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THE INSECTICIDAL ACTIVITY OF TEA TREE OIL (MELALEUCA ALTERNIFOLIA) AGAINST THE COMMON PEST IN MUMMIES (DERMESTES MACULATUS)

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Abstract

Egyptian mummies show different signs of deterioration caused by insects, such as missing parts, gaps and accumulated dust. Dermestes maculatus is one of the serious pests that cause damage to Egyptian mummies. To assess the insecticidal activity of tea tree oil against the larvae of the museum insect pest Dermestes maculatus (isolated from Egyptian mummies) we tested it under different concentration and treatment times by the bioassay methods. Our results showed that tea tree oil diluted in ethanol was highly toxic to Dermestes maculatus larvae. Insecticidal activity depended on both concentration and exposure time. By increasing the concentration level and the exposure time we obtained a higher mortality rate.

Keywords: *mummy; tea tree oil; Dermestes maculates; biological activity.*

Introduction

Insect infestation is a major threat to the preservation of organic objects. Museums expend significant efforts to minimize the damage and prevent the spread of insect infestations within their collections. Many objects, because of their material composition and construction, present difficulties in visually detecting whether insects are hidden within, as does determining the type of insect, its lifecycle stage and its habits. Recognizing the presence of an active insect infestation requires trained eyes and experience. It is rare to find adult insects or larvae, usually objects are suspected of insect activity when remnants such as larval or pupal casings, frass or recent exit holes are found [1].

Ideally, before any mummy is considered for exhibition, a thorough exam, including CT scans, needs to be conducted to determine the stability and conservation needs of that mummy. If insect damage or other kinds of decomposition are found, a treatment needs to be started right away to stop any further degradation. However, there are several methods used for controlling insect pests in museums, yet not all of those methods are suitable for Egyptian mummies.

In recent years, there have been increasing and concerted efforts at developing plant based insect toxicants that are environmentally friendly. Plant materials have been known to be relatively inexpensive, readily available, safe, bio-degradable and to have broad spectrum applications. However, most studies focused on toxicants used for agricultural products of plant

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origin. Only a few detailed studies of the efficacy of using edible tropical plant species to kill the notorious pest, *D. maculatus*, that poses serious problems for mummies in tombs and in storage were available [2].

The tea tree is native to the northern coast of New South Wales, Australia. This is a world famous Australian product, used for thousands of years by the aborigines to help alleviate cuts, bites, burns and other skin ailments [3; 4]. Tea tree oil itself is clear, and colorless to pale yellow. The most active compounds in this oil are terpinen-4-ol, terpinene, 1,8-cineole and terpinolene. The International Standards Organization, ISO 4730, mandates a minimum concentration of 30% for terpinen-4-ol and a maximum concentration of 1,8-cineole of 15% in the oil.

The oil of the tea tree brings together over 100 different compounds and is globally recognized as a natural medicinal product. It has antiseptic (five times stronger than the usual household disinfectants), dermatological (prevents dry skin) and anti-fungal benefits and can also be used to fight infections/infestations (effective against head lice, ticks, etc.). Its oil is considered to have some of the best natural antiseptic/antifungal properties in the world. Tea tree oil is active against a wide range of bacteria: *Escherichia coli, Propionibacterium acnes, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Salmonella typhimurium, Streptococcus pyogenes, Helicobacter pylori*, etc. Tea tree oil has also gained widespread recognition for its therapeutic use in fungal and microbial infections but is not yet registered for use in the medical profession and its novel medicinal activity has yet to be explored on textile substrates [5]. However, little work has been done to assess it against the mummies pest *Dermestes maculatus.*

The aim of this paper was to assess the insecticidal activity of tea tree oil against the larvae of insect pests in mummies (*Dermestes maculatus*) under different concentrations and treatment times.

Materials and Methods

Insects

The Dermestidae are generally oval, small in size, the largest species reaches 0.8 mm in length. These chunky beetles have pale grey/brown markings which are formed of minute scales. They roll over on their backs with their legs folded and lie still, feigning death. Females lay up to 150 eggs from which small hairy larvae hatch within about 3 weeks. The larval stage lasts from 5 to 15 weeks, depending on temperature and food type. Larvae are hairy 'woolly bears' and have urticating properties. The pupal stage lasts from 2 weeks to 2 months. The beetles enter the pupal shell towards the end of year, during winter and emerge the following spring. The hide beetle, *Dermestes maculatus*, is a small, carrion-eating insect. Natural aggregations vary in size depending on the food source, but on a small carcass they typically range from one to thirteen adults. Males possess a pheromone gland on the base of their abdomen that elicits an aggregation response in both sexes. In the laboratory, the species mates and females commence oviposition within 24h following their first mating. Females are capable of ovipositing throughout their 4–6 month life span and will, given the opportunity, re-mate. Males prefer to mate with virgin females; however, they will also mate with non-virgins. Females will re-mate with the same male, although less readily than with novel males. To date there has been no assessment of the skew in male or female reproductive success when individuals are maintained in aggregations [6].

Tea Tree Oil

Tea tree oil was purchased from the local market (*Abd El Rahman M.Harraz, Agricultural seeds, spices and medicinal plants co.*, Cairo, Egypt). It has a light spicy, rather pungent smell and is very pale in color with a watery viscosity, soluble in ethanol. The main chemical components of tea tree oil are α-pinene, β-pinene, sabinene, myrcene, α-phellandrene,

α-terpinene, limonene, 1,8-cineole, y-terpinene, p-cymene, terpinolene, linalool, terpinen-4-ol and α-terpineol.

Quantitative Bioassay

This process requires great sensitivity and accuracy in the processes of extraction and purification of a component isolated from overlapping material, which may also have a toxic effect in the results of total toxic response. The precision in the selection of laboratory samples is very important, so that the larvae chosen were of similar age, in order to obtain a high degree of response. To estimate the quantitative response, increasing and graded concentrations were used in order to obtain the critical toxic concentrations [7].

Insect culture:

Several unsexed adults of *Dermestes maculatus* were obtained from rats which were mummified by using the second and third mummification processes. They were transferred into jars containing smoked fish, to initiate new colonies and to form a number of larvae to be used for the experiment. The cultures were kept at ambient conditions (25^{o}C and 65%RH). Individual selections were made for our bioassay. Larvae of *Dermestes maculatus* of third instar were chosen to give sensitive and accurate results.

Preparation of Sample, and Oil With Acaricidal Activity

20g of tea tree oil were dissolved in 10 ml of ethanol to make the basic solution and then multiple dilutions from the basic solution were taken in geometric sequences.

Test on Dermestes maculatus larvae:

A 1 g sample of the fish was weighed into each of the disinfected Petri dishes and mixed with different concentrations. Ten larvae of *Dermestes maculatus* of the third instar were introduced into each of the Petri dishes containing different concentrations and the fish. The Petri dishes with the fish sample without any of the treatment and solvents served as the reference sample. The concentration of mortality, $LC_{25,50,75,90,95,99}$, r, RR, toxicity index, slope values and fiducially limits were estimated by using a software package ″LD-P line″, copyright of Dr. Ihab M. Bakr from the Plant Protection Research Institute.

Results and Discussions

The mortality response of acetone extract in Table 1 shows that the highest percentage (100%) of death was recorded with the sixth concentration (1600mg/g) after one hour of the test, followed by the fifth concentration (1000mg/g), the fourth concentration (800mg/g), the third concentration (400mg/g), the second concentration (200mg/g) and the first concentration (100mg/g), which produced 100% mortality after 3hrs, 12 hrs, 1 day, 2 days and 4 days respectively. There was no death evidence in the control sample.

The lethal concentration LC $_{25,50,75,90,95\ \&\ 99}$ slope values, correlation coefficient (r), resistance ratio (RR) and toxicity index were then calculated (Table 2, Fig. 1). As shown in Table 2, tea tree oil diluted in ethanol for all investigated times was active. The LC $_{25,50,75,90,95\ \&\ 99}$ values decreased with the number of days.

Concerning the correlation coefficient (r), we found that the correlation coefficient values were positive and strong during all test periods, except on the second and third days when it was positive and average. With regard to the resistance ratio (RR), we found that the resistance ratio of *Dermestes maculatus* larvae to tea tree oil diluted in ethanol decreased with increasing duration of exposure, reaching 1 on the third day.

The relationship between time and death rates (LT) using different concentrations was studied (Table 3, Fig. 2). It was noted that death rates increased with increased exposure time to each concentration such as in the first concentration (LT_{25} 16.47h and LT_{99} 255.82h), in the second concentration (LT_{25} 5.23h and LT_{99} 100.19h), in concentration three (LT_{25} 1.24h and

LT_{99} 28.85h), in concentration four (LT_{25} 0.75h and LT_{99} 18.63h) and in concentration five (LT_{25} 0.29h and LT_{99} 2.25h). The decrease in death time by increasing the concentration was also tabulated in Table 3, such as $LT_{25,}$ for first concentration it was 16.47h and $LT_{25,}$ for the fifth concentration it was 2.25h.

Table 1. Comparative mortality response of selected tea tree oil concentrations on the larvae of leather beetles: *Dermestes maculates*

Sampling time	Mortality average % (Σ Replic. $_{1\text{-}3}$/3)/Conc.						
	Control	C_1 0.05mL 100mg/g	C_2 0.1mL 200mg/g	C_3 0.2mL 400 mg/g	C_4 0.4mL 800 mg/g	C_5 0.5mL 1000 mg/g	C_6 0.8mL 1600mg/g
0.0 time	0	0	0	0	0	0	0
1 h	0	0	0	20	37	87	100
3 h	0	3	17	57	70	100	100
6 h	0	7	30	77	87	100	100
12 h	0	10	47	93	100	100	100
1 day	0	33	73	100	100	100	100
2 days	0	60	100	100	100	100	100
3 days	0	83	100	100	100	100	100
4 days	0	100	100	100	100	100	100

Table 2. Probit analysis data of selected tea tree oil with ethanol concentrations on the larvae of leather beetles: *Dermestes maculates*

Time	LC (mg/g) (between brackets upper and lower limits)						Slope	r	RR	Index
	25	50	75	90	95	99				
1 h	503.116	695.234	960.714	1285.349	1529.95	2121.1802	4.802 +/- 0.4163	0.9202 (tabulated 0.95)	12.3	8.16
3 h	240.9333 (124.41-309.583)	383.689 (236.89-563.55)	611.03 (438.71-1054.74)	928.862 (746.540-1897.407)	1193.43 (1016.96-2720.70)	1909.621 (1790.08-5425.887)	3.3377 +/- 0.2145	0.9394 (tabulated 0.811)	6.76	14.79
6 h	173.1124 (80.8802-223.745)	283.917 (166.71-435.62)	465.6437 (330.919-880.6472)	726.8523 (594.802-1711.251)	948.8025 (835.087-2576.278)	1563.9994 (1550.52-5647.862)	3.1392 +/- 0.2216	0.9622 (tabulated 0.811)	5.00	19.99
12 h	141.7029 (125.885-156.267)	198.664 (181.73-216.72)	278.5231 (253.824-310.6318)	377.5194 (335.756-438.6303)	452.8738 (395.092-541.7543)	637.1176 (533.724-808.6048)	4.5965 +/- 0.3817	0.9757 (tabulate d 0.811)	3.50	28.57
1 day	91.0843 (76.5404-103.496)	131.740 (117.73-145. 30)	190.5435 (172.587-214.0076)	265.6158 (233.937-315.6769)	324.0272 (278.288-401.7126)	470.4315 (382.773-635.6246)	4.2084 +/- 0.4356	0.9046 (tabulate d 0.811)	2.32	43.08
2 days	78.0293	93.456	111.9326	131.667	145.1034	174.112	8.6089 +/- 2.8266	0.7186 (tabulate d 0.811)	1.65	60.73
3 days	55.9663	71.1869	90.5469	112.4366	127.991	163.2006	6.4562 +/- 2.9040	0.7186 (tabulate d 0.811)	1	100

Table 3. LT of tea tree oil with ethanol concentrations on leather beetle larvae: *Dermestes maculatus*:

Line name	LT (hour) (between brackets upper and lower limits)						Slope	r	RR	index
	25	50	75	90	95	99				
C1	16.471 (9.305-21.958)	30.512 (20.103-45.566)	56.5229 (41.8951-98.0196)	98.452 (78.90-200.845)	137.229 (114.09-311.63)	255.824 (224.55-720.806)	2.519 +/- 0.160	0.904 (tabulated 0.755)	65.90	1.517
C2	5.2272 (3.4701-6.7287)	10.152 (7.365-13.281)	19.7165 (15.0736-27.1835)	35.8347 (27.8324-53.4479)	51.2363 (39.724-81.007)	100.1859 (76.2309-179.4432)	2.340 +/- 0.136	0.9557 (tabulated 0.755)	21.93	4.561
C3	1.242 (0.9663-1.5173)	2.5186 (2.125-2.9287)	5.1074 (4.4093-5.9933)	9.6506 (8.041-12.0751)	14.123 (11.364-18.616)	28.8471 (21.4878-42.4188)	2.197 +/- 0.166	0.9689 (tabulated 0.707)	5.44	18.38
C4	0.7477 (0.5366-0.9587)	1.5405 (1.2408-1.8405)	3.1736 (2.7012-3.7534)	6.0826 (5.0364-7.7003)	8.9777 (7.1567-12.094)	18.6328 (13.5944-28.6998)	2.149 +/- 0.190	0.9172 (tabulated 0.707)	3.33	30.065
C5	0.2927	0.4632	0.7329	1.1077	1.4183	2.2549	3.384 +/- 1.217	0.6648 (tabulated 0.707)	1	100

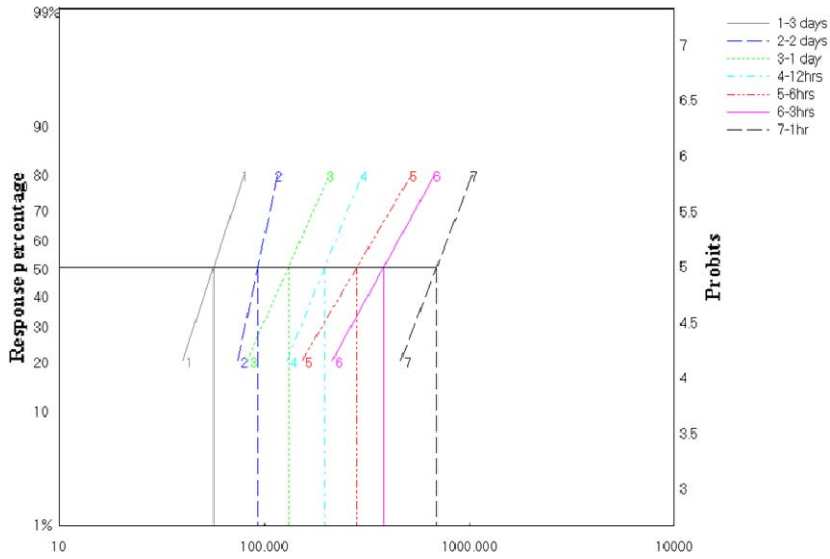

Fig. 1. Ldp lines of tea tree oil concentrations on leather beetle larvae: *D. maculatus.*

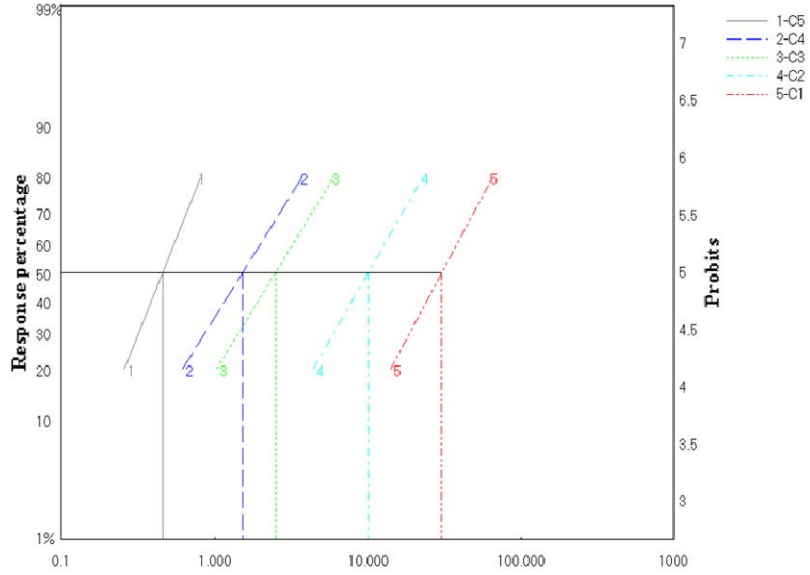

Fig. 2. LT_{50} of tea tree concentrations on leather beetle larvae: *D.maculatus.*

Conclusions

From our present research we concluded the following:

- The activity of tea tree oil against 4th instars larvae of *Dermestes maculatus* was assessed in the laboratory.
- The mortality response of tea tree oil diluted in ethanol showed that the highest effect was yielded from the sixth concentration, while the first concentration was less effective.

- The data presented in the tables above revealed the effectiveness of the tested substance, when used at different concentrations and after successive post-treatment periods. The results we obtained obviously indicated that the tested substance had a toxic effect on 4th instars larvae.

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