



Toxicological potential of four plant extracts impregnated on packaging materials against red flour beetle

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Abstract

The red flour beetle is an important secondary insect and cause severe damage to the food commodities. The use of chemical insecticides in the grain storage facilities leads to problems of resistant development in insect, residues and pollution in the environment. The present study was conducted as an alternative to chemical insecticides, to evaluate the toxicity of packaging materials treated with different medicinal plant extracts against the red flour beetle *Tribolium castaneum*. The experiments were carried out to evaluate the toxicity of leaf extracts of four medicinal plants Kuppaimeni, neem, tulsi and thyme against red flour beetle, *T. castaneum*. All the aqueous extracts of medicinal plants had effects on mortality to *T. castaneum* adults. It was observed that the effect on mortality increased with increased in the concentration of the plant extracts. The results showed that extracts of all the four plants had direct toxic effect on red flour beetle. The jute bags treated with thyme extracts showed lower lethal time (LT₉₉ of 113.48 and 99.27min), whereas tulsi leaf extract possessed the lowest toxic effect (LT₅₀ of 31.00 and 29.67 min). The effect of cloth bags treated with thyme extracts (8%) depicts the lowest LT₅₀ and LT₉₉ of 31.01 and 113.65 min and neem extract also showed similar effect at 10% concentration. Stigmasterol was the most active component obtained in the GCMS analysis of thyme extracts. These results revealed that packaging materials treated with medicinal plant extracts can be used to manage red flour beetle.

Keywords: insect mortality, adult, lethal dose, *T. castaneum* (Herbst), packaging material

Introduction

Proper storage of food commodities is an important area of research as they are readily vulnerable to the attack of insects. Insect infestation is a very serious issue that affects about 14% of during storage and 50% of total crops (Kulkarni *et al.*, 2009) [16]. Thus, stored product insects has got tremendous impact on the food safety as they adversely affect both quality and quantity resulting in significant decrease in volume, weight loss and also damage during germination (Phillips and Throne, 2010; Nadeem *et al.*, 2012; Ukeh *et al.*, 2012; Dumitru *et al.*, 2020) [31, 25, 7]. It was reported that a significant increase in humidity and temperature supports insect infestation leading to microbial growth (Semeao *et al.*, 2012; Keskin and Ozkaya, 2013; Padin *et al.*, 2013; Malhi *et al.*, 2021) [41, 14, 27, 18].

Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the most destructive insects of stored food products (Mishra *et al.*, 2012b; 2012a) [21, 22]. It is a secondary insect and external feeder, mainly found in processed or milled food products that is associated with grains, cocoa, nuts, dried fruits and spices (Rees, 2018) [35]. The synthetic insecticides and fumigants are used to manage *T. castaneum*. The use of chemical insecticides in the grain storage facilities leads to problems of resistant development in insect, residues and pollution in the environment. These may also lead to lethal effects on non-target organism's toxicity to users. Pérez *et al.* (2010) [30] reported that the curative method of disinfestation of stored products is the

chemical method that uses indiscriminate doses of chemicals that has adverse effects on environment and human health.

The newer alternative to chemicals were attempted and the use of plant extracts is one among them that are insecticidal, non-toxic and safe for environment and human health (Choupanian *et al.*, 2017; Ahmed *et al.*, 2019; Mangang *et al.*, 2020) [4, 2, 19]. The active components of plant extracts possess the potential to form natural insecticides which will be safe to mankind (Abreu-Villaca and Levin, 2017) [1]. The urge for using natural insecticides is the need of the era as mankind start focusing on organic farming, since the use of synthetic insecticides for managing pest leads to degradation of nature (Pretali *et al.*, 2016) [33]. It was reported that various essential oils from Kingdom Plantae are rich in complex volatiles and secondary metabolites (Dehsheikh *et al.*, 2020) [6]. Natural insecticides were proven effective against various stored product insects (Sagheer *et al.*, 2011) [38]. The insecticidal action of the botanical extracts depends on the type and characteristics of the insects and the compounds present in the specific botanical used. In general, insecticidal action of botanicals can be categorized into six types; repellent, feeding deterrent/ antifeedant, toxicant, growth retardant, chemosterilant, and attractant (Rajashekar *et al.*, 2012).

The investigations of insecticides from botanicals have risen drastically with the interests in eco-friendly insecticides and have been proved to be a promising alternative to synthetic

Insecticides (You *et al.*, 2014; Pandey *et al.*, 2018; Wang *et al.*, 2018). The plants taken for the analysis such as Neem, Tulsi, Kuppaimeni and Thyme are widely available in India and has proven effective against insect infestation (Saleem *et al.*, 2018). The direct application of these botanical extracts on the food product result in the production of benzoquinone, making it unfit for human consumption. Thus, the treating of packaging material with medicinal plant extracts is an alternative method for prevention of insects onto the stored food commodities. In this context, the study was conducted to evaluate the toxicity of packaging materials treated with different medicinal plant extracts against the red flour beetle, *T. castaneum*.

Materials and methods

Insect rearing

The insect cultures of *T. castaneum* used in the study was obtained from the stock culture which was maintained in the post-harvest department of IIFPT. Wheat flour was used as culture media for *T. castaneum*. The uniform aged adults were collected from the stock culture and released in each of the plastic jars (30nos. of *T. castaneum*) containing wheat flour (30g) and covered with muslin cloth. After 24 hrs, adults were sieved out of the media to achieve homogeneous population, as adults of *T. castaneum* lays their eggs within a day. The media along with the eggs, was kept under the optimum conditions of temperature and relative humidity of $32\pm 3^{\circ}\text{C}$ and $70\pm 4\%$, respectively. Adults used for the study were obtained after a time period of 28-35 days as illustrated by (Islam and Talukder, 2005) [13].



Fig 1: Insect culturing: (a) Insects reared in different glass jars (b) an adult *T. castaneum*

Plant materials

The fresh leaves of four medicinal plants (Kuppaimeni, neem, tulsi and thyme) were collected from Thanjavur, Tamil Nadu, India. These plant extracts were used for impregnating packaging materials and were evaluated for their toxicity against adults of *T. castaneum*.

Preparation of plant extracts

The leaves were first weighed (50g), ground (mortar and pestle) and soaked in equal quantity of water. The soaked leaves were kept overnight at 4°C and filtered using a muslin cloth. The filtrate was collected and re-filtered using Whatman filter paper (No.1). The extract was subjected to get stock solution as described by Sagheer *et al.* (2013) [37]. Similarly, the procedures were followed to obtain acetone extract whereby acetone was used instead of water.

Bioassay to evaluate the toxic effect of plant extracts treated on packaging materials against *T. castaneum*

These experiments were conducted using two types of packaging material: jute and cloth bags. These packaging materials were cut into discs of 9cm diameter to fit in the

petri plates (9cm). These discs of packaging materials were treated with plant extracts (8 and 10% concentration). Each treated discs were placed in separate petri plates. (Mullen *et al.*, 2012). The adults of *T. castaneum* (30 Nos.) were released in each petri plate and closed. The experiment was repeated thrice. The mortality data were recorded after 12, 24, 48 and 72 hours exposure time. If the beetles show no movements on touching with brush, they were considered dead (Othira *et al.*, 2009) [26].

GC-MS analysis

The Gas Chromatography Mass Spectroscopy (GC-MS) technique is a screening technique to analyse the extracts qualitatively and quantitatively by providing spectral output of all the components that gets separated from the sample (Hori *et al.*, 2019) [12]. The sample was freeze dried (Lark 4kg model) at pressure and temperature combination of 0.100Pa and -72°C . The dried sample (1g) and ethanol (25ml) were mixed in a volumetric flask (100ml) and vortexed for 15 minutes. The contents were centrifuged (Make: Remi; Model: R-8C BL) at 10000 rpm and the supernatant liquid was injected in the GC (Make: Bruker; Model: Scion 436-GC). The obtained mass spectra from the GC-MS were compared with the NIST library and the results were depicted as given by Mangang *et al.* (2020) [19].

Statistical Analysis

The bioassay experiments were laid out in Minitab software. Turkey's test was conducted to check the significance and determine the insecticidal plant having the highest mortality (Mangang *et al.*, 2020) [19]. Reported means and standard deviation are of original data given in percentage. Polo plus software was also used to find Lethal Time (LT_{50} and LT_{99}) of the medicinal plants (Minj *et al.*, 2021; Tiwari *et al.*, 2021) [20, 42].

Results and Discussion

Effect of jute and cloth bags treated with extracts on the mortality of *T. castaneum*

The effect of jute bags treated with Kuppaimeni extracts (8%) on the adults of *T. castaneum* were observed to possess LT_{50} and LT_{99} of 40.63 and 119.76 min, respectively (Table 1). The time taken to obtain the 50 and 99 per cent mortality was lower in jute bags treated with Neem extract (LT_{50} and LT_{99} of 32.80 and 104.29 min) at 8% concentration. Attaining the desired lethal time in a shorter period suggest higher effectiveness in achieving the desired mortality. In a similar pattern, the effect of jute bags treated with neem at 10% concentration showed higher effectiveness (LT_{50} and LT_{99} of 30.25 and 96.80 min). The result also depicts that the increase in concentration of extracts reduces lethal time. The jute bags treated with thyme required more time to achieve 99% mortality (LT_{99} of 113.48 and 99.27min) next to neem at 8 and 10 per cent concentrations. These results revealed that jute bags treated with neem extracts possess higher toxicity to *T. castaneum* when compared with other medicinal plant extracts. Morsy *et al.* (2018) reported the similar trend using neem extract coated paper for packaging of wheat flour and results showed higher mortality as the neem extract concentration increases. However, jute bags coated with Tulsi showed lowest LT_{50} of 31.00 and 29.67 min at 8 and 10 per cent concentration, respectively, but required more time to achieve 99 % mortality (Table 1).

Table 1: Lethal dose (min) for 50% (LT50) and 99% (LT99) mortality of the adult *T. castaneum* during exposure to Jute bags treated with medicinal plant extract

Medicinal plant extracts	Concentration exposed (%)	LT ₅₀ (min)	LT ₉₉ (min)	Slope ± SE	x ² (df)
Kuppaimeni	8	40.63 (36.07 - 45.59)	119.76 (95.48-170.45)	4.95±0.43	14.95(10)
	10	36.13 (32.01 -40.62)	104.76 (83.99 -147.01)	5.03±0.42	15.51(10)
Neem	8	32.80 (28.92 - 36.97)	104.29 (82.73 -148.36)	4.63±0.39	14.93(10)
	10	30.25 (27.66 - 33.00)	96.80 (81.38 -122.10)	4.61±0.38	6.21(10)
Tulsi	8	31.00 (27.41- 34.91)	117.18 (91.64- 168.20)	4.03±0.34	12.38(10)
	10	29.67 (26.01- 33.63)	106.94 (83.29- 155.97)	4.18±0.35	14.33(10)
Thyme	8	33.18 (30.28- 36.26)	113.48 (94.15 to 146.20)	4.36±0.37	8.02(10)
	10	31.34 (28.31 to 34.58)	99.27 (81.72 to 130.44)	4.65±0.39	10.05(10)

The effect of cloth bags treated with thyme extracts at 8% concentration depicts the lowest LT₅₀ and LT₉₉ of 31.01 and 113.65 min, respectively when compared with cloth bags treated with other medicinal plant extracts (Table 2). However, the time taken to reach LT₅₀ (28.57) and LT₉₉

(97.20) was faster in cloth bags treated with Neem extract at 10% concentration. The results in current findings proved that cloth bags treated with Thyme extracts is more effective at 8% concentration.

Table 2: Lethal dose (min) for 50% (LT50) and 99% (LT99) mortality of the adult *T. castaneum* during exposure to Cloth bags treated with medicinal plant extract

Medicinal plant extracts	Concentration exposed (%)	LT ₅₀ (min)	LT ₉₉ (min)	Slope ± SE	x ² (df)
Kuppaimeni	8	39.32 (34.69 - 44.61)	154.57 (116.93 - 237.67)	3.91±0.35	13.01(10)
	10	33.76 (29.57 - 38.34)	100.13 (79.04 -145.36)	4.93±0.41	17.77(10)
Neem	8	33.24 (30.32 - 36.37)	116.53 (96.23 - 151.08)	4.27±0.36	9.72 (10)
	10	28.57 (26.03 - 31.24)	97.20 (81.08 -123.97)	4.37±0.36	6.46(10)
Tulsi	8	35.49 (29.72 -42.21)	144.94 (102.13-268.81)	3.81±0.33	23.49(10)
	10	32.04 (28.37- 36.04)	112.82 (88.93- 160.53)	4.25±0.36	12.98(10)
Thyme	8	31.01 (28.20 to 34.00)	113.65 (93.49 to 148.01)	4.12±0.35	8.54(10)
	10	29.61 (26.37 to 33.11)	107.27 (85.55 to 148.45)	4.16±0.34	11.33(10)

Phytochemicals present in Thyme aqueous extract

The toxicity reports revealed that the extracts of Thyme and neem showed lowest lethal time to get more mortality. The GCMS report for neem extracts were earlier reported in previous paper while studying the repellent effects of neem against *T. castaneum*. Hence, GSMS analysis for Thyme extracts were carried to find out the active compounds responsible for the toxicity to insects. The aqueous extract of thyme after the GC-MS analysis showed the presence of

Stigmasterol with the highest peak area (26.73%) which may be responsible for the mortality of adult insect. Earlier study by Gade *et al.* (2017) [9] proved that Stigmasterol possess acetylcholinesterase inhibitory activity which is responsible for larvicidal and repellent properties in mosquitoes. This was followed by gamma-Sitosterol (22.73%) and Taraxasterol (15.40%) which are all secondary metabolites.

Table 3: Major Phytochemicals found after GC-MS analysis of thyme aqueous extract

Retention time (min)	Phytochemical	Molecular formula	Molecular weight	Peak area (%)
17.41	Phytol	C ₇ H ₁₁ NS	141.23	7.93
18.63	dl-.alpha.-Tocopherol	C ₁₂ H ₅ N	173.25	13.07
20.85	Ergost-5-en-3-ol, (3.beta.)-	C ₁₂ H ₁₈	162.27	9.27
21.28	Stigmasterol	C ₁₄ H ₂₂ O	206.32	26.73
22.15	gamma.-Sitosterol	C ₂₁₆ H ₃₂	224.42	22.73
22.38	Benzo [h] quinoline, 2,4-dimethyl-	C ₁₂ H ₂₄ O ₂	200.31	4.87
23.52	Taraxasterol	C ₁₇ H ₃₄ O	284.50	15.40

Mankind invented packaging material to keep the food safer for a longer period. 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.*, 2018) [5, 40]. The use of coating of these repellent extracts on packaging materials will be helpful in order to prevent insect infestation (Mullen *et al.*, 2012) [24].

The toxicity of different essential oils and their components were previously studied against stored-product insects (Paranagama *et al.*, 2003) [29]. Muhammad Bilal (2018) reported that the packaging materials treated with *A. indica*,

Eucalyptus globulus and *Ocimum basilicum* showed maximum mortality of 30.07, 26.82 and 20.54% respectively in polyethylene. According to Islam and Talukder (2005) [13], the leaf powder of *A.indica* has certain degree of toxicity against *T. castaneum*. Plant derivatives, neem seed extract (100 µg/insect) showed higher toxicity (53.13 % mortality) to red flour beetles. *Eucalyptus camaldulensis* essential oil shows a wide range of biological activity against pathogens including fungi, insects, bacteria, mites and some weeds. Although its vapor forms are too much toxic towards a number of microorganisms as well as insects, hence they could commercially use as fumigants for stored products and packaging materials, thus help in the prevention of insect infestation (Batish *et al.*, 2008) [3].

A. indica was proved very much effective by the researchers from various parts of the world. They recorded the success of *A. indica* plant extract to control the *T. castaneum* without causing any adversative effect on the surrounding environment (Saeed *et al.*, 2016)^[36]. So, degradable form of packaging with these medicinal plants will be cost effective, may also abate environment pollution and reduced health hazards. The results of present study supported to develop insect resistant packaging materials with medicinal plant extracts for safe storage of food commodities without any health hazards and environmental pollution.

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Conflict of interest

The authors have no conflict of interest to declare.

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