REPELLENT AND ANTIFEEDANT ACTIVITIES OF EUPHORBIA THYMIFOLIA (LINN.) AND MANILKARA HEXANDRA (ROXB.) AGAINST RHYZOPERTHA DOMINICA (FAB.)

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ABSTRACT *Plants have been regarded as an alternative source of pest controlling agents as reported from several ethnobotanical studies. In the present study, the aqueous extracts of Euphorbia thymifolia (whole plant) and Manilkara hexandra (leaf) belonging to families Euphorbiaceae and Sapotaceae respectively, were evaluated for repellent and antifeedant activities against Rhyzopertha dominica adults. At the highest concentration of 10% and after 24 hours of exposure time, the aqueous extracts of Euphorbia thymifolia and Manilkara hexandra showed 83.33% and 93.33% repellency respectively while the extract of their combination exhibited the highest repellency of 96.67% against adults of R. dominica. In case of feeding deterrency, all the treatments achieved 100% feeding deterrency at the highest concentration of 10%. The effectiveness of all the extracts varied with concentration and produced a dose dependent impact on R. dominica. Thus, the selected plants were found to have the potential to be used as grain protectants.*

Keywords:*Repellent, antifeedant, Euphorbia thymifolia, Manilkara hexandra*

Introduction

Insect pests of stored grain are a serious problem throughout the world as they cause severe damage to a major portion of the farmers' crop production. One of the major primary pests of stored grain is the lesser grain borer Rhyzopertha dominica (F.) (Coleoptera; Bostrichidae). The insect is capable of flight and has a broad host range. It feeds internally on stored food products such as wheat, maize, rice, pulses, etc. due to high potential to infest stored products in both larval and adult stages. This pest causes qualitative and quantitative damage to stored products resulting in huge economic loss to farmers. It decreases the nutritive value of stored grain reducing their amino acid contents and make them unfit for human consumption. The control of this pest relies extremely on the use of synthetic chemical insecticides and fumigants, which increase the cost of application and cause toxicity to the users if precautions are not taken carefully. Uncontrolled application of pesticides or grain protectants in last some decades produced environmental and health hazards as well as resulted in the development of some resistant strains of pests. On the other hand botanical pesticides are bio-degradable, cause less or no toxicity to non target organisms, not hazardous to human health and environment. Unlike insecticides, which mostly kill insects, plant ingredients are known to suppress the feeding and breeding behaviour of insects in many ways in addition to direct mortality (Jilani, 1984).

Keeping in view the negative impact of chemical pesticides and the advantages of botanical pesticides, the present work was carried out to examine the repellent and antifeedant activities of Euphorbia thymifolia (Family:Euphorbiaceae) and Manilkara hexandra (Sapotaceae). Antifeedants, sometimes referred to as "feeding deterrents" are defined as chemicals that inhibit feeding or disrupt insect feeding by rendering the treated materials unattractive or unpalatable (Munakata, 1997; Saxena et al., 1988). The repellents are desirable chemicals as they offer protection with minimal impact on the ecosystem, as they drive away the insect pest from the treated materials by stimulating olfactory or other receptors (Rajashekar et al., 2012).

Material and methods

Rearing of Rhyzopertha dominica (Fab.)

The culture of R. dominica was collected from infested wheat grains and identified on the basis of their morphology under stereozoom microscope which was compared with the available literatures and web resources.Wheat grains (300 g) having no infestation were disinfected at 55˚C temperature in hot air oven for 3-4 hours followed by 10 days conditioning in laboratory and filled in a plastic container of one litre volume. Then 200 beetles were released into it and reared in the laboratory at 28 ^{o}C±1 ^{o}C, 60%±5% Relative

humidity.

Collection and authentication of the plants

Euphorbia thymifolia plant material was collected from the village, 'Nitardi', and Manilkara hexandra plant material was collected from 'Santosh nursery', Shujalpur located in Shajapur district, Madhya pradesh, during the month of December, 2016. Further plant materials were identified and voucher specimens were submitted in 'Herbarium', Department of Botany, Dr. Hari Singh Gour University, Sagar, M.P. and the registration numbers allotted to Euphorbia thymifolia and Manilkara hexandra specimens are Herbarium number P1 (bot/BG/201198) and P2 (bot//BG/201199) respectively. The plant materials were thoroughly washed under tap water and dried under shade at room temperature for about 15 days. The dried plant samples were powdered by mechanical grinder and sieved to give particle size 40- 100 mm. The powders were stored in polythene bags at room temperature before extraction.

Preparation of extract

Euphorbia thymifolia (whole plant) and Manilkara hexandra (leaves) dried and powdered materials (50 g each) were extracted with 'Hot continuous percolation method' (Soxhlet extraction) separately. The temperature was maintained at 70°C. The extraction was carried out using water as a solvent. The extracts were filtered through a paper filter (Whatman, No.1) and evaporated to dryness under reduced pressure by the rotary evaporator. The obtained crude extract was stored in dark glass bottles for further processing. For the experiments, the required concentrations were prepared by diluting the extracts in distilled water.

Evaluation of repellent activity

The repellent activity of aqueous extracts of Euphorbia thymifolia Linn. (whole plant) and Manilkara hexandra Roxb. (leaf) at different concentrations (2.5, 5.0 and 10.0 %) was evaluated separately and in combination (in equal proportion of 1:1 v/v) against stored grain pest Rhyzopertha dominica adults following 'Area Preference method' described by Obeng-Ofori et al. (1998) with some modifications. The test areas were 9 cm diameter filter-paper discs cut into half. Each extract (1ml) was uniformly applied to one half paper-discs while the other half disc was treated with distilled water only (control). The half-paper discs were then air dried for about 30 minutes to evaporate the solvent completely and full discs re-made by attaching the half-paper discs with adhesive paper tape. Each remade filter paper was fixed at the bottom of 9 cm diameter petri dish. Then, 20 adult insects (8-10 days old) were released at the center of the filter paper disc. Small amount of wheat flour was also introduced on both the sides to avoid the chances of death due to starvation. For the experiment, three replications for each treatment were used.

The number of insects present on the control (C) and the testing (T) parts of the filter paper was recorded after 24 h, 48 h and 72 h, respectively and percentage repellency was calculated by counting the insects in untreated half.

1.The value of percent repellency (PR) was calculated as follows:

$PR\ (\%) = [(C - T)/(C + T)] \times 100$

2. Class of % repellency, (Liu and Ho, 1999);

Class- 0: 0, >0.01- < 0.1%, Class-I: 0.1-20.0%, Class-II: 20.1-40%.0, Class-III: 40.1-60.0%, Class-IV: 60.1-80.0%, Class-V: 80.1-100.0%.

Evaluation of antifeedant activity

Antifeedant activity was studied as the method described by Keita et al. (2001) with some modifications. Wheat grains (10g) were treated with the aqueous extracts of Euphorbia thymifolia (whole plant), Manilkara hexandra (leaf) separately and in combination (in equal proportion of 1:1 v/v). For all the treatments, three different concentrations (2.5%, 5% and 10%) were used and control consisted of grains treated with distilled water only. Grains in the treated and the control part were air dried for about 1 hour to evaporate the solvent completely and filled into small plastic vials. Twenty adult Rhyzopertha dominica were introduced into each plastic vial which were then covered with fine cotton clothes held with rubber bands for proper ventilation and also to prevent the insects from escaping out. Three replications for each treatment were made. After 15 days of feeding period, grains were weighed, and weight loss was measured. The percentage of antifeedant index (A.I.) was calculated by this formula:

$\%A.I. = (C-T) \times 100/C$, where C is the mean weight loss in control group and T is weight loss in treatment groups.

Data analysis

Means and standard errors (SEM) were calculated from the data obtained from the experiments. One way analysis of variance (ANOVA) was then carried out followed by Tukey's Honestly Significant Difference

(HSD) post hoc test to compare and separate the means.

Results

Table:1 Percent repellency of plant extracts to R. dominica

Treatments	PR % Mean ±SE			
	Concentration	After 24 hours of exposure	After 48 hours of exposure	After 72 hours of exposure
Euphorbia thymifolia Linn. ($T_{1)}$	2.5%	50.00±5.77^{cde} (III)*	40.00±5.77^{cde} (II)	36.67±3.33^{def} (II)
	5%	60.00±5.77^{bcde} (III)	56.67±6.67^{bcde} (III)	53.33±3.33^{cdef} (III)
	10%	83.33±8.82^{abcd} (V)	73.33±3.33^{abcd} (IV)	66.67±3.33^{abcdef} (IV)
Manilkara hexandra Roxb. ($T_{2)}$	2.5%	70.00±5.77^{abcde} (IV)	66.67±8.82^{abcde} (IV)	60.00±10.00^{bcdef} (III)
	5%	83.33±6.67^{abcd} (V)	80.00±5.77^{abcd} (IV)	76.67±3.33^{abcde} (IV)
	10%	93.33±3.33^{abc} (V)	86.67±3.33^{abc} (V)	86.67±6.67^{abcd} (V)
Combination of T_1+T_2	2.5%	76.67±6.67^{abcde} (IV)	73.33±6.67^{abcd} (IV)	73.33±8.82^{abcde} (IV)
	5%	90.00±5.77^{abc} (V)	83.33±3.33^{abcd} (V)	86.67±8.82^{abcd} (V)
	10%	96.67±3.33^{abc} (V)	93.33±3.33^{abc} (V)	93.33±6.67^{abc} (V)

Values are expressed as Mean±SE (Standard error), obtained from three replications. Mean values followed by different letters within a column are significantly different ($p \leq 0.05$) from each other using ANOVA followed by Tukey's HSD post hoc test.

*Roman numbers in parenthesis are representing class of repellency.

Table:2 Percent antifeedant index of plant extracts against R. dominica

Treatments	Concentration	Mean weight loss (in g)	% Mean Antifeedant index
Euphhorbia thymifolia (T_1)	2.5%	0.78±0.05^{bc}	79.60±1.28^{defg}
	5%	0.54±0.05^{bcd}	85.85±1.25^{bcde}
	10%	0.00±0.00^{cd}	100.00±0.00^{a}
Manilkara hexandra (T_2)	2.5%	0.92±0.04^{bc}	76.04±1.08^{fg}
	5%	0.66±0.07^{bc}	82.90±1.96^{bcdef}
	10%	0.00±0.00^{cd}	100.00±0.00^{a}
Combination of extracts (T_1+T_2)	2.5%	0.69±0.04^{bc}	81.94±1.06^{cdef}
	5%	0.46±0.05^{bcd}	87.93±1.29^{bcd}
	10%	0.00±0.00^{cd}	100.00±0.00^{a}
Control		3.84±0.37^{a}	

Values in the table are expressed as mean ± SEM obtained from three replications. Mean values followed by different letters within a column are significantly different ($p \leq 0.05$) from each other using ANOVA followed by Tukey's HSD post hoc test.

Discussion

The results of repellent activity presented in table-1 revealed that all the treatments caused significant repellency at the highest concentration of 10% during all the exposure periods and maximum repellency was reported after 24 hours. No regular trend for variation in repellency was observed throughout on increasing exposure time whereas at all the exposure periods, repellency enhanced with increasing concentration of extracts. Similar observations were made by Jahromi et al. (2012) while working towards the repellent activity of the natural garlic emulsion found that maximum percent repellency was recorded at

highest concentrations and no significant effect of time factor was reported.

It is clear from table-2 that wheat grains treated with 2.5% and 5% aqueous extracts of Euphorbia thymifolia exhibited less weight loss as compared to Manilkara hexandra and less weight loss indicates high antifeedant activity. Maximum loss in weight of wheat grains was reported from the control treatment to which all the treatments differed significantly ($p \leq 0.05$). At the highest extract concentration of 10% all the treatments were equally effective in causing feeding deterrency. Overall, Manilkara hexandra was more effective in causing repellency against R. dominica as compared to Euphorbia thymifolia. On the other hand Euphorbia thymifolia showed better feeding deterrency than Manilkara hexandra. The plants in combination produced an additive effect than they produced individually.

Secondary compounds from plants include alkaloids, terpenoids, phenolics, flavonoid, saponins and other chemicals can affect insects in several ways. They may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, and antifeedants or modify ovipositor (Abbott, 1925). The qualitative and quantitative estimation of phytochemicals previously done by Sisodiya et al., (2017 & 2018) have revealed the presence of active principles in the aqueous extracts of Euphorbia thymifolia (whole plant) and Manilkara hexandra (leaf). Thus, it is clear from the obtained data that both the plants were effective as repellents and feeding deterrents to R. dominica which is due to the presence of bioactive phytochemicals called secondary metabolites. The active principles present in the plants inhibit insect feeding behaviour or make the food inedible for the insect resulting in feeding deterrence which leads to starvation and ultimately death of insect.

Conclusion

The use of plant products as insecticides in stored grain protection is found to be cost effective and eco-friendly. Compared to synthetic repellents and antifeedants, plant based products are assumed to be safer for human and are easily biodegradable. The selected plants have good potential to be used as stored grain protectants from the tested insect pest. The development of novel molecules from plants for the management of insect pests of stored grain is the need of the hour. Hence, the present study can be beneficial in serving as a guideline for developing effective measures to control Rhyzopertha dominica Fab.

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