REPELLENT ACTIVITY OF SOME SELECTED AROMATIC PLANT EXTRACTS AGAINST RUST-RED FLOUR BEETLE, TRIBOLIUM CASTANEUM (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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Abstract

In recent year's botanical pesticide have received increasing attention in developing countries due to continued damaging of grains associated to post harvest insect pest. Sustainable agriculture through better ecological approaches in pest management is the stimulus for further research in alternate pesticides against conventional pesticides, which lead to increased research in plant sources. Hence in the present study, Methanol based plant extracts of *Cinnamomum tamala*, *Rosmarinus officinalis and Pelaragonium graveolens* was assessed against the stored product pest (*Tribolium castaneum*) of processed food commodities. Periodic analysis for the repellence was done by impregnating half-filter paper disc at various concentrations (2.5, 5, 10 %). The experimental trials showed significant repellent effects at 24 h exposure of *Cinnamomum tamala* (98.5±3.78 %) followed by *Pelaragonium graveolens* (96.4±6.43 %) and *Rosemary officinalis* (94.6±8.3 %). Overall, the *Cinnamomum tamala* extracts were proved to be most effective repellent followed by *Pelaragonium graveolens*. Results suggest a safer potential of these natural extracts towards disturbing the biology and invasion of stored product pest, *T. castaneum*. Results also indicate a definite potential of these extracts towards incorporation of these extracts in pest management programs and towards optimizing food security through utilizing them as bio-pesticides.

Keywords: Cinnamomum tamala, Rosmarinus officinalis, Pelaragonium graveolens, Tribolium castaneum, bioactivities, contact toxicity, repellency.

1. Introduction

India is a storehouse of diversified green wealth where enormous varieties of vegetables, cereals, oil seeds, pulses and horticultural plants are available. It has this multiplicity because of its different climatic conditions found among separate geographic regions. The presence of such diversified plants results in a variety of its natural enemies. Stored foods are destroyed by different groups of insect pests, especially by beetles, moths and mites (Rajendran and Sriranjini, 2002).

Among them, red flour beetle *Tribolium castaneum* Herbst (Coleoptera Tenebrionidae) is one of the most widespread and destructive pests of stored products, feeding on different stored-grain and grain products (Mishra *et al.*, 2012 a; 2012 b and Weston and Rattlingourd, 2000). Rust-red flour beetles have an extremely large appetite for a variety of foods, such as food products stored in soils, warehouses, grocery stores, and houses including meal, crackers, beans, spices, pasta dried pet food, dried flowers, chocolate, nuts and seeds, and even dried museum specimens (Via, 1999 and Weston and Rattlingourd, 2000). Also, they are particularly abundant in cereal products, in wheat and flour (Aitken, 1975; Hodges *et al.*, 1996). When they occur in large number, red flour beetles secrete a chemical mixture that includes quinones, which are carcinogenic, thereby affecting product quality (Hodges *et al*., 1996).

Protecting stored grain and seeds against insect pests are one of the major challenges in post-harvest processes. These stored commodities are vulnerable to insects attack and the quality of food is deteriorated. It is necessary to conserve the stored food grains reserves so that the supply food remains continuous and the prices of food grains and derived products remain stable (Jahromi *et al*., 2014: Nadeem *et al*., 2012 and Talukder, 2006). Loss in weight and germination ability of grains is a severe problem, especially due to pitiable sanitation along with poor storage facilities that encourage stored pests attack, disease causing organisms and increase in temperature and humidity of the stored products (Keskin and Ozkaya, 2013; Padin *et al*., 2013; Phillips and Throne, 2010; Semeao *et al.,* 2012 and Upadhyay and Ahmad, 2011).

Since stored-grain insect pests become widespread throughout the world through human activity and seed transportation, they are considered to have evolved adaptations to different stored foodstuffs. Generally, the control of this pest species relies on chemicals, fumigants, phosphine and residual grain protectants. However, fumigation, by far the most effective method of grain and grain product disinfestations, that has serious limitations (Mills, 1983; Bell and Wilson, 1995; Bell, 2000; Caddick, 2004). Nowadays, incremental necessity has been occurred to find out alternatives to chemicals. Biological control is a novel method to replace chemicals. Bio pesticide based products could help in crop protection thereby increasing crop yields. For these reasons, development of new novel bio pesticides has continued to increase rapidly since mid-1990s. In short, bio pesticides play a vital role in crop protection, meeting societal needs and sustaining the Earth.

Therefore, the present study was conducted to investigate the repellency potential of methanolic extracts of leaves of *Cinnamomum tamala*, *Rosmarinus officinalis, Pelaragonium graveolens* against the rust-red flour beetle- *T. castaneum*.

2. Materials and Methods

Experiments were performed at Department of Zoology, Bharathiar University, Coimbatore during the year 2016 -2017.

2.1. Plant Materials Collection

The plant materials were collected in and around Mathuramalai Hills of Coimbatore (11.0168ºN latitude, 76.9558º E longitude) and Kodaikanal Hills, Tamil Nadu. (10.2381º N latitude, 77.489º E longitude). The scientific name and family of each plant are given below in the Table 1 and shown in the Fig.1,2 and 3.

Table 1. List of plants and their family and the parts used for Powder

S.No	Scientific Name	Family	Parts used
1	*Cinnamomum tamala*	Lauraceae	Leaves
2	*Rosmarinus officinalis*	Lamiaceae	Leaves
3	*Pelaragonium graveolens*	Geraniaceae	Leaves

Fig. 1. Leaves and Powder form of *Cinnamomum tamala*

Fig. 2. Leaves and Powder form of *Rosmarinus officinalis*

Fig. 3. Leaves and Powder form of *Pelaragonium graveolens*

2.2. Insect Collection

Collection of test insect was done from the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Collection of the food samples were done in the go downs and grain shops of Coimbatore. The collection was made after on the spot sieving of infested food commodities and shown in fig. 4

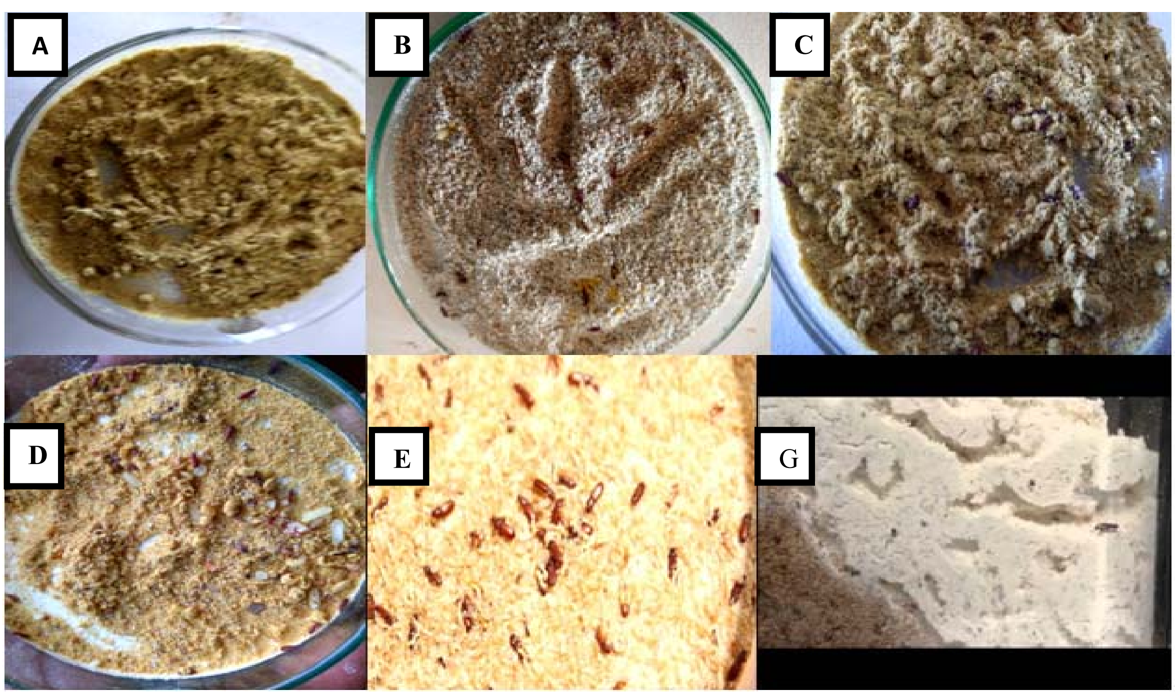

Fig.4. Various food stuffs infested with *Tribolium castnaeum* (A) Coriander powder (B) Finger millet (Ragi) flour C) Pearl millet flour D) Wheat grains E) Sorghum flour F) Rice Flour

2.3. Insects Rearing

The red flour beetles were maintained in the dark cabinet of an incubator at 28 - 30^{0}C and 60 -70 % relative humidity. The insects were reared in glass containers (0.5 L) containing sterilized wheat flour at 12-13 % moisture content mixed with yeast (10:1, w/w). Insects used in the experiments were about 15 days old adults. Adult beetles were released for egg laying and after 5 days removed from the flour through sieving. The eggs were allowed to hatch and develop under uniform conditions. The homogenous population was achieved after 25 - 35 days as given by Islam and Talukder (2005) and it is shown in fig.5.

Fig.5. Plate showing the homogenous population of *Tribolium castaneum* after 20 days

2.4. Extract Preparation

Grinding of plant material was done in an electric mixer. The dried powered leaves (500 g) of *Cinnamomum tamala, Rosmarinus officinalis* and *Pelaragonium graveolens* was percolated using 95 % methanol solution (1000 ml) in a closed container on a orbital shaker at 120 rpm for 48 h. It was filtered and the filtrates was then concentrated at reduced pressure to dryness using rotary evaporator at 45^{o}C (Sagheer *et al*., 2013). The solvent was evaporated under reduced pressure to obtain the crude extract (105.0 g).

2.5 Experiment for percent repellency

The repellent activity of different concentrations (2.5, 5 and 10 %) of *Cinnamomum tamala*, *Rosmarinus officinalis* and *Pelaragonium graveolens* against *T. castaneum* adults was evaluated using the area preference method (Zhang *et al*., 2011 and C-x You *et al*., 2017). The filter paper (10 cm in diameter) was cut into two equal pieces. One piece was uniformly treated with 500 µL of testing solution as a testing part, and the other piece was dealt with 500 µL of methanol as a control part. The filter papers were air dried for about 1 hour to evaporate the solvent completely, and full discs were subsequently remade by attaching testing part to control part with cello tape. Each remade filter paper was tightly fixed on the bottom of a 10 cm diameter Petri dish side by side. For each test, ten adults (20 days old) insects were released at the center of the filter paper disc which was then covered with a lid to avoid the escape of insects. Small amount of insect diet (wheat flour) was also introduced on both the sides to reduce the chances of death due to starvation factor. Each tested concentration was replicated five times and the experiment was repeated three times. The number of insects presented 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. The obtained data were statistically analysed for one way ANOVA using software Statistica 7.0.

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

PR (%) = [(C − T)/(C + T)] × 100

2. Index of repellency (IR) was calculated by following formula:

IR = 2T / T + C (Mazzonetto, 2002).

The repellency index was classified as:

IR: values <1 repellency; 1 neutral; >1 attractant

3. Results

The repellent activity of methanol extract of three different plants (*Cinnamomum tamala, Rosmarinus officinalis* and *Pelaragonium graveolens*) were measured and results are presented in Table 2, 3 and 4 and it is shown in fig.6 and 7.

The concentration tend to affect the repellency as highest was noted in case of *Cinnamomum tamala* (95.85 ± 2.76 %) while the lowest was recorded for *Rosmarinus officinalis* (89.81 ± 4.70). The concentrations have a definite impact on repellent action because of increase in active metabolites present in the extracts. The PR values showed that the plant extracts exhibited various repellent activities against *T. castaneum*. It was found that *C. tamala* exhibited the significant repellent activity against *T. castaneum*. For example, at the concentration of 10 %, the PR value of *C. tamala* was 98.64 at 24 h after exposure, comparable to that of the control. At relatively lower concentration of 2.5 %, the PR value of *C. tamala* was higher than that of the control, methanol at 24 h after exposure and the PR values of 5 % concentration of *R. officinalis* and *P. graveolens* were higher than that of the control at 24, 48 and 72 h after exposure. Index of repellency was calculated for plants samples tested and was found to be 0.14, 0.29 and 0.86 respectively for *C. tamala, P. graveolens* and *R. officinalis.* The control value was greater than 1 value which was considered to be an attractant.

Table 2. Repellent effect of three plant extracts at three concentrations (2.5, 5 and 10 %) against *T.castaneum* at 24 h exposure

Repellency % ± Standard Error			
Concentrations (%)	***Cinnamomum tamala***	***Rosmarinus officinalis***	***Pelaragonium graveolens***
2.5	91.50 ± 3.19	85.25 ± 2.49	90.37 ± 4.82
5	93.03 ± 5.75	87.55 ± 3.68	92.41 ± 2.46
10	95.85 ± 2.76	89.81 ± 4.70	94.22 ± 5.19

Table 3. Repellent effect of three plant extracts at various exposure periods (24, 48 and 72 h) against *T.castaneum at* 10 percentage concentration

	Repellency % ± Standard Error			
Time h	**Control**	***Cinnamomum tamala***	***Rosmarinus officinalis***	***Pelaragonium graveolens***
24	10.6 ±2.3	97.00 ± 3.19	94.83 ± 5.75	95.92 ± 2.76
48	0.00	92.37 ±5.21	88.65 ± 3.68	93.01 ± 3.70
72	0.00	96.67±8.7	86.5±6.2	91.52 ± 4.19

Table 4. Mean comparison of the percent repellency of crude extract of different plant extract against *T. castaneum* as a function of time and concentrations

Name of the plant	**PR % Mean ±SE**								
	Treatment with plant extracts at 24 h in different concentration (%)			**Treatment with plant extracts at 48 h in different concentration (%)**			**Treatment with plant extracts at 72 h in different concentration (%)**		
	2.5 %	**5 %**	**10 %**	**2.5 %**	**5 %**	**10 %**	**2.5 %**	**5 %**	**10 %**
Cinnamomum tamala	90.3±2.31	92.5±5.76	98.5±.3.78	84.5±.89	86.9±7.54	93.6±3.65	82.65±6.87	88.2 ±5.66	96.67±8.7
Rosmarinus officinalis	86.5±6.63	91.5±6.82	94.6±3.86	82.8±3.21	84.7±6.43	87.6±3.24	78.4±5.85	84.8±6.57	89.5±6.22
Pelaragonium graveolens	89.3±7.52	93.7±8.36	96.4±6.43	83.6±7.81	85.5±3.92	92.6±5.52	80.3±3.94	87.9±6.49	90.6±2.84

Mean values (± SE) of five replicates of three trials having 10 insects each. $P \leq 0.01$.

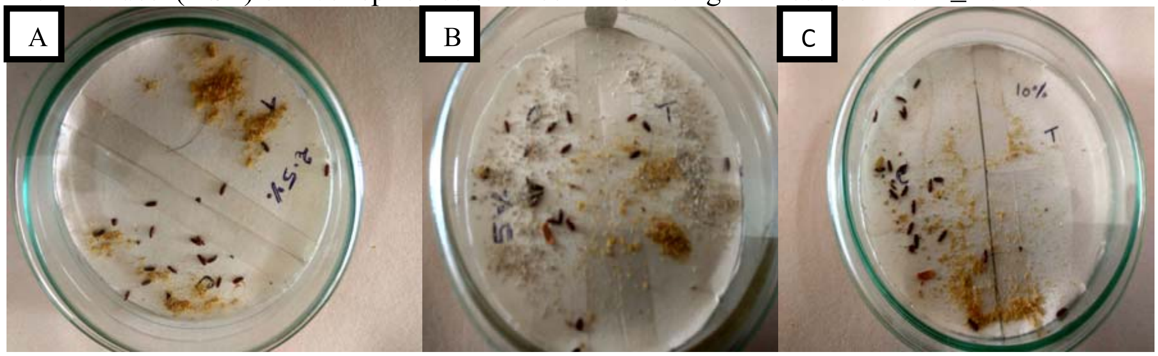

Fig. 6. : Repellent effect of plant extract at three concentrations (A) 2.5, (B) 5 and C) 10 % against *T.castaneum* at 24 h exposure

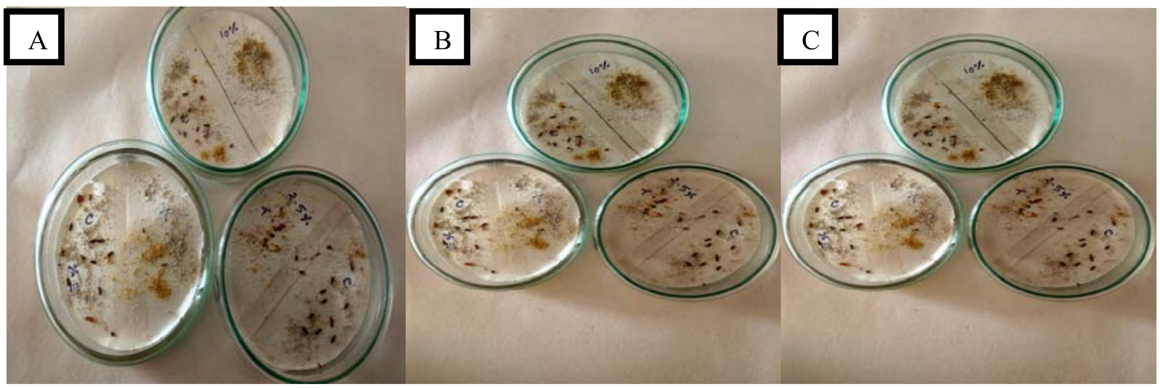

Fig 7: Repellent effect of three plant extracts at various exposure periods (A) 24 h, (B) 48 and (C) 72 h against *T .castaneum at* 10 percentage concentration.

4. Discussion

Massive post-harvest losses sustaining in the developing world due to physical, nutritional and quality deterioration of stored grains that are caused by insects and the detrimental impact of these losses on food security are well known (FAO, 1999).

In the recent years, there has been a renewed interest amongst scientists to study the bioactivity of plant extracts against stored-grain insect pests (Benzi *et al*., 2009 and Dubey *et al*., 2008). Our experiments proved a significant impact of plant extracts on the test insect that cause damage to processed and stored commodities. Chaubey (2007) determined the repellent impact of the *N. sativa* and *T. ammi* and found a significant impact of these extracts. Similar results were concluded by Sagheer *et al.* (2011, 2013) while working on the effects of different repellent plant extracts towards the repellent action. In this investigation, crude leaf extract of *Cinnamomum tamala, Rosmarinus officinalis* and *Pelaragonium graveolens* showed good potential as repellent agents to adults of *T. castaneum*. In the repellence tests it was verified that the highest rates of repellency corresponds to *Cinnamomum tamala.*when compared to the other two plant extracts. Our experiment also supports that the potential of the plant extracts to cause repellency with the increase in concentration. As the concentrations of the extracts increased there was an increase in the percentage of repellency values. Jahromi *et al.* (2014) also worked towards the repellent effects of the natural garlic emulsion on the percent repellency. Serial concentrations were made and maximum percent repellency was shown at highest concentrations. But, the results are different for the time factor because in their experiment there is no significant effect of time. It may be contributed towards the method used for experiments. They utilized the olfactometer method while in our case; the area preference method was used which showed a progressive decrease in repellency over the time factor. Our study confirms the findings of several studies which demonstrated the highly repellent effect of selected plant species against stored-product beetles (Alok-Krishna *et al*., 2005 and Zia *et al*., 2011).

Generally, the repellency of most extracts varied with concentration and indicated a dose dependent behavioral response of *T. castaneum*. Our result corroborates the previous observations on the repellent effect of essential oils and plant volatiles on other insect species such as *T. confusum*. It has been reported that essential oils of *M. chamomilla* exhibited strong repellent action against *Oryzaephilus surinamensis* (L) and *T. castaneum* (Al-Jabr, 2006). Some botanicals were highly effective when they were applied on the grains, (Zapatan and Smagghe, 2010) while others caused more mortality by topical application. On the other hand, the high insecticidal effect of different extracts against *T. castaneum* in topical application method was also reported (Tripathi *et al*., 2003; Suthisut *et al*., 2011).

5. Conclusion

The repellent activities of three plant species were studied against *T. castaneum*. *C. tamala* exhibited highest repellent activity against *T. castaneum* with IR values of 0.14 and IR values for *P. graveolens* and *R. officinalis* was 0.29 and 0.86 respectively. Future research should be focused on isolation and identification of compounds responsible for the repellent activity. This could be useful in the field application of these extracts and strategies should be worked out ways to increase the repellent action of these extracts. Application method should also be depicted in this regard to promote the sustainable practice towards managing stored product pests.

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