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Irengbam Barun Mangang
Indian Institute of Food Processing Technology, Pudukkottai Road, Thanjavur, Tamil Nadu, India

Abhinav Tiwari
Indian Institute of Food Processing Technology, Pudukkottai Road, Thanjavur, Tamil Nadu, India

R Meenatchi
Indian Institute of Food Processing Technology, Pudukkottai Road, Thanjavur, Tamil Nadu, India

M Loganathan
Indian Institute of Food Processing Technology, Pudukkottai Road, Thanjavur, Tamil Nadu, India

Correspondence
M Loganathan
Indian Institute of Food Processing Technology, Pudukkottai Road, Thanjavur, Tamil Nadu, India

Comparative repellency study of novel insect repellent cloth bags treated with the plant extracts against *Tribolium castaneum*

Irengbam Barun Mangang, Abhinav Tiwari, R Meenatchi and M Loganathan

Abstract
Red flour beetle (*Tribolium castaneum*) is a globally serious stored pest that invades the grains through small openings available in the packaging material. Among the novel methods to manage this pest is the development of insect repellent cloth bag. In the current research, the cloth bags treated with Malathion, aqueous extract of papaya leaves and garlic bulbs were checked for its efficacy against the adults of *Tribolium* species. In the olfactometer, sachets coated with the aqueous extract of papaya leaves showed the most repellent behaviour (92.5%). This result is followed by garlic bulb showing repellent behaviour comparative to Malathion (90%). In the insect management unit, the readings were taken at 2, 8 and 24 hours of treatment at a constant concentration of 26ml/544cm^2 (i.e. 0.05ml/cm^2). All of the treatments gave comparatively relevant repellency (>70 %) when compared with Malathion in the insect management unit. However, the cloth bag treated with the aqueous extract of garlic bulb showed reasonable results of 76–83 per cent repellency against *T. castaneum* when compared with malathion (73-87%).

Keywords: Cloth bag, insect repellent, *Tribolium castaneum*, garlic bulbs, Malathion

1. Introduction
Mankind invented packaging material to keep the food safer for a longer period of time. In the food industry, different types of packaging materials serve as a barrier to protect the food materials against infestation by insects. However, adults of *Tribolium* species, *Oryzaephilus* species which are called invaders, can enter through small openings (> 0.71mm) in the packaging material (Chung *et al*., 2011; Scheff *et al*., 2016) [2, 7]. This gives adverse effects to the food commodity leading to contamination, development of yellowish colour and off-smell which makes the food commodity unfit for consumption (Ali *et al*., 2014) [1]. People lean towards chemical pesticides only for controlling the pest but interest in plant based products as a source of chemical-free pesticide is increasing. Also, the undesirable impacts of chemicals and increasing chemical resistance have demanded for an alternative method to control the pests and avoid invasion (Reddy *et al*., 2016) [6].
Willis (2017) [9] reported that 59.37 per cent out of 30000 plants which are documented have medicinal use. Many researchers have performed various studies on medicinal plants for checking the properties and their effectiveness as repellent, anti-feed ant, attractant, ovicidal or larvicidal against different storage insects. Still the use of chemical insecticides in godowns and storage houses continues, leaving residues in the stored products and developing resistance in the insects. To overcome the use of chemical insecticides, extensive research is yet to be done.
Neem leaves and DEET were compared for its repellent behaviour against *Sitophilus oryzae*, *Tribolium castaneum*, *Cryptolestes ferrugineus*, and *Oryzaephilus surinamensis.* The report showed that neem leaf was less effective (Hou *et al*., 2004) [4]. In another research study by Wong *et al*. (2005) [10], it was reported that citronella treated carton boxes repelled 50 per cent of the penetrators observed in control cartons. Licciardello *et al*. (2013) [5] also reported that polypropylene coated with the essential oils of citronella, oregano and rosemary showed high repellent activity up to 87 per cent. Song *et al*. (2018) [8] microencapsulated cinnamon oilusing polyvinyl alcohol and it is coated on the polypropylene film. This resulted in the protection of the grains from Indian meal moth. These research studies on the use of plant extracts as a source of repellent by incorporating with the packaging material created the idea of testing medicinal plant extracts as repellent.

In the current research, two medicinal plant extracts will be impregnated in the cloth bag and their effectiveness will be evaluated by comparing with a chemical insecticide.

2. Materials and methods

2.1. Insect culture

T. castaneum adults were reared on wheat flour in the laboratory at 25-30˚C in a glass container covered with muslin cloth. The culture was maintained to get uniform age adults for conducting experiments.

2.2. Extracts

The aqueous extract of papaya leaves (*Carica papaya*) and garlic bulbs (*Allium sativum*) were selected for the current research. They were collected from the local areas of Thanjavur. Papaya leaves were ground separately in a pulveriser and weight to level the quantity with distilled water at 1:1 ratio in a 500ml beaker. The beaker was then partially sealed with aluminium foil and kept at 4˚C for 24 hours. On the next day, muslin clothes were used to filter it and papaya leaf extract was collected in a vial. Similar procedure was followed for preparing the extracts from garlic bulbs.

2.3. Malathion dose

Malathion is a recommended insecticide used for the management of stored product insects. Hence, it is used for comparing with the plant extracts. According to the details given by Darboucosa (2017) [3], the recommended dose for using Malathion is 9/2 ounce per gallon of water. Therefore, the dilution was made at 3.5 per cent (1.75 ml of Malathion in 50 ml of distilled water).

2.4. Impregnation bioassay

Cloth bags with the size of 17 × 16 cm were prepared for conducting the experiments. They were treated in hot water followed by shade drying for 7 hours. Preliminary studies were conducted to find out the amount of liquid chemicals and extracts to be sprayed. It was found that the optimum amount of liquid required for the complete coverage of the outer layer of cloth bag was 26ml. Hence, the repellency test was planned to perform at a concentration of 26ml/544cm^2. The cloth bags were sprayed separately with the extracts of garlic bulb, papaya leaf and Malathion at the fixed concentration followed by shade drying for 2 hours.

2.5. Repellency test with olfactometer

The olfactometer used in this research is a four arm acrylic chamber with a single drop point for insect and vaccum, discretely. It also consists of an air pump, a vacuum unit and silicon connecting tubes. The air used is purified by activated carbon filter. Sachets sized 3.3 × 3.3 cm were made from the treated packaging materials, with the treated side as the outer part. It was filled with 4g of barnyard millet. The sachets coated with garlic bulb, papaya leaf, Malathion and untreated were placed in each arms, with 40 numbers of *T. castaneum* released in the middle of the chamber. The readings were taken when all the insects dispersed from the middle of chamber to the arms and the experiment was replicated three times.

2.6. Testing of insect repellent packaging material using Insect management unit

The effectiveness of the insect repellent packaging material (cloth bags) was observed in the insect management unit which is made up of acrylic (2 mm thickness). The treated bag was placed on one end and the untreated on the another end (Licciardello *et al*., 2013) [5]. It was replicated thrice for each extracts and chemical separately, for cloth bags. The readings were taken at 2, 8 and 24 hours' interval. The percentage repellency (R) was calculated using the formula below.

$$R = \frac{Ut}{Total\ number\ of\ insects\ released} \times 100$$

Where,

Ut = Number of insects in the side of the control bag

2.7. Statistical analysis

The data was subjected to statistical analysis using ANOVA and DMRT test was conducted to find out the most significantly different data ($p<0.05$).

3. Results and discussion

3.1. Repellent effect of plant extracts against *T. castaneum* in an olfactometer

The aqueous extract of papaya leaves and garlic bulbs were tested for its repellent effect against *T. castaneum* and compared with Malathion in an olfactometer. The repellent effect was checked by counting the number of insects present in each arms of the olfactometer after all *T. castaneum* get dispersed from the main chamber. The results showed that the aqueous extract of papaya leaves have the most significantly different repellent effect of 92.5 per cent (Fig. 1). It is followed by Malathion and garlic bulbs extract with 90 per cent repellency for both.

Table 1: Repellent effect of plant extracts against *T. castaneum*

Treatments (Quantity: 1mL/21.78cm^2)	Number of insects repelled (Mean ± S.D)
Garlic bulbs	36.00 ± 0.00^a
Papaya leaves	37.33 ± 1.15^a
Malathion	36.00 ± 2.00^a
Control	10.67 ± 2.30^b

All the data were significant at $p<0.05$.

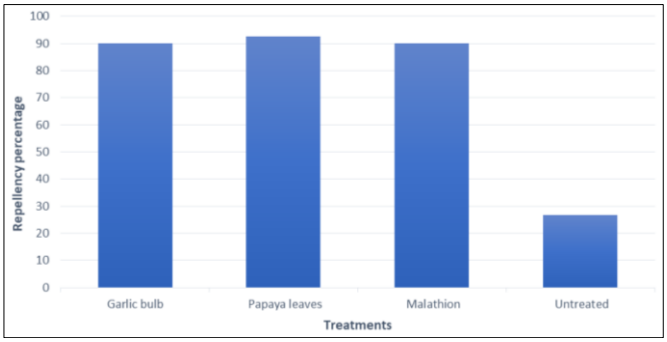

Fig 1: Repellent effect of plant extracts against *T. castaneum* in the olfactometer

3. Repellency effect of extracts against *T. castaneum*

The cloth bags treated with papaya leaves aqueous extract was checked for its repellency by counting the number of insects in the treated side of the box including those which invades the bag at 2, 8 and 24 hours against *T. castaneum.* It showed a repellency of more than 70 per cent. Likewise, cloth bags were treated with garlic bulb aqueous extract and its repellency against *T. castaneum* was evaluated. The result showed comparatively higher repellency of 76-84 per cent. Malathion treated cloth bag was tested for its repellent

activity against *T. castaneum.* The results showed the highest significantly different repellency of 73-87 per cent by Malathion treated cloth bag. The result depicts that the repellency of the extracts increases as exposure time increases (Table 2.).

Table 2: The repellent effect for all the treatments at three different time intervals

Treatments (Quantity: 26mL/544cm^2)	Repellent effect (Mean ± S.D.)		
	2 hours	8 hours	24 hours
Garlic bulb aqueous extract	83.33 ± 2.88^a	76.67 ± 7.64^a	78.33 ± 2.88^b
Papaya leaves aqueous extract	70.00 ± 5.00^b	76.67 ± 2.88^a	76.67 ± 5.77^b
Malathion	73.33 ± 2.88^b	75.00 ± 5.00^a	86.67 ± 2.88^a

The data in the first and third column differ significantly at $p<0.05$ while the data in the second column does not differ significantly at $p<0.05$.

Among all the treatment it was found that garlic bulb treated cloth bags showed the highest repellency of more than 83 per cent after 2 hours of treatment against *T. castaneum* (Table 2). It was followed by Malathion. This repellent effect of garlic bulb may be due to the presence of bioactive component, diallyl disulphide and diallyl sulphide (Zhao *et al*., 2013; Yang *et al*., 2012) [12, 11].

All the treatments gave a repellency ranging from 75-77 per cent after 8 hours of treatment against *T. castaneum.* The result clearly depicts that there is no significant difference between the treatments (Table 2).

After conducting the treatment for 24 hours it was found that Malathion repelled the highest amount of *T. castaneum* with 86.67 per cent repellency (Table 2). However, garlic bulb and papaya treated cloth bags also repelled more than 75 per cent of *T. castaneum.*

4. Conclusion

It can be concluded that, during the repellency study using insect management unit, garlic bulb showed a comparatively good result by repelling more than 76 per cent of *T. castaneum* which is comparable with the chemical insecticide. The repellency of garlic bulb ranges from 76-84 per cent. Furthermore, in the study of insect repellency by using an olfactometer, garlic bulbs and Malathion showed similar results (90% repellent). Additional research can be done to entrap the aqueous extract of garlic bulbs using techniques like microencapsulation and give a slow release profile, giving a key to the future of insecticide free storage of food grains.

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6. References

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