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EVALUATION OF CEDAR WOOD OIL (*CEDRUS LIBANI A*. RICH) FOR THE CONTROL OF COMMON EGYPTIAN MUMMIES' INSECT PEST (*DERMESTES MACULATUS*)

Gomaa Abdel-Maksoud[1], Abdelrahman Elamin[2*], Fathy Afifi[3]

[1]*Conservation Department, Faculty of Archaeology, Cairo University, Giza, Egypt*
[2]*Conservation Center, Grand Egyptian Museum, Ministry of Antiquities, Egypt*
[3]*Pesticides Chemistry and Plant Protection Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*

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**Corresponding author: Abdelrahman Elamin (elamin_555@hotmail.com)*

ABSTRACT

Egyptian mummies show different signs of deterioration caused by insects. *Dermestes maculatus* is one of the serious pests that cause damage to Egyptian mummies. To assess the insecticidal activity of cedar wood 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 cedar wood oil diluted in ethanol had a toxic effect on 4th instars larvae of *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, Cedar wood oil, Bio-deterioration, *Dermestes maculatus*, Bioactivity, Conservation

1. INTRODUCTION

Egyptian mummies (human, animal and birds) have provided an extensive source of stored meat products for insect attack since the time of their deposition, and it can be difficult to ascertain when the faunas entered the material. Egyptian mummies, because of their material composition and construction, present difficulties in visually detecting whether insects are hidden within, as does 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 (Koestler et al., 2000).

The leather beetle (hide beetle) *Dermestes maculatus* is known as a common pest of Egyptian mummies which feeds on hides, skins, feathers and horns. Its natural diet is rich in proteins and lipids, and larvae as well as adults are attracted to lipoidal components such as triglycerides, long-chain fatty acids, and their methyl esters which serve also as phagostimulants. The insects display a remarkable adaptation, growing and developing on very high dietary quantities of fat, whereas other insects would be adversely affected by this. The latter phenomenon is most likely associated with the lipid-rich habitat of *D. maculatus* (Cohen, 1974).

A brief review of the different species recorded as museum pests follows. A survey of bio-deterioration of museum collections showed that the leather beetle, *Dermestes vulpinus* (*D.maculatus*) is predominant. The dermestid beetles, (Coleoptera: Dermestidae) cause damage to a variety of museum objects throughout the world, many of them being cosmopolites. An increase in the international exchange of museum objects has resulted in a greater distribution range of many species of dermestid beetle (Zaitseva, 1987).

There are several types of dermestid beetles were isolated from many Egyptian mummies. The aspects of deterioration which caused by these beetles were stated. An x-ray of the mummy of Pennsylvania, which dated to the first or second century AD, found that it was a mass of holes covered with beetles. A study of the body of Horemkenesi revealed that his feet had been badly eaten by carrion beetle larvae (El-Mahdy, 1991). Panagiotakopulu (2001) mentioned that *Drmestes ater* was also found by Neolitzky in 1911 on a mummy from the Predynastic (4000–3500 B.C) cemetery near Naga-ed-der in Upper Egypt. The most problematic part of Horemkenesi mummy is the reconstruction of the initial steps of the excavation. Body rigidity combined with the fragility of the bandages due to chemical erosion and to extensive damage by carrion beetle left the mummy difficult to handle (Dawson, 2002). Buckland et al. (2009) mentioned that both John Atkinson (1825) and Frederick Hope (1834) reported *Dermestes maculatus* Deg. from the head of a mummy from Thebes, brought to England by Wilkinson.

The use of chemicals to protect museum collections against damage by pests has a long tradition. However, concern over the health and safety implications of the use of chemicals in the museum environment, effectiveness, and potential adverse effects on museum specimens and associated materials has led to a reappraisal of collection protection policies. Therefore, this has focused attention on alternative methods of controlling and eradicating pests from museums. There are now well reported alternatives which focus on a nontoxic approach which is beneficial to both the collections and the general public. These concerns have led to the development of non-chemical treatments such as freezing, heating and the use of gamma, infrared and microwave radiation and controlled atmospheres. However, these physical techniques mainly focus on the direct treatment of known or suspected infestations rather than prevention and although welcome do not provide resistance to future attacks. These problems have highlighted the need for the development of new types of pesticides alternatives (Zhang, 2012). As an attempt to use the essential oil instead of commercial pesticide in the conservation process of mummies, Abdel-Maksoud and El-Amin (2013) added teat tree essential oil to the completion paste for the missing part of a gazelle mummy, that dates to the Late Egyptian Period, for protecting the organic components of the paste from any bio-deterioration in the future.

Some efforts searching for the new types of pesticides have been focused on the plant extracts. The use of medicinal plants as a source for relief and illness is ancient and well documented in the written documents of the early civilization, but it is without question an art as old as mankind. Medicinal plants usually produce many natural products, such as phenols, flavonoids, quinons, tannins, alkaloids, saponins, sterols, and volatile essential oils. These secondary metabolites have various functions, including antimicrobial, insecticide and appetite suppressant properties. Additionally, natural products are generally easily biodegradable. Therefore, they do not tend to persist in the environment (Abdel-Maksoud et al., 2014).

The widespread use of plants oils in ancient Egyptian ages indicates that the embalmers were aware of the special properties of those oils that allow them to protect mummies against future damage. Cedar wood oil was one of those oils which used in Ancient

Egypt (Abdel-Maksoud and El-Amin, 2011). Iskander (1980) mentioned that in the second method of mummification, oil of cedar was injected into the body through the anus, which was afterwards stopped up to prevent the liquid from escaping. Amoros and Vozenin-Serra (1998) mentioned that coniferous material (in the form of sawdust) came from the cedar tree and was found in mummies dating to different periods (Eleventh, Twelfth, Eighteenth, Nineteenth, and Twenty-first Dynasties and the Greco-Roman period). Taconis (2005) noted that in the First Intermediate Period, evisceration was practiced, either by incision of the abdominal wall or by means of an enema of cedar oil.

The genus *Cedrus* includes coniferous evergreen species and is distributed in four geographically separated regions: a-Algeria and Morocco, b-Cyprus, c-Lebanon, Syria and Turkey and d-Afghanistan and the Himalayas. Cedrus was first described as a genus by Trew 1757 and most of the taxonomists agree now on the four species classification arrangement: *Cedrus atlantica* Mannetti (1844) in Morocco and Algeria, *C. brevifolia* Henry in Cyprus, *C. libani* A. Rich (1823) in Lebanon, Syria and Turkey and *C. deodara* D. Don (1830) in Afghanistan and India (Scaltsoyiannes, 1999; Qiao et al., 2007)

Lebanon Cedar, *Cedrus libani* A.Rich., is significant from the historical, cultural, aesthetic, scientific, and economic perspectives. It is presently found primarily in the Taurus Mountain range of Asia Minor, Turkey. Historical records indicate extensive and magnificent forests of Lebanon cedar also occurred in Syria and Lebanon; however, heavy cutting, burning, and goat grazing for the past 5.000 years have left only small populations in Syria and Lebanon. Wood of the Lebanon cedar is highly resistant to decay and atmospheric conditions, durable and has a peculiar smell and color. The wood of the eastern red cedar, *Juniperus virginiana* L. aromatic cedar has been long thought to possess insecticidal or repellent properties (Sweetman et al., 1953).

Early studies demonstrated that cedar wood and its shavings killed various stages of the clothes moth, *Tineola bisselliella* Hummel, and black carpet beetle, *Attagenus unicolor* Brahm. Cedar oil volatiles are lethal to larval *T. bisselliella*. Milled aromatic cedar ßake board repelled but did not kill *Blattella germanica* while *Periplaneta americana* and *P. fuliginosa* were neither repelled nor killed (Heike and Silverman, 2001). The present study was conduced to screen the insecticidal activity of cedar wood oil against the larvae of the common mummies' pest (*Dermestes maculatus*). This aims to provide suitable alternative to the used traditional control methods in museums.

2. MATERIALS AND METHODS

2.1. *Leather beetle (Dermestes maculatus)*

The Dermestidae are generally oval small in size, the largest species 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' which have urticating properties. The pupal stage lasts 2 weeks -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 (Jones et al., 2006).

2.2. *Cedar Wood Oils (Cedrus libani A. Rich)*

Cedar oil purchased from *(El Naser Pharmaceutical Chemicals Co.* Cairo, Egypt), Light yellow to pale brown viscous liquid; Wt Per ml/20^{o}C: 0.95 g and soluble in ethanol. Major constituents are Essential oil (3-carene, limonene, myrcene, α-pinene, β-pinene, α-pinene, camphene, β-phellandrene, a-thujene, terpinolene,α-terpinene, γ-terpinene, p-cymene, and ocimene, monoterpenes, sesquiterpenes, atlantol (Li,2000). Effective as a compound on mummy's body α-pinene, β-myrcene, limonene, terpinolene,α-terpinene, γ-terpinene (Geron et al., 2000). Cedar oil is used in pet care products to repel fleas and ticks (Craiga et al., 2004).

2.3. *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 members is very important so that the larvae chosen were of similar age in order to obtain the high degree of response. To estimate the quantitative response, increasing and graded concentrations were used in order to obtain the critical toxic concentrations.

2.4. Insect Breeding

Several unsexed adults of *D. maculatus* were obtained from the rats which were mummified using the second and third mummification processes. These were transferred into jars containing smoked fish to initiate new colonies and to form numbers of larvae used for the experiment. The cultures were kept at ambient conditions (25°C and 65%RH). Individual selections were made for bioassay. Larvae of *D. maculatus* of third instar were chosen to give sensitive and accurate results.

2.5. Preparation of Cedar Wood Oil Having Insecticidal Activity

31.6 mg of cedar wood oil was dissolved in 100 ml of ethanol to make the basic solution, and then multiple dilutions from the basic solution were taken in the form of geometric sequences.

2.6. Test on D.maculatus Larvae

Sterilized Petri dishes were prepared for using in the test. 1 g sample of the fish was weighed into each of Petri dishes and mixed with different concentrations. Ten larvae of *D. maculatus* of the third instar were introduced into each of the Petri dishes containing different concentrations and the fish. The Petri dishes without any of treatment and solvents with fish sample served as the control. Concentration of mortality, $LC_{25,50,75,90,95,99}$, r, RR, index, slope values and fiducially limits were estimated by using a software package "LD-P line"", copyright of Dr. Ihab M. Bakr, Plant Protection Research Institute. (Note: LdP Line is an application that calculates probit analyses according to Finney, which is used to illustrate the relation between stimulus and response in toxicological and biological studies. With the help of LdP Line one has the possibility to calculate correlation coefficients, make relative studies among several treatments and calculate the resistance ratio and significant intervals. Also, one is able to preview the relations between stimulus and response in toxicological studies. Moreover it saves the data in a file and re-opens, re-edits, and re-calculates them any time later).

3. RESULTS AND DISCUSSIONS

The effect of cedar wood oil diluted in ethanol on *Dermestes maculatus* 4th Instars larvae was studied. Six selected concentrations were used [31.6 ppm (0.0316mg/g), 63.2 ppm (0.0632mg/g), 126. ppm (0.1264mg/g), 252.8 ppm (0.2528mg/g), 505.6 ppm (0.5056mg/g), 1011.2 ppm (1.0112mg/g)]. Mortality response to cedar wood oil showed that the highest percentage (100%) of death was recorded with the sixth concentration (1.0112mg/g) within one hour of the test, while the first concentration (0.0316 mg/g) was less effective, which caused 100% mortality only after 12 days. There was no death rate in the control sample.

The LC 25,50,75,90,95 & 99 slope values, correlation coefficient (r), resistance ratio (RR) and index toxicity were calculated (Fig. 1). Cedar wood oil from all investigated stages was active. $LC_{25,\ 50\ \&\ 75}$ decreased with the number of days whereas we find that there is fluctuation between high and low in $LC_{90,95\&99}$ increasing in the first seven days and returning to normalcy in the eighth and ninth days. This fluctuation may be attributed to the presence of some individuals that had a higher resistance to the concentrations used. This resistance faded away by the eighth and ninth days. With regards to the slope values, the data revealed that the slope values of LD-p lines gradually decreased by the period after the cedar wood oil treatments. The correlation coefficient (r), which defines the relationship between the concentrations and ratios of death, was positive and strong in all tested days. Toxicity index values were increased with exposure, so the superior efficiency of cedar wood oil was shown by the ninth day (100%). The resistance ratio (RR) was found to have decreased with increasing duration of exposure, reaching 1 by the ninth day.

In Fig. 2 the relationship between time and death rates with different concentrations was studied. It was noted that death rates increased with increasing exposure time in each concentration such as in the first conc. (LT_{25} 86.5hr and LT_{99} 457.3hr), in the second concentration (LT_{25} 63.05hr and LT_{99} 475.43hr), in concentration three (LT_{25} 59.09hr and LT_{99} 426.37hr), in concentration four (LT_{25} 16.79hr and LT_{99} 575.68hr), and in concentration five (LT_{25} 0.29hr and LT_{99} 2.25hr). The decrease of time with increasing concentration was stated, such as LT_{25} for the first concentration was 86.5hr and LT_{25} for the fifth concentration was 0.2927hr. Despite the decrease of slope values with increased concentration, this was not achieved with the fifth concentration. This difference was also recorded when comparing the results of the correlation coefficient, where it was found positive and strong with all concentrations except the fifth concentration which was positive and average (0.60).

These results are in agreement with the finding of previous reports which indicated that the insecticidal activity of *Illicium verum* and *Melaleuca alternifolia* against *D. maculatus* was dependent upon both concentration and exposure time (Bo Zhang, 2012; Abdel-Maksoud et al., 2013)

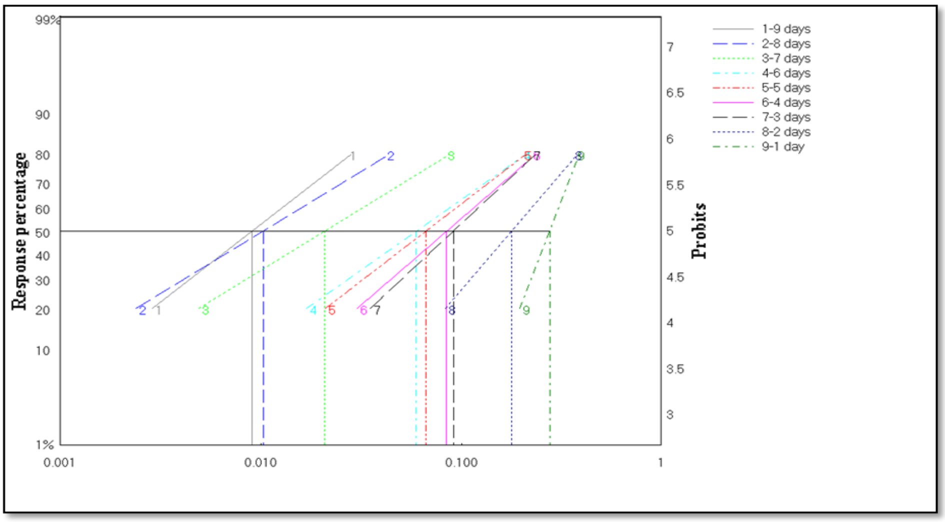

Figure 1. d-p lines of cedar wood oil with ethanol concentrations on leather beetle larvae: D.maculatus.

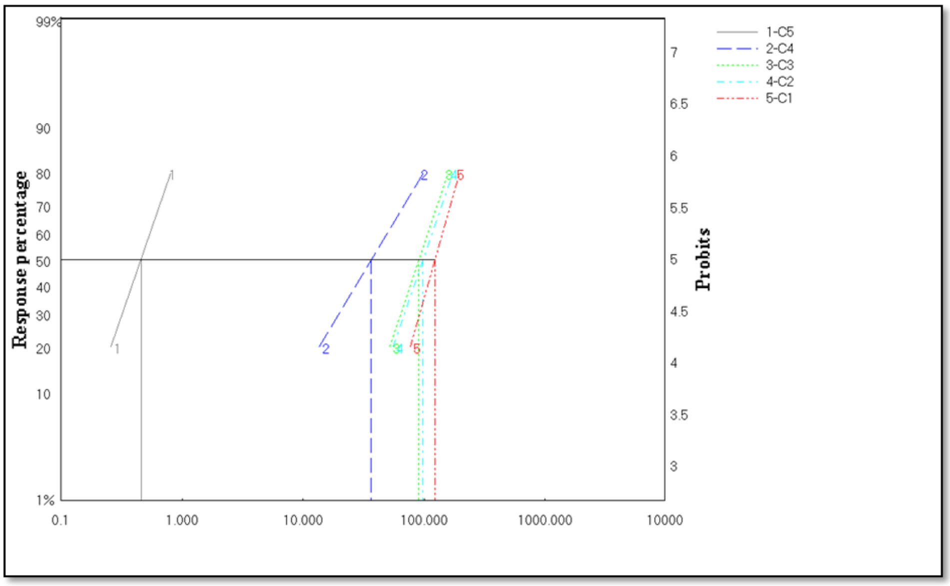

Figure 2. LT_{50} of cedar wood oil with ethanol concentrations on leather beetle larvae: D. maculatus.

4. CONCLUSIONS

The activity of cedar wood oil diluted in alcohol which was used against 4th instars larvae of *Dermestes maculatus* were assessed in the laboratory. Both the concentration of the cedar wood oil and the exposure time were the key factors affecting the mortality of *D. maculatus*. With the increasing of the concentration of the cedar wood oil and the exposure time, higher mortality was obtained. The Petri dishes without any of treatment and solvents with fish sample served as the control. There was no recorded death rate in the control sample. The mortality response of *cedar wood oil* showed that the highest effect was yielded from sixth concentration, while the first concentration was less effective. The obtained results obviously indicated that cedar wood oil had a toxic effect on 4th instars larvae.

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