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Laboratory evaluation of herbal extracts for a pest of stored foodstuffs: *Tribolium castaneum*: Coleoptera (Tenebrionidae)

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Abstract
Plant materials such as essential oils and extracts are useful pest control tools. Damage of stored commodities by the bruchid beetles are of great concern in India. The objective of this study, therefore, was to determine promising herbal method for replacing chemical pesticides in pest control programs without affecting the quality. Experiments were carried out to evaluate the toxicity herbal plants against red flour beetle. The results showed that herbal extracts *Ricinus communis* leaf extract, *Eucalyptus globulus* leaf extract and *Citrus reticulate* peel extract against the larvae and adults. These herbal extract shows a strong to moderate toxicity at a different concentration on bruchid beetle *Tribolium castaneum. R. communis* leaf extract outperformed all treatments during 7 days of exposure (mortality rate 90.9% at 10% concentration and 45.5% at 2.5% concentration, respectively).The cross linked *Citrus* extract also has significant insecticidal activity.

Keywords: essential oil, herbal extract, management, mortality, insecticidal activity

Introduction
Agricultural cultures are the most important food source for millions of people around the world. Methyl bromide and phosphine were two of the main fumigants used against storage pests worldwide [1, 2] but ultimately even highly concentrated phosphine formulations failed to kill insects as storage pest resistance developed in some locations in India and Australia. The control of these insects mainly depends on the continuous application of synthetic insecticides which, in addition to direct toxicity, result in environmental disturbances, increased application costs, pest resurgence, pest resistance, and lethal effects on non-target organisms. [3] Therefore, the risks associated with using these products have led to the development of environmentally friendly alternatives. Over the past two decades, agrochemical companies have made strides in studying natural products for the development of new insecticides. Evidence of this is the number of organic plants. Therefore, naturally derived insecticides are being proposed as reasonable alternatives to synthetic ones and are rapidly increasing among the biopesticides in the botanical pesticide markets. [4, 5] In this context, plant studies are invested in the isolation or identification of secondary metabolites that may have an insecticidal, repellent or anti-taste effect against insects, and therefore some research has shown that extracts botanicals have several properties related to it allow them to consider them as biopesticides [11-13] in alternative strategies aimed at limiting the use of synthetic pesticides; Since these biopesticides are biodegradable [6], they are considered products with a low environmental impact. The diversity in its composition and mix of compounds enhances its insecticidal effectiveness and reduces the development of tolerance and resistance to these products [7]. Therefore, in the present studies, the effectiveness of some native plant extracts and powders, i.e. Citrus peel, castor bean leaves and eucalyptus leaves tested against mealworms.

Materials and Methods
Plant materials and sampling
Fruit peel of Kinnow (*Citrus reticulata*), Castor leaves (*Ricinus* communis) and *Eucalyptus globulus* leaves were collected from different parts of Jodhpur district and dried in shade at room temperature for studying their insecticidal activity against *Tribolium castaneum.*

Preparation of herbal extract
Soxhlet equipment was used for making ready the extract of the assorted pulverized leaves/peel.15 g powder (Figure 1) of every plant leaves/peel was unbroken within the thimble and extraction was administrated by victimization 200 ml of dissolving agent as a solvent during a spherical bottom flask that was heated with electrical heating mental [8]. Dissolving agent was gaseous by employing a water bathtub at 60°C till solely solid crude extracts were left in the beakers. These solid extracts were thought-about to be one hundred pc pure active

ingredients and their stock solutions were ready by dissolving them in acetone to form desired concentrations tested in the experiments. [9] The extracts were keeping at 4°C within the icebox before application.

Fig 1: Plant powder used against test insect *Tribolium castaneum*

Insect bioassays

Laboratory Maintenance of the Experimental Insect

The adult arthropod genus castaneum was collected from troubled grains purchased from an area market associated dropped at the laboratory. The culture of red flour beetle, T. castaneum was raised on the sterilized bajra floor in a setup at 25±1°C temperature and 70±5% ratio at the laboratory of Lachoo Memorial College of science & technology (Autonomous), (Figure 2) Jodhpur Rajasthan. The jars were lined with muslin, secured with an elastic to forestall the insect' escape. The insects were kept in the jars (Figure 3) for about 3 to 4 d to lay eggs. After oviposition, the insects were transferred to new jars of wheat flour using a camel hair brush to lay more eggs and then the flour combined with that in the original jar. Complete emergence of adult beetles was achieved when 30-35 d and these adults were employed in toxicity bioassays.

Fig 2: BOD incubator to study various levels of temperature & RH under laboratory condition

Fig 3: Stock culture maintenance of *Tribolium castaneum* under laboratory condition

Mortality tests

This test carried out against adults, adopting the methodology used by Khani [10] method with slight modifications: 12 grams of crushed Bajra grains were taken in 90 mm Petri plates with small holes for aeration purposes. The different concentrations of each plant extracts [14-16] were prepared: 2.5%, 5%, 7.5% and 10% using acetone (Figure 4). After treatment, all the petri plates were kept in an incubator at room temperature to provide storage like conditions. The mortality of insects was observed at every 48hr intervals upto 15 days.

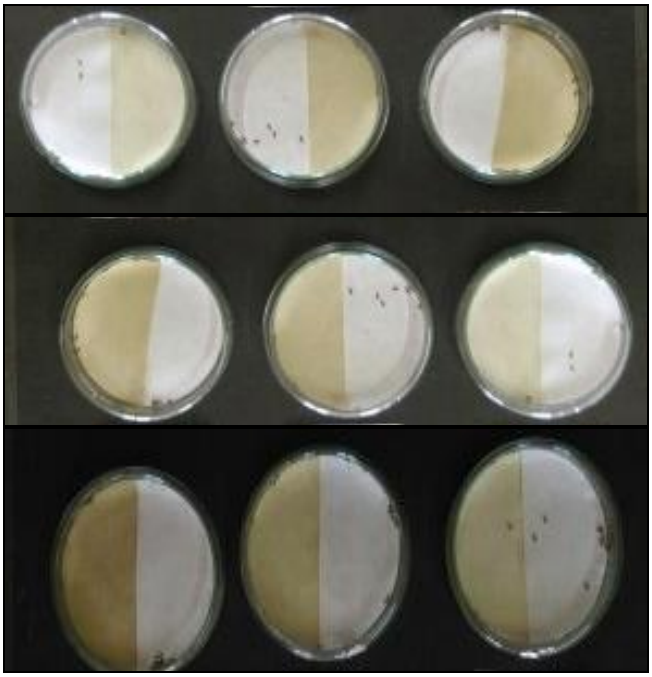

Fig 4: Different concentrations of herbal extract (2.5%, 5%, and 10%) using acetone against *Tribolium castaneum*

Statistical Analysis

The mortality data was corrected by using Abbott's formula and subjected to probit analysis. The LC_{50}, LC_{90}, and LT_{50} values of each tested sample were calculated statistically through the respective probit regression equation.

Results

The different concentrations of the leaf extracts of Ricinus communis (2.5%, 5%, 7.5%, and 10%) showed potent insecticidal activity against adult Tribolium castaneum compared to the control (Table 1). The leaf extract at a concentration of 10% induced mortality of 90.9%. and 45.5% test insect death was observed at a concentration of 2.5%, indicating an increase in mortality with increasing concentration. Adults of *Tribolium castaneum* mortality were directly proportional to the concentrations (2.5%, 5%, 7.5%, and 10%) of citrus reticulate peel extract during a 7-day exposure (Table 2). The highest concentration (10%) caused 96.3% mortality, while 2.5% of the dose caused very low mortality (25.9%) at day 7 post-dose. The LC50 and LC95 values were 3.92% and 10.72%, respectively, over a 1-week exposure in adults. The insecticidal effects of *Eucalyptus globulus* leaf extract against Tribolium castaneum after 9 days exposure (Table 3) are concentration-dependent and induced the highest mortality of 96.2%. at a concentration of 10%, followed by mortality of 71.7%, 50.9% and 34% at doses of 7.5%, 5% and 2.5%, respectively.

Table 1: Toxicity of *Ricinus communis* extract against *Tribolium castaneum* after 7 days of exposure

Label	Concentration (%)	Total no. of insects	No. of insects killed	Mortality (%)	Corrected mortality	Log Concentration (x)	Probit
C1	2.50	60.00	30.00	50.00	41.5	0.40	4.89
C2	5.00	60.00	39.00	65.00	61.8	0.70	5.30
C3	7.50	60.00	49.00	81.67	80.0	0.88	5.84
C4	10.00	60.00	55.00	91.67	90.9	1.00	6.34
Control	0	60.00	5	8.33			

Regression Equation: y = 2.3495x + 3.8452

Chi Square: 1.986; LC50: 3.066%; LC95: 16.706%

Table 2: Toxicity of *Citrus reticulata* extract against *Tribolium castaneum* after 7 day exposure

Label	Concentration (%)	Total no. of insects	No. of insects killed	Mortality (%)	Corrected mortality	Log Concentration (x)	Probit
C1	2.50	60.00	20.00	33.00	25.9	0.40	4.35
C2	5.00	60.00	38.00	63.00	59.3	0.70	5.23
C3	7.50	60.00	53.00	86.67	85.2	0.88	6.04
C4	10.00	60.00	58.00	96.67	96.3	1.00	6.79
Control	0	60.00	6	10			

Regression Equation: y = 3.9583x +2.6638
Chi Square: 2.092; LC50= 3.923%; LC95: 10.72%

Table 3: Toxicity of *Eucalyptus globulus* extract against *Tribolium castaneum* after 9 days of exposure

Label	**Concentration (%)**	**Total no. of insects**	**No. of insects killed**	**Mortality (%)**	**Corrected mortality**	**Log Concentration (x)**	**Probit**
C1	2.50	60.00	25.00	41.67	34.0	0.40	4.59
C2	5.00	60.00	34.00	56.67	50.9	0.70	5.02
C3	7.50	60.00	45.00	75.00	71.7	0.88	5.57
C4	10.00	60.00	58.00	96.67	96.2	1.00	6.78
Control	0	60.00	7	11.67			

Regression Equation: y = 3.2973x + 3.0405
Chi Square: 10.939; LC50: 3.999%; LC95: 15.149%

Discussion

The botanical extracts of plants can influence the most important metabolic, biochemical, physiological and behavioral functions of insects and concluded that it is the possibility to use essential oils or its main components as ecologically secure nature resources against stored bass pests and products of its facility [17-19]. The results of *Eucalyptus globulus* leaf extract are in close agreement with the findings of that evaluated 7 different plant extracts and found that *Eucalyptus globulus* possesses insecticidal properties against *Tribolium castaneum*. Similarly, *Eucalyptus globulus* essential oil was found to be toxic to Corcyra cephalonica larvae. Toxic effects of Eucalyptus leucoxylon essential oil have also been shown by against T. confusum and T. cateneum and against T. confusum. From current research, it is clear that phytochemicals are likely to be the future has substitutes for chemical insecticides in controlling pests in stored grain. Also concluded that botanical extracts might show promise to protect stored grain from beetle pests.

Conclusion

After analyzing the present investigations, it is hereby concluded that the extracts of *Ricinus communis*, *Eucalyptus globulus*, and *Citrus reticulata* fruit peel possess significant insecticidal activity and have the potential to be used as an alternative in granaries against *Tribolium castaneum* and possibly against other stored grain insect pests. The extract of *Ricinus communis* leaves is the best among the plants evaluated in present studies and can be employed as an integral part of integrated pest management strategies to reduce the harmful impacts of the chemical pesticides. Considering the insecticidal activity and abundant availability of castor in wild rural and urban areas there is a great potential for further investigation and practical use in pest management programs. [20-21].The Citrus fruit peel can also be made a part of integrated pest management as it will be helpful in reducing the fruit peel waste which is capable of polluting the environment. Additionally, know plant is extensively grown and relished in sub-tropical India therefore, there will always be a high availability of the fruit peel.

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