terpenoids, alkaloids, glycoside, phenols, tannins etc., which play a major role in plant defense and cause behavioural and physiological effects on insects. Thus, in recent years research is being focused on evaluating and developing botanical insecticides in view of their relative safety to the environment. The present study evaluates the toxicity of hexane plant extracts from some plants against *S. oryzae* under laboratory conditions.

MATERIALS AND METHODS

The experiments were conducted during 2019-2021, at the Natural Pesticide Laboratory (9.925°N, 78.119°E), Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai, Tamil Nadu. Stock culture of *S. oryzae* was collected from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore and maintained in the laboratory (30± 2° C; 70± 5%RH0. Maize grains were kept in hot air oven at 40°C for 48 hr to eliminate possible insect infestation from the field. Adult weevils (200 nos.) were released in plastic jars (1 l capacity) filled with clean, undamaged and sterilized 500 g of whole maize grains and covered with perforated plastic lids, and kept undisturbed until adult emergence. The F1 generation of the weevils emerged from the culture were used for the bioassay. All experiments were carried out under the same insect rearing conditions. A total of 1 1 botanicals were evaluated in comparison with *Acorus calamus*

1% as treated check, acetone as positive control and untreated check as negative control.

The botanicals viz., *Citrus aurantium, Curcuma longa, Eucalyptus globulus Lantana camara, Mentha spicata, Murraya koenigii, Ocimum sanctum, Ricinis communis, Tagetes erecta* and *Vitex negundo* were obtained from the healthy, mature and succulent leaves/ rhizomes/ flowers. The plant parts were washed with distilled water and air dried for a week under shade at room temperature of 29-31°C and macerated into fine powder using an electric grinder and sieved through sieve, with 0.1 mm pore size, to obtain uniform particle size. The leaf powders were stored separately in plastic containers and placed in a dark place until used for extraction. Dried leaf/ rhizome/ flower powders (100 g) were separately soaked in 1000 ml hexane (non-polar solvent) and stirred in a magnetic stirrer at 2000 rpm for 2 hr and left to stand for 24 hr. Later, the contents were carefully filtered through Whatman No.1 filter paper. The extract was concentrated in rotary vacuum evaporator at 40°C under reduced pressure to get the crude hexane extract. The residue obtained was weighed and % recovery yield was estimated. The residue was stored in amber coloured glass vials (wrapped with aluminium foil) and maintained in a refrigerator (4 °C) until bioassay.

The contact toxicity of hexane extract at 5% was evaluated by treating circular discs of Whatman No. 1 filter paper (9 cm dia) and exposed to 10 weevils in petri dish, covered with vaseline coated lid. Each treatment was replicated thrice, and observations on dead insects made after 24, 48 and 72 hr of treatment and expressed as % mortality (Ashamo and Akinnawonu, 2012). The oral toxicity was evaluated by thorough coating of the maize seeds (10 g) with 5% hexane extract and five pairs of adults were released. The treated seeds were placed in plastic jars and secured with cotton cloth and rubber bands. The number of dead weevils was recorded after 3, 5, 7, 9 and 15 days of treatment (Rani Selva et al., 2019) and expressed as % mortality; any mortality in control was corrected using Abbott formula (1925). The fumigant effect of hexane extracts (5%) was evaluated by impregnating the filter paper discs (1.5 cm dia.) and it was attached to inner surface of screw cap of glass vials (25 ml) after evaporating the solvent. Ten weevils were released into the vials with 20 maize seeds separately. The neck of the vials was blocked with nylon cloth to avoid direct contact of insects with paper disc (Jayakumar et al., 2017). Mortality was observed after 24, 48 and 72 hr exposure. Each treatment and control were replicated thrice. The repellent activity of botanical extracts was investigated by the method described by Talukdar and Howse (1994). The repellency treatment was replicated thrice and *S. oryzae* present on the treated and untreated halves was recorded at hourly interval from one to 24 hr. Data was expressed as % repulsion (PR) following the formula (Valsala and Gokuldas, 2015).

$$\% \text{ repellency } (\%) = \frac{NC-NT}{NC+NT} \times 100$$

Where, NC = % of weevils present in the control half, and NT = % of weevils present in the treated half. Based on % repellency, the concentrations were grouped into different classes. The repellency class 0 included 0.01-<0.1% repellency, 0.1-20 is grouped under class I, 20.1-40 is grouped under class II, 40.1-60 is grouped under class III, 60.1-80 is grouped under class IV and 80.1-100 is grouped under class V. All the experiments were done in completely randomized design (CRD). The % mortality and repellency determined was transformed to arcsine values and statistically analyzed for ANOVA using SPSS for windows (version 16) software. Grouping of data were done by using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results revealed that the all the botanicals exhibited toxicity against *S. oryzae*, while the treated check *Acorus calamus* 1% was efficient than the other treatments. The phytochemical recovery using hexane (non-polar solvent) from the selected botanicals ranged from 1.01 to 5.35%; leaves of *E. globulus* yielded the maximum of 5.35% followed by 3.43% in *C. longa* rhizome and 3.20% in *C. aurantium* leaves; *M. spicata* extract showed maximum contact toxicity (80.00%) after 72 hr of treatment, and it was followed by *V. negundo*, *O. sanctum* and *E. globulus* (70.00%) (Table 1). Earlier Kumar et al. (2009) reported that *M. arvensis* essential oil caused 100% contact toxicity at 200µl l^{-1} against *Callosobruchus chinensis*. Earlier reports on *V. negundo* showed that 6% acetone extract was toxic against *S. granarius* (72.22%) (Rahman et al., 2003), leaf aqueous extracts 10% against *C. chinensis* in black gram (Jayaraj et al., 2019) and admixture of *V. negundo* leaf powder 2% against *C. maculatus* (36.10%) (Manju et al., 2019). Hexane extracts of *Ocimum suave* caused mortality of *S. zeamais* (Opiyo, 2020) *E. globulus* oil caused mortality of *S. oryzae* at 2 µl/ 500 cm^3 (Patil et al., 2019).

Table 1. Toxicity of hexane extracts of certain botanicals against *S. oryzae* adults

Treatments	Mean mortality (%)			Insect repellency (%)							
	Contact toxicity @ 72 hrs	Oral toxicity @15 DAT	Fumigation toxicity @ 72 hrs	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr	Mean Repellency	Class repellency
C.aurantium 5%	63.33 (52.77)^{c}	73.33 (59.00)^{d}	66.67 (54.78)^{ab}	56.67 (48.84)^{bc}	50.00 (45.00)^{cd}	56.67 (48.84)^{cde}	63.33 (52.77)^{bc}	63.33 (52.77)^{bc}	63.33 (52.77)^{def}	58.89 (50.17)^{fg}	III
C. longa 5%	63.33 (52.77)^{c}	76.67 (61.21)^{cd}	60.00 (50.77)^{b}	50.00 (45.00)^{cd}	56.67 (48.84)^{bcd}	56.67 (48.84)^{cde}	63.33 (52.77)^{bc}	66.67 (54.78)^{bc}	66.67 (54.78)^{cde}	60.00 (50.83)^{efg}	III
E. globulus 5%	70.00 (56.99)^{c}	70.00 (56.99)^{d}	66.67 (55.06)^{ab}	60.00 (50.76)^{bc}	63.33 (52.77)^{bc}	66.67 (54.78)^{bc}	63.33 (52.86)^{bc}	66.67 (54.78)^{bc}	73.33 (59.00)^{bc}	65.56 (54.16)^{cd}	IV
L. camara 5%	66.67 (54.78)^{c}	76.67 (61.21)^{cd}	63.33 (52.77)^{ab}	53.33 (46.92)^{bcd}	56.67 (48.92)^{bcd}	46.67 (43.07)^{e}	60.00 (50.85)^{bcd}	56.67 (48.84)^{c}	63.33 (52.77)^{def}	56.11 (48.56)^{g}	III
M. spicata 5%	80.00 (63.43)^{b}	90.00 (71.56)^{b}	70.00 (56.79)^{ab}	63.33 (52.77)^{b}	63.33 (52.77)^{bc}	66.67 (54.78)^{bc}	70.00 (56.79)^{b}	70.00 (56.99)^{bc}	70.00 (56.79)^{bcd}	67.22 (55.15)^{bc}	IV
M. koenigii 5%	63.33 (52.77)^{c}	80.00 (63.92)^{bcd}	66.67 (54.77)^{ab}	36.67 (37.22)^{e}	50.00 (44.99)^{cd}	46.67 (42.99)^{e}	46.67 (43.07)^{d}	60.00 (50.85)^{c}	60.00 (50.76)^{ef}	50.00 (44.98)^{h}	III
O. sanctum 5%	70.00 (56.99)^{c}	86.67 (68.85)^{bc}	70.00 (56.99)^{ab}	63.33 (52.77)^{b}	70.00 (56.99)^{ab}	70.00 (56.79)^{b}	73.33 (59.00)^{b}	76.67 (61.21)^{b}	70.00 (56.78)^{bcd}	70.56 (57.25)^{b}	IV
R. communis 5%	66.67 (54.78)^{c}	73.33 (59.00)^{d}	66.67 (54.77)^{ab}	53.33 (46.92)^{bcd}	56.67 (48.84)^{bcd}	63.33 (52.77)^{bc}	70.00 (56.79)^{b}	66.67 (54.78)^{bc}	76.67 (61.21)^{ab}	64.44 (53.55)^{cde}	IV
T. erecta 5%	66.67 (55.07)^{c}	86.67 (68.85)^{bc}	63.33 (52.85)^{ab}	53.33 (46.92)^{bcd}	53.33 (46.92)^{cd}	60.00 (50.85)^{bcd}	60.00 (50.76)^{bcd}	70.00 (56.79)^{bc}	73.33 (59.00)^{bc}	61.67 (51.87)^{def}	IV
T. erecta 5%	60.00 (50.76)^{c}	73.33 (59.20)^{d}	70.00 (56.99)^{ab}	43.33 (41.15)^{de}	43.33 (41.15)^{d}	50.00 (45.00)^{de}	50.00 (44.99)^{cd}	56.67 (48.84)^{c}	56.67 (48.84)^{f}	50.00 (45.00)^{h}	III
V. negundo 5%	70.00 (56.79)^{c}	90.00 (71.56)^{b}	73.33 (58.99)^{a}	53.33 (46.92)^{bcd}	56.67 (48.84)^{bcd}	56.67 (48.84)^{cde}	60.00 (50.85)^{bcd}	70.00 (56.79)^{bc}	73.33 (59.00)^{bc}	61.67 (51.87)^{def}	IV
A. calamus 1% (Standard check)	100.00 (89.50)^{a}	100.00 (89.50)^{a}	93.33 (77.54)^{a}	83.33 (66.14)^{a}	80.00 (63.43)^{a}	90.00 (71.56)^{a}	86.67 (68.85)^{a}	93.33 (77.54)^{a}	100.00 (89.50)^{a}	88.89 (72.83)^{a}	V
Acetone (Control)	0.00 (0.66)^{d}	0.00 (0.66)^{e}	0.00 (0.00)^{c}	-	-	-	-	-	-	-	
Untreated check	0.00 (0.66)^{d}	0.00 (0.66)^{e}	0.00 (0.00)^{c}	-	-	-	-	-	-	-	
Mean	60.00	69.76	59.29	55.83	58.33	60.83	63.89	68.06	70.56	62.92	-
SED	2.95	3.58	4.10	2.49	3.68	3.32	3.88	3.84	2.47	1.33	

Figures in parentheses arcsine transformed values; In a column, mean followed by same letter not significantly different from each other; DMRT ($p \leq 0.05$); SED: Standard Error of the difference.

Extracts of *M. spicata* and *V. negundo* 5% showed maximum oral toxicity (90.00%) followed by *O. sanctum* and *T. erecta* leaf extract (86.67%) after 72 hr of treatment. Demetre et al. (2021) reported that 5% essential oil of *M. arvensis*, *O. sanctum* and *O. basilicum* showed 100, 99.00 and 97.0% mortality of *S. granarius*, respectively after 24 hr of treatment (Table 1). Oil from *V. negundo* leaves showed high overall toxicity to the adults of *S. zeamais* (Liu et al., 2009). Soujanya et al. (2016) showed that the *V.negundo* acetone leaf extract caused 73.30% mortality of *S. oryzae; O.sanctum* 2% recorded 36.50% mortality of *S.oryzae* (Deb et al., 2015); and methanol: hexane blend extract of *O. basilicum* was most effective against *S. zeamais* (Ouko et al., 2017). Bandana Bhau et al. (2010) reported that the admixture of *T. erecta* leaf powder caused 78.00% mortality of *S. oryzae* adults in rice. Maximum fumigant toxicity was exhibited by *V. negundo* 5% (73.33%) and it was statistically on par with the treated check *A.calamus* followed by *M. spicata*, *O. sanctum* and *T. erecta* flower extract (70.00%), after 72 hr of treatment (Table 1). Kathirvelu and Senthoor Raja (2015) reported that the *V. negundo* extracts caused 70.00% fumigant toxicity. Kathirvelu et al. (2012) found that the biotablets made of *V. negundo* performed better in causing mortality. The present results agree with those of Kathirvelu and Senthoor Raja (2015) who reported that the *M. piperita* acetone extracts caused 63.00% mean mortality and 70.00% mortality of weevils after 5th day of fumigation; *O. canum* and *O. basilicum* caused 80.00% mortality. Ouko et al. (2017) stated that the hexane extracts of *O. basilicum* had potent toxic activity against *S. zeamais*.

The results on the repellency of hexane extracts against *S. oryzae* showed that maximum repellency was found in *O. sanctum* 5%- 70.56% followed by *M. spicata* 5%- 67.22%. Srinivasan et al. (2003) reported that the *O. sanctum* extracts displayed 90.00% repellency against *S. oryzae* in stored maize. Kumar et al. (2009) observed that the repellent activity of *M. arvensis* was to an extent of 85% at 200µl l^{-1} against *C. chinensis* after 30 min of exposure (Table 1). These confirm the contact, oral and fumigant toxicity and repellent effect of *M. spicata, V. negundo*, *O. sanctum* and *E. globulus.* The contact toxicity due to *Mentha* spp. treatment is attributed to the presence of carvone, which was identified in our GC-MS-MS analysis (data not included). The contact toxicity due to *Mentha* spp. treatment is attributed to the presence of carvone identified in our GC-MS-MS analysis (data not included). Carvone were reported to present in *M. spicata* and possessed contact toxicity effect on *Tribolium castaneum* (Mansoori et al., 2020), and contact and fumigant toxicity on *S. zeamais* (Herreraa et al., 2014). Many studies have shown maximum toxicity of ketones against *Sitophilus* in contact and fumigant tests (Lee et al., 2003; Tripathi et al., 2003; Liu et al., 2011; Germinara et al., 2012). Present GC-MS-MS analysis revealed that *V. negundo* and *O. sanctum* possessed the bioactive principle β-caryophyllene, as shown by Liu et al. (2010). β-caryophyllene is responsible for the contact toxicity to *S. oryzae,* and the adult was most sensitive to it with the LD50 of 0.159 µl/cm^2 (Chaubey, 2012). Caryophyllene and germacrene D exhibited ovipositional suppression on *Callosobruchus maculatus* (Adebayo and Gbolade, 1994).

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