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REPELLENCY OF THREE EXTRACT OF THREE INDIGENOUS PLANTS AGAINST ADULTS OF *Tribolium castaneum* HERBST (COLEOPTERA: TENEBRIONIDAE)

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Abstract

The present investigation was aimed to assess the impact of crude extracts of certain plants such as *Ocimum sanctum, Cardiospermum halicacabum, Mentha longifolia* was tested for their repellant activity against *Tribolium castaneum* in the laboratory condition. Among the extracts tested the ethanol extracts showed remarkable repellent activities than hexane and ethyl acetate at 200µg/ml. The finding of the present study indicates that the possible role of utilization of plant and plant associated products to control the coleopteran beetle T. *castaneum* as an alternate strategy to the chemical pesticides.

Keywords: *Ocimum sanctum, Cardiospermum halicacabum, Mentha longifolia* , Ethanol, Ethyl acetate ,*Hexane* ,*Tribolium castaneum*, Repellency.

Introduction

In South East Asian countries especially in India, grain storage loss by insects have been estimated to be 2.5% (Ahamed 1983). *Tribolium castaneum* (Herbst.) (Coleoptera : Tenebrionidae), has been observed as a common pest of stored wheat and other cereals all over the world. Since, both quality and quantity are seriously damaged by it (Hussain *et al.*, 1996). Lot of chemical pesticides are available today to control this insect pest are all causing serious side effects because of their long persistence and non-degradability. Hence, scientific community started search of safer chemicals as an alternative to the synthetic pesticides. Botanical insecticides are considered as alternatives the synthetic chemicals for being bio-degradable, target specific, non-hazardous to human health and environment and leaving no toxic residue in nature (periera and wholgemuth 1982). In order to keep these stored grain products free from pest attack, various synthetic chemicals have been used. Synthetic pesticides are currently the method of choice to protect stored grain from insect damage. but continuous or heavy uses of synthetic pesticides has created serious problems arising factors such as direct toxicity to parasites, predators, pollinators, fish and man. it also develops pesticides resistance (Zetter 1991) susceptibility of crop plant to insect pests (Piementel, 1977) and increased environmental and social cost.

Materials and Methods

Collection and extraction of plant material

A fresh leaf of the selected plants was collected in and around Bommidi Village (11°59′0″N 78°14′0″E), Dharmapuri District, Tamil Nadu, India. The plant materials were thoroughly washed with running tap water to remove particles and shade dried at room temperature (27±2◦C; R.H. 75±5%). After shade drying, the leaves were powdered by using electrical blender. 100 grams of the dry powder was sequentially extracted with 100 ml of hexane, ethyl acetate and ethanol. After 48 hrs the crude extracts were individually filtered using Whatmann No.1 filter paper in 500ml pre-sterilized amber bottle using a rotary vacuum evaporator, and then they were condensed to powder form by placing them in desiccators. The crude extracts were stored in refrigerator for further studies. The extracts obtained were dissolved in the corresponding pure solvent at the time of experimentation.

Collection of test organism

Stored product pest**,** *T. castaneum* was procured from the local market and warehouses from the infested gram and have been continuously cultured on the same food source. The stock culture was maintained in the laboratory in the dark atmosphere at 28 ± 2℃ and 70%-80% relative Humidity. The eggs were collected on black filter papers by placing T. *castaneum* adults on the filter papers after 48 h.

Bioassay

Repellency was assessed according to the method described by (Xie *et al.,*1995) with some modifications. A bioassay system consisting of 4 glass jars and 3 plants extract treatments, 1 negative control was connected together at their rims by means of 10 cm nylon mesh tube. A 5 cm diameter circular hole was cut at the middle of the mesh for introduction of the test insects. Twenty unsexed adult insects were introduced into the nylon mesh tube through the circular hole by means of 5 cm diameter funnel. Samples (100 g) of black gram was separately mixed with the individual extract and their mixture in the glass jars at concentrations of 12.5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml and 200µg/ml (0.5µl extract / sample grains) and kept at room temperature (28 ± 2^{o}C; 75± 5% RH) for 12 h so as to allow the solvent to evaporate completely. An appropriate amount of acetone was used as a negative control. Experiments were replicated five times. After 3 h, the contents of beetles at each treated or control diet was counted and the repellency (%) was calculated by following formula:

$$\text{Repellency (\%)} = \frac{\text{C} - \text{E}}{\text{T}} \text{X}100$$

Where,

C is the insect numbers in the negative control jar,
E is the insect numbers in extract treated jar and
T is the number of total insects.
C, E and T were the mean data of 5 replicates.

Results

Repellent activity of different solvent extracts of three plant extracts against the adult *T. castaneum*

Repellent activity of different solvent extracts of *Ocimum sanctum* was tested with different concentrations against the adult *Tribolium castaneum* and the data pertaining to the experiments are presented in table 1 and fig.1. Data clearly indicates that among the 12.5µg/ml concentration ethanol extract showed 66.22±4.22% repellent activity followed by 59.41±4.55and 56.43±4.77% repellent activity by ethyl acetate and hexane respectively. Whereas, at 25µg/ml ethyl acetate 65.78±3.37%repellent activity and ethanol extracts 73.47±6.44%repellent activity showed. Similarly, 79.36±7.0, 70.92±1.2 and 67.11±5.15% repellent activities were recorded at 50 µg/ml; 90.90±6.10, 82.64±6.5 and 75.95±6.07% repellent activities were recorded at 100 µg/ml and100.00±0.00, 96.15±3.0 and 96.15±8.44% repellent activities were recorded at 200 µg/ml of ethanol, ethyl acetate and hexane extracts respectively. Repellent activity of different solvent extracts of *Cardiosperumum halicacabum* was tested with different concentrations against the adult *Tribolium castaneum* and the data pertaining to the experiments are presented in table 2 and fig 2. Data clearly indicates that among the 12.5µg/ml concentration ethanol extract showed 65.70±2.92% repellent activity followed by 61.23 and 54.71% repellent activity by ethyl acetate and hexane respectively. Whereas, at 25µg/ml ethyl acetate and ethanol extracts were showed similar repellent activity (72.30%). Similarly, 81.10±5.55, 75.95±2.55, 70.05±5.75% repellent activities were recorded at 50 µg/ml; 90.09±5.14, 80.38±7.00, 81.10±2.90% repellent activities were recorded at 100 µg/ml and 100.00±0.0, 99.00±2.00 and 94.33±8.66% repellent activities were recorded at 200 µg/ml of ethanol, ethyl acetate and hexane extracts respectively. Repellent activity of different solvent extracts of *Mentha longifolia* was tested with different concentrations against the adult

Tribolium castaneum and the data pertaining to the experiments are presented in table 3and fig3. Data clearly indicates that among the 12.5µg/ml concentration ethanol extract showed 69.44±6.89% repellent activity followed by 61.23±3.11and 56.81±3.89% repellent activity by ethyl acetate and hexane respectively. Whereas, at 25µg/ml ethyl acetate 72.30±5.12 and ethanol extracts 75.64±5.44 showed similar repellent activity. Similarly, 82.64±9.77, 75.95±3.78 and 62.11±2.33% repellent activities were recorded at 50µg/ml; 89.12±5.00, 80.38±2.19 and 65.57±6.24% repellent activities were recorded at 100 µg/ml and 100.00±0.00, 99.00±1.90 and 76.33±8.72% repellent activities were recorded at 200 µg/ml of ethanol, ethyl acetate and hexane extracts respectively.

Discussion

The present study was investigated for repellent activity of three plant extract tested against *Tribolium castaneum*. Maximum repellency was recorded in ethanol extract of *Ocimum sanctum*, *Cardiospermum halicacabum* and *Mentha longifolia* at higher concentration level. Insect repellent activity has been found in many plant species (Tripathi *et al*., 2000; Sighamony *et al*.,1894; Scheare 1984). Several authors have reported that plant essential oils are potential alternatives to current stored grain pests because of their low toxicity to warm-blooded mammals and their high volatility (Shaaya *et al.*,1991; Shaaya *et al.*,1993; Li *et al.*, 2001). In the present study, it has been observed that the rate of repellant activity was increased with the increase with the concentration of the plant extract. Earlier, several authors have also reported that, the increased concentrations of the extract applied resulted with less damage in the stored grains (Amin *et al*. 2000). Abdul *et al*.,(2011) reported that the complete repellency (100% protectant) was only occured with *S.oryzae* when treated with the highest concentration (0.80 µg/cm2) of the plant extracts. Our study clearly reveals that the possible utilization of the chemicals present in the selected plant extract (s) could be used as a component in the Integrated Pest Management programme in the near future.

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Table 1: Repellent activity of different solvent extracts of *Ocimum sanctum* against the adult *Tribolium castaneum*

Concentrations tested (µg/ml)	Extracts tested		
	Hexane	Ethyl acetate	Ethanol
12.5	56.43±4.77 [a]	59.41±4.55 [a]	66.22±4.22 [a]
25	61.34±3.33 [b]	65.78±3.37 [b]	73.47±6.44 [b]
50	67.11±5.15 [c]	70.92±1.2 [c]	79.36±7.0 [c]
100	75.95±6.07 [d]	82.64±6.5 [d]	90.90±6.10 [d]
200	96.15±8.44 [e]	96.15±3.0 [e]	100.00±0.00 [e]

Values are mean ±S.D of five replications. Different alphabet in the columns are statistically significant (*p<0.05)*

Table 2: Repellent activity of different solvent extracts of *Cardiosperumum halicacabum* against the adult *Tribolium castaneum*

Concentrations tested (µg/ml)	Extracts tested		
	Hexane	Ethyl acetate	Ethanol
12.5	54.71±2.66 [a]	61.23±2.75 [a]	65.70±2.92 [a]
25	66.18±6.10 [b]	72.30±5.66 [b]	72.30±4.13 [b]
50	70.05±5.75 [c]	75.95±2.55 [c]	81.10±5.55 [c]
100	81.10±2.90 [d]	80.38±7.00 [d]	90.09±5.14 [d]
200	94.33±8.66 [e]	99.00±2.00 [e]	100.00±0.0 [e]

Values are mean ±S.D of five replications. Different alphabet in the columns are statistically significant (*p<0.05)*

Table 3: Repellent activity of different solvent extracts of *Mentha longifolia* against the adult *Tribolium castaneum*

Concentrations tested (µg/ml)	Extracts tested		
	Hexane	Ethyl acetate	Ethanol
12.5	56.81±3.89 [a]	61.23±3.11 [a]	69.44±6.89 [a]
25	59.80±5.08 [b]	72.30±5.12 [b]	75.64±5.44 [b]
50	62.11±2.33 [c]	75.95±3.78 [c]	82.64±9.77 [c]
100	65.57±6.24 [d]	80.38±2.19 [d]	89.12±5.00 [d]
200	76.33±8.72 [e]	99.00±1.90 [e]	100.00±0.00 [e]

Values are mean ±S.D of five replications. Different alphabet in the columns are statistically significant (*p<0.05)*

Figure 1: Repellent activity of different solvent extracts of *Ocimum sanctum* against the adult *Tribolium castaneum*

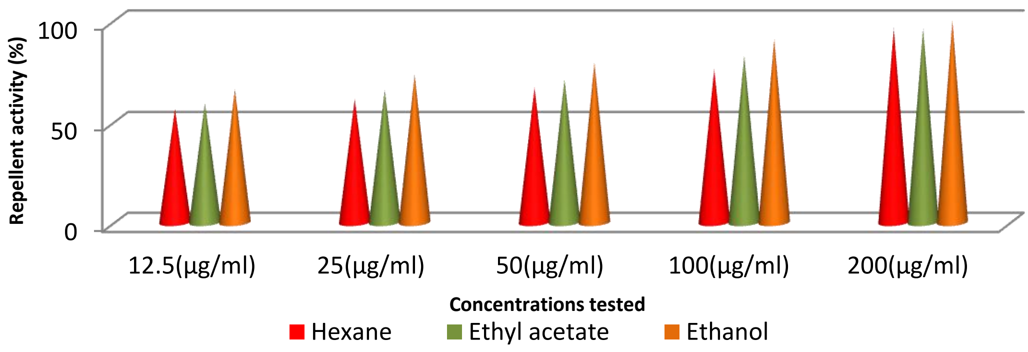

Figure 2: Repellent activity of different solvent extracts of *Cardiospermum halicacabum* against the adult *Tribolium castaneum*

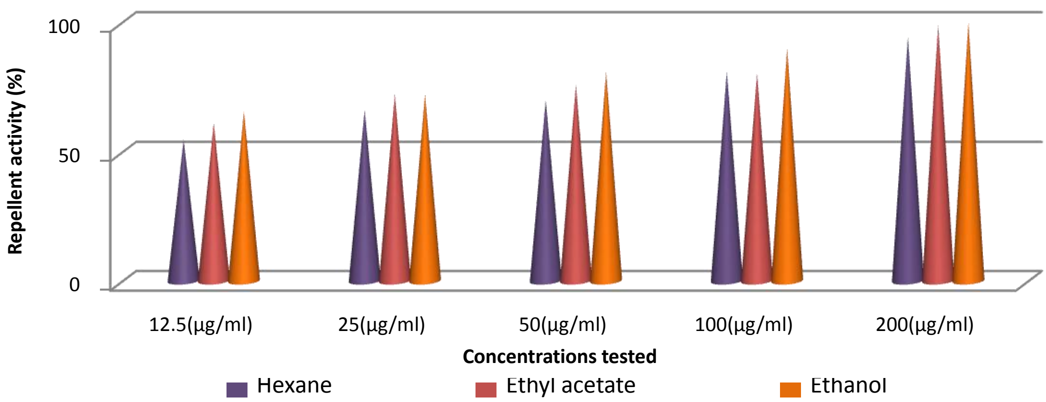

Figure 3: Repellent activity of different solvent extracts of *Mentha longifolia* against the adult *Triboliumcastaneum*