RESEARCH ARTICLE

Evaluation of Ethyl Acetate Extract of Some Botanicals against Rice Weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae) in Stored Maize

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ABSTRACT

In the present study, botanicals (11 nos.) were extracted using ethyl acetate solvent (mid-polar) and evaluated for their fumigant toxicity and repellent activity at 5% concentration against rice weevil, *Sitophilus oryzae* L. in comparison with *Acorus calamus*.The results revealed that all the botanicals were effective against *Sitophilus oryzae* compared to untreated control. *Mentha spicata* 5% ethyl acetate extract exhibited 83.33% fumigant toxicity after 72 hours of treatment with maximum repellency rate of 76.11%. *Ocimum sanctum* displayed 80.00% fumigant toxicity with the repellency rate of 76.11% and grouped under Class IV. *Vitex negundo* 5% treatment caused 83.33% fumigant toxicity, and it was statistically on par with the *M. spicata* and the repellency rate was 72.78 against *S. oryzae*. *Curcuma longa* caused effective repellency rate of 75.56% and was grouped under Class IV. Hence, it is concluded that 5% ethyl acetate extract of *M. spicata, V. negundo* and *O.sanctum* were toxic against *S. oryzae* in stored maize.

Keywords: *Sitophilus oryzae*; *Ethyl acetate extracts*; *Botanicals; Fumigant toxicity*; *Repellency*

INTRODUCTION

Plant metabolites can be classified into two main classes, primary and secondary metabolites. Primary metabolites are essential for the continued existence of the organism by which they are produced, but secondary metabolites mainly affect other organisms. Secondary metabolites include terpenoids, alkaloids, glycoside, phenols, tannins etc., which play a significant role in plant defense and cause behavioral and physiological effects on insects. Among them, terpenoids are the largest group of naturally occurring compounds, which are further subdivided into various classes; out of them, monoterpenes and sesquiterpenes are the main components (Batish *et al*., 2008). Plant vital compounds exhibit a wide range of biological activity against crop pests and may act as contact insecticides, oviposition deterrents, antifeedants, repellents, fumigants or they can influence the behavior and growth rate of insect pests.

Regarding *S. oryzae,* both grub and adults of rice weevils cause damage to the grain. They are internal feeders, and the entire development cycle occurs within the kernel. Adults can fly and easily distribute themselves throughout a storage facility because of their flight ability. They may also infest grain while the crop is still standing in the field, especially if the harvest is delayed and mild temperatures. Because of this fact, it is essential to inspect incoming loads for this pest when the loads are coming directly from the field. One pair of *S. oryzae* can reproduce about one million of its species within a period of 3 months under favorable conditions (Thomas *et al*., 2002). The prevention of losses in stored products due to insects is of paramount importance.

Thus, unless control measures are taken, heavy infestations may take place. Additionally, the kernel damage caused by *S. oryzae* grub enables other external feeders to infest the damaged grain; hence the damage increases rapidly. Further, it is high time to protect the grains in an ecofriendly way. It is the need of the hour in the present trend of livelihood to keep pace with our future generation to tackle storage problems. Keeping this in view, this study aims to focus on the utilization of plant metabolits as a bioinsecticide.

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MATERIAL AND METHODS

The experiment was conducted at Natural Pesticide Laboratory. Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai during 2019.

Test insects

The insect culture was maintained in laboratory at room temperature 30 ± 2 °C and 70±5% relative humidity. They were reared in the laboratory to obtain the first generation (F1) for use in bioassays. The maize seeds were dried in hot air oven at 40 °C for 48 hours to eliminate possible insect infestation from the field. About 200 numbers of adult weevils (mixed sex) were released in plastic jars (1lit capacity) filled with clean, undamaged and sterilized 500gms of whole maize seeds and covered with perforated plastic lids to allow gas exchange.

Procurement of raw materials for extraction of natural insecticides

Totally 11 botanicals were selected for evaluation in comparison with *Acorus calamus* as treated check, acetone as positive control and untreated check as a negative control. The plant extracts were obtained from the healthy, mature and succulent leaves/rhizomes/flowers of the selected plants. The leaves of *Eucalyptus globulus, Lantana camara, Murraya koenigii, Ricinis communis* and *Vitex negundo* were collected in and around the campus, *Tagetes erecta* leaves and flowers were collected from farmer's field, leaves of *Citrus aurantium* were collected from Thadiyankudisai, the rhizome of *Curcuma longa,* leaves of *Ocimum sanctum* and *Mentha spicata* were purchased from the local market. The collected samples were dried under shade at room temperature of 29 °C to 30 °C for 10 days and ground into fine powder using an electric grinder and sieved through a mesh sieve (0.1 mm pore size) to get uniform particle size. The leaf powders were kept separately in plastic containers in a dark place until used for extraction.

Preparation of Ethyl Acetate extracts

The 100g of dried powder was separately macerated in 1000 mL ethyl acetate (mid-polar solvent). Then the mixture was stirred for 2 hours in a magnetic stirrer at (2000 rpm) and left to stand for 24 hours. After 24 hours, the contents were carefully filtered through Whatman No.1 filter paper. The extract was concentrated using a rotary evaporator at 40 °C under reduced pressure to get the crude ethyl acetate extract. The residue obtained as weighed and stored in glass vials and maintained in a refrigerator (4 °C) until bioassays.

Fumigant toxicity

Five percent concentration of ethyl acetate extracts was prepared earlier by diluting with acetone, and filter paper discs of 1.5 cm diameter were impregnated with different treatments (2 μL/ cm^3) separately. The filter paper discs were allowed to evaporate the solvent for 2 min. After that, the filter paper discs were attached to the inner surface of the screw cap of glass vials (25 mLcapacity) separately. Ten numbers of 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. The cap of each vial was screwed tightly and kept at 28 ± 2 °C temperature. Mortality was observed after 24, 48 and 72 hours of exposure. Each treatment and control was replicated three times (Huang *et al*., 2000; Pandey *et al*., 2011; Jayakumar *et al*., 2017). Mortality was considered when the weevil did not respond to gentle pressure using a fingertip. Percentage insect mortality was calculated using the corrected formula of Abbott (1925).

Repellency test

The repellency effect of ethyl acetate extract of botanicals was tested by applying 1 mL of 5% concentration uniformly on one half of the filter paper and acetone was applied on another halves, which was used as an untreated check. A 9 cm diameter of filter paperwas cut into two halves and used for the experiment. Both the treated and untreated half circles were allowed to dry to evaporate the solvent. The entire disc of the treated and untreated half circles was remade by attaching with adhesive tape from the lower side and placed on the Petri dishes. Ten newly emerged adult weevils were released in the middle of the two halves and were covered with the lid. The inner side of the lid was coated with vaseline to prevent the weevil staying on the lid. The repellency treatment was replicated three times. *S. oryzae* present on the treated and untreated halves was recorded at every one-hour interval from one to twenty-four hours. The per cent repellent activity of plant extracts was investigated by the method described by Talukdar and Howse (1993, 1994). Data from all treatments was converted to express percentage repulsion (PR) by the following formula (Valsala and Gokuldas, 2015).

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

Where,

NC = Percentage of weevils present in the control half

NT = Percentage of weevils present in the treated half

Based on per cent repellency, the concentrations were grouped into different classes.

Class Repellency rate

Repellency Rate (%)	Class
0.01-<0.1	0
0.1-20	I
20.1-40	II
40.1-60	III
60.1-80	IV
80.1-100	V

Statistical analysis

The experiment was laid at Completely Randomized Block Design (CRD). The per cent mortality and repellency were determined, transformed to arcsine values and statistically analyzed by using SPSS software (version 16) to carry out analysis of variance (ANOVA). Grouping of data was done by using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results revealed that all the botanicals exhibited toxicity against *S. oryzae* compared to untreated check and acetone control, while the treated check *Acorus calamus* was efficient than the treatments.

Phytochemical recovery

The phytochemical recovery using ethyl acetate (Midpolar solvent) from the selected botanicals was found to range from 2.50 to 43.45 per cent (Table 1). Among the tested botanicals, the leaves of *Lantana camara* yielded the maximum of 43.45 per cent followed by 10.20 per cent in *C. longa* rhizome and 9.30 per cent in *R. communis* leaves.

Fumigant toxicity

The results on the fumigant toxicity of ethyl acetate extracts against *S. oryzae* is presented in Figure 1. The treated check *A.calamus* performed better and recorded the highest mortality (96.67%) of *S. oryzae* at 1% concentration 72 hours after treatment. Among the test botanicals, 5% of *M.spicata* and *V. negundo* 5% extracts showed more fumigant toxicity (83.33%) 15 days after treatment, followed by *O. sanctum* 5% (80.00%) 15 days after treatment.

The current results were corroborating with the findings of Ainane *et al*. (2019), who reported that the 2μL/cm^3 concentration of *M. spicata* essential oils caused 100% mortality, *M.piperita* caused 96.66% and *M.pulegium* caused 86.66% mortality against *S.granarius* and for *S.oryzae* the *M.spicata* caused 100% mortality, *M.pulegium* caused 96.66% mortality and *M.piperita* caused 90.00% mortality and for *S.zeamais*, the *M.spicata* and *M.piperita* caused 100% mortality and for *M.pulegium* showed 86.66% mortality after 24 hours of exposure.

Ainane *et al*. (2019) reported that the 1.5μL/cm^3 concentration of *M. spicata* oils showed 83.33% mortality *M.piperita* showed 76.66%, *M. pulegium* 70% mortality against *S. granarius* and for the *S.oryzae* *M. spicata* oil caused 53.33%, *M.piperita* 56.66% and *M. pulegium* 56.66% mortality after 24 hours of exposure. The average (1.5μL/cm^3) concentration of *M.spicata*, *M.piperita* and *M.pulegium* oils caused 23.33%, 50.00% and 50% mortality against *S. zeamais*. Demeter *et al*. (2021) reported that 5% of *M.arvensis* essential oils showed 100% mortality against *S.granarius*. and *O. sanctum* (99.00%) mortality against *S.granarius* at 24 hours after treatment and *O.basilicum* essential oils showed 97.70% mortality at 24 hours after treatment against *S.granarius*. El-Salam (2010) observed that 100% mortality of *C.chinensis* after 3 days exposure to *O.basilicum* EO at 1.0 mL/38.5mL air.

Table 1. Recovery of bioactive compounds from botanicals using ethyl acetate compounds

S. No.	Common Name	Scientific Name	Family	Plant Parts	Extraction recovery %
1	Sour orange	*Citrus aurantium* L.	Rutaceae	Leaves	2.62
2	Turmeric	*Curcuma longa* L.	Zingiberaceae	Rhizome	10.20
3	Tasmanian blue gum	*Eucalyptus globulus* Labill	Myrtaceae	Leaves	7.80
4	Big sage	*Lantana camera* L.	Verbenaceae	Leaves	43.45
5	Spear mint	*Mentha spicata* L.	Lamiaceae	Leaves	4.15
6	Curry tree	*Murraya koenigii* Sprengel	Rutaceae	Leaves	4.75
7	Basil	*Ocimum sanctum* L.	Lamiaceae	Leaves	5.75
8	Castor	*Ricinis communis* L.	Euphorbiaceae	Leaves	9.30
9	Marigold	*Tagetes erecta* L.	Asteraceae	Leaves	2.95
10	Marigold	*Tagetes erecta* L.	Asteraceae	Flower	2.51
11	Indian privet	*Vitex negundo* L.	Lamiaceae	Leaves	2.50

Saad *et al*. (2018) estimated that the LC_{50} value of eugenol against *S.oryzae* was >100µL/L air at 24-hour exposure. Shaaya *et al*. (1997) showed that the LC_{50} values of *O.basilicum* essential oil against *S.oryzae* was >15 µL/L air. Bhavya *et al*. (2018) who reported that the 479 µL/L air of *O.tenuiflorum* essential oil was required for 50% mortality for 24 hours after treatment.

Table 2. Repellency of ethyl acetate extract of different plant materials against adults of S. *oryzae*

Treatments	Repellency (%)						Mean Repellency (%)	Class Repellency
	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr		
T1-	56.67±0.00	63.33±5.77	70.00±0.00	66.67±5.77	63.33±5.77	73.33±5.77	65.56	IV
C.aurantium	(50.76)^{bcde}	(52.77)^{def}	(56.79)^{bcd}	(54.78)^{cd}	(52.77)^{c}	(59.00)^{b}	(54.48)^{de}	
T2-	63.33 ±5.77	73.33 ±5.77	76.67 ± 5.77	76.67 ± 5.77	83.33 ± 5.77	80.00 ± 0.00	75.56	IV
C.longa	(54.78)^{bc}	(59.00)^{bc}	(61.21)^{bc}	(61.21)^{bc}	(66.14)^{b}	(63.43)^{b}	(60.96)^{b}	
T3-	56.67±5.77	53.33±5.77	60.00± 10.00	60.00±0.00	70.00±0.00	73.33±5.77	62.22	IV
E. globulus	(46.92)^{de}	(46.92)^{f}	(50.85)^{d}	(50.76)^{d}	(56.79)^{bc}	(59.00)^{b}	(51.87)^{e}	
T4-	56.67±5.77	66.67±5.77	70.00±0.00	76.67±5.77	76.67±5.77	73.33±5.77	70.00	IV
L. camara	(52.77)^{bcd}	(54.78)^{cde}	(56.79)^{bcd}	(61.21)^{bc}	(61.21)^{bc}	(59.00)^{b}	(57.63)^{cd}	
T5-	63.33 ±10.00	73.33 ±5.77	80.00 ±0.00	80.00 ±10.00	83.33 ±5.77	76.67 ±5.77	76.11	IV
M. spicata	(56.99)^{b}	(59.00)^{bc}	(63.43)^{ab}	(63.92)^{ab}	(66.14)^{b}	(61.21)^{b}	(61.79)^{b}	
T6-	56.67 ±0.00	56.67±5.77	60.00±10.00	66.67±5.77	63.33±5.77	73.33±5.77	62.78	IV
M. koenigii	(45.00)^{e}	(48.84)^{ef}	(50.85)^{d}	(54.78)^{cd}	(52.77)^{c}	(59.00)^{b}	(51.87)^{e}	
T7-	60.00± 5.77	76.67±5.77	76.67±5.77	76.67±5.77	83.33±5.77	83.33±5.77	76.11	IV
O. sanctum	(54.78)^{bc}	(61.21)^{ab}	(61.21)^{bc}	(61.21)^{bc}	(66.14)^{b}	(66.14)^{b}	(61.79)^{b}	
T8-	63.33± 0.00	60.00 ± 0.00	70.00±0.00	70.00 ±0.00	63.33 ±5.77	73.33 ±5.77	66.67	IV
R.communis	(50.76)^{bcde}	(50.76)^{def}	(56.79)^{bcd}	(56.79)^{bcd}	(52.77)^{c}	(59.00)^{b}	(54.48)^{de}	
T9-	56.67±11.55	60.00±0.00	66.67± 5.77	66.67±5.77	73.33±5.77	76.67±5.77	66.67	IV
T. erecta	(48.93)^{cde}	(50.76)^{def}	(54.78)^{cd}	(54.78)^{cd}	(59.00)^{bc}	(61.21)^{b}	(54.91)^{de}	
T10-	60.00±5.77	60.00±10.00	60.00±10.00	60.00±0.00	63.33±11.55	73.33±5.77	62.78	IV
T. erecta	(46.92)^{de}	(50.85)^{def}	(50.85)^{d}	(50.76)^{d}	(52.86)^{bc}	(59.00)^{b}	(51.87)^{e}	
T11 -	60.00 ± 0.00	70.00 ± 0.00	76.67 ±5.77	73.33 ±5.77	76.67 ±5.77	80.00 ±0.00	72.78	IV
V. negundo	(50.76)^{bcde}	(59.79)^{bcd}	(61.21)^{bc}	(59.00)^{bc}	(61.21)^{bc}	(63.43)^{b}	(58.74)^{bc}	
T12- *A.calamus*	83.33±5.77	83.33±5.77	86.67±5.77	86.67±5.77	93.33±5.77	96.67±5.77	88.33	V
(Standard check)	(68.85)^{a}	(66.14)^{a}	(68.85)^{a}	(68.85)^{a}	(77.54)^{a}	(83.52)^{a}	(72.29)^{a}	
Mean	61.39	66.39	71.11	71.67	74.44	77.78		
SED	2.85	2.67	3.29	3.09	4.25	3.73		

*Mean values of three replications are represented as mean ± standard deviation; Figures in the parentheses are arcsine transformed values; In a column, the mean followed by the same letter are not significantly different from each other, DMRT ($p \leq 0.05$); SEd: Standard Error of the difference.

Abdelgaleil *et al*. (2016) reported that the *Vitex agnus-castus* essential oil caused 50% mortality at the concentration of 39.85 mg L^{-1} against *S.oryzae*. Sahaf *et al*. (2008) *V.pseudo-negundo* oil was toxic at the highest concentration of 925.9 µL/L air caused 100% mortality in 12 hours after treatment against *S.oryzae*. 370.4 µL/L air concentration caused 50% mortality at 12 hours of exposure and 90% mortality caused 24 hours of exposure, against *S.oryzae*.

Repellent activity

The results on the repellency effect of ethyl acetate extracts against *S. oryzae* is presented in Table 2. The treated check *A.calamus* performed better in causing the highest mean repellent effect 88.33% (class V) of *S. oryzae* at 1% concentration. Among the test botanicals, *M.spicata* and *O.sanctum* showed maximum mean repellency 76.11% (class IV), followed by *C.longa* 75.56% mean repellency, but statistically on par with the *M. spicata* and *O.sanctum followed* by *V. negundo* leaf extract showed (72.78%) mean repellency.

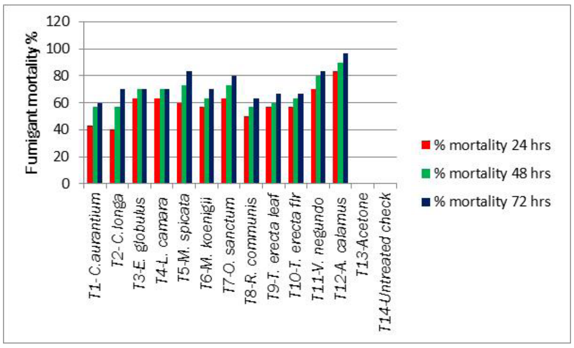

Figure 1. Fumigant toxicity of Ethyl Acetate extract of different plant materials against adults of S. oryzae.

The current results were corroborating with the findings of Khani *et al*. (2017), showed that *M. piperita* oil exhibited 95.0% repellency against *S.oryzae*. *M.spicata* oil showed 100% repellency at 0.025 µL/mL air concentration. Ouko *et al.* (2017) reported that the methanolic extracts of *O. basilicum* showed 72.5% repellent activity at 5th hour of exposure at 25% concentration, a slightly higher repellent effect of 82.5% at 50% concentration after 3 hours of exposure, and greater repellence activities of 80% at 75% concentration and 87.5% at 100% concentration of extracts, against *S. zeamais* after 1hour of exposure. Soujanya *et al*. (2016) reported that the *V.negundo* extracts showed that 67.00% repellency after 6 hrs of exposure.

CONCLUSION

Results suggested that the effectiveness of leaf extract of *M. spicata* against rice weevil(*S.oryzae.,* a major storage insect pest of cereals like rice, maize, wheat, sorghum and barley. *M.spicata* ethyl acetate extract was found to possess both insecticidal and repellency effects against *S. oryzae* and it can reduce the dependency on chemical pesticides against *S.oryzae* in stored maize.

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