Essential oils: a potential addition to integrated pest management strategies against adult varied carpet beetle, Anthrenus verbasci, in natural science collections

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Essential oils: a potential addition to integrated pest management strategies against adult varied carpet beetle, *Anthrenus verbasci*, in natural science collections

Kayleigh PEACE[1], Dimitrios E. BAKALOUDIS[2], Amanda CALLAGHAN[1], Ivan CAÑADA LUNA[3], Graham J. HOLLOWAY[1]
[1]*School of Biological Sciences, HLS Building, Whiteknights, University of Reading, UK*
[2]*School of Forestry and Natural Environment, University Campus, Aristotle University of Thessaloniki, Greece*
[3]*Palma, Mallorca, Spain*

Abstract

The varied carpet beetle, *Anthrenus verbasci* (L.), is a major pest in museums containing artifacts or specimens of animal origin, e.g., natural science collections. Integrated pest management techniques are deployed to prevent *A. verbasci* access and controlling the pest should they be found on valuable artifacts. Several synthetic chemicals have been used in the past in the fight against *A. verbasci* and other insect pest species but the use of these chemicals (such as naphthalene and dichlorvos) is now banned following health concerns. Attention is turning towards natural compounds in the search for natural, safe alternatives. As well as having active ingredients against adult *A. verbasci*, it is also essential that any useful products found fall within museum budgets. Here we examine the repellent properties of four 'off-the-shelf' essential oils: clove, lemon, lavender, and eucalyptus. All four essential oils exhibited some repellent property, but *A. verbasci* found lavender and eucalyptus oils the most repellent, offering the possibility that some easy to obtain and inexpensive natural products might have a role to play in museum IPM.

Key words: museum, IPM, Dermestidae, control, alternative techniques.

Introduction

It is likely that animal-derived artifacts in human habitations have always been beset by beetles in the family Dermestidae (Peacock, 1993). Many Dermestidae eat keratinous animal products as larvae, making animal material such as wool or skins very attractive, especially in warm, dry human habitations (Peacock, 1993). This also describes the content of many natural history collections and most likely accounts for the reason Linnaeus named *Anthrenus museorum* (L.) the museum beetle, in the belief that it was responsible for the destruction of many animal collections. We now know that although Dermestidae are prevalent in museums, *A. museorum* is very rarely found inside buildings, at least in the UK (Holloway and Pinniger, 2020). Many museums battle continually against, for example, Dermestidae and Lepidopteran moths (e.g., Tineidae) that find taxidermy, dried insect collections and artifacts made from animal hairs attractive (Pinniger, 2015; Querner, 2015).

In temperate regions of the world, *Anthrenus* species feature prominently and both the varied carpet beetle, *Anthrenus verbasci* (L.), and the Guernsey carpet beetle, *Anthrenus sarnicus* Mroczkowski, are serious pests (Armes, 1988; Pinniger and Lauder, 2018). In warmer climates additional *Anthrenus* species infest natural history collections including *Anthrenus flavipes* Le Conte (Kumar *et al*., 2013; Holloway and Bakaloudis, 2021) and *Anthrenus coloratus* Reitter (Veer *et al*., 1991; Nardi and Háva, 2019).

Insect damage prevention is addressed in the larger natural history collections by scientists who specifically manage insect activity in and around collections. This is known as integrated pest management (IPM) which in museums has evolved into a well-developed process (Pinniger, 2015; Querner, 2015) following the philosophy that it is better to keep insects away from collections than it is to control them *in situ*. Nevertheless, insects do often attack specimens, so IPM has in the past deployed a range of chemical options to manage outbreaks as part of overall strategies. Over time, many of the chemicals used have been removed from circulation for human health or environmental reasons (Batterman *et al*., 2012; Okoroiwu and Iwara, 2018). Dichlorvos, an organophosphate pesticide, was used extensively against moth and beetle activity (Linnie and Keatinge, 2000) and naphthalene was regularly and successfully used to protect pinned insect collections from insect attack (Linnie and Keatinge, 2000). Luckily synthetic pesticides such as cypermethrin-based formulations are still available to use in museums (Vondráček *et al*., 2018).

In the search to find alternatives to synthetic chemicals to control pest insects, attention has turned to plant-based compounds (botanicals) (Oyeniyi *et al*., 2015a; 2015b; Said and Pashte, 2015; Suleiman and Rugumamu, 2017; Oladipupo *et al*., 2019). Plants produce a wide range of secondary metabolites to protect against herbivory (Harborne, 1982) and there now exists a large body of research focusing on the role that some of these chemicals could play in protecting stored commodities (Said and Pashte, 2015). A well-known example of a botanical with insecticidal properties is neem *Azadirachta indica*. In India, leaves from the neem tree are used to protect manuscripts and books against damage from book lice (Psocidae) (Perumal and Wheeler, 1997). Neem oil is an effective method of control against stored product beetle pests (Jenkins *et al*., 2003; Boeke *et al*., 2004) and has paved the way for botanical pesticides, many of which have low

risk to human health, and few adverse environmental effects (Khater, 2012; Sola *et al*., 2015).

The search for natural and more environmentally friendly solutions to insect control has led to investigations into the efficacy of essential oils (EOs) as potential chemical control agents. EOs are produced commercially for their odour and contain natural compounds including hydrocarbon molecules, such as terpenes and sesquiterpenes, and other oxygenated molecules, such as esters, aldehydes and phenolic compounds (Nerio *et al*., 2010). EOs are extracted through distillation either via steam or water or by cold pressing the plants and then combined with a carrier (e.g., hemp *Cannabis sativa*) oil for use. They are generally considered to be safer for human handling than many synthetic pesticides used to control insect pests (Trongtokit *et al*., 2005) and research in this area is developing rapidly (Ilboudo *et al*., 2010; Caballero-Gallardo *et al*., 2012; Olivero-Verbel *et al*., 2013; Hernandez-Lambraño *et al*., 2015; Titouhi *et al*., 2017). Many of the studies looking at essential oils as insecticides have focussed on Tenebrionidae, Curculionidae and Bruchidae beetles; fewer have tested EO activity against Dermestidae (e.g., Bakr *et al*., 2010; Feroz, 2020). The purpose of the current study was to examine whether a variety of 'off-the-shelf' products offered the capacity to protect natural science specimens against adult *A. verbasci*.

Materials and methods

Anthrenus verbasci

A. verbasci is very common in the countryside in Mediterranean countries, and active earlier in the year than in the UK. Over 1000 *A. verbasci* were collected from Pollença, Mallorca 23-27 April 2019 and a similar sized sample was collected from Thessaloniki, Greece 6-8 May 2019. *A. verbasci* were collected by knocking them from the Apiaceae plants on which they were feeding into trays, and then aspirated using a pooter. In the laboratory, insects were fed on tissue paper soaked in sugar water. Insects were kept at 4 °C until ready for use.

Essential oils

Pure aromatic EOs were purchased online from Lagunamoon™ Beauty Ltd. The steam distilled EOs tested were eucalyptus (*Eucalyptus globulus*), clove (*Syzygium aromaticum*), lemon (*Citrus limon*) and lavender (*Lavandula angustifolia*). These EOs were selected because they are easy to obtain and relatively inexpensive. The major components in eucalyptus EO are monoterpenes, principally eucalyptol (1,8-cineole) (80+%) (Limam *et al*., 2020). Clove EO is dominated by eugenol (~80%), an allylbenzene, and β-caryophyllene (~8%) (Fuentes *et al*., 2020). Lemon EO consists largely of terpenes, but also sesquiterpenes and aldehydes (Evans, 2009). The commonest terpenes in lemon EOs are limonene (~70%), β-pinene (~8.5%), and γ-terpinene (8.5%) (Clarke, 2008). Lavender EO is a complex cocktail of nearly 50 compounds. The principal ingredients are 1,5-dimethyl-1-vinyl-4-hexenylbutyrate (43.73%), 1,3,7-octatriene, 3,7-dimethyl- (25.10%), eucalyptol (7.32%), and camphor (3.79%) (Hui *et al*., 2010).

The EOs in the present study were carried in hemp oil. To test whether hemp oil had any repellent effect in the absence of EO, hemp oil was used as a control.

Choice chambers

The choice-chamber apparatus is illustrated in figure 1. Four identical apparatus were available for the study. Each apparatus consisted of five Perspex chambers 7 cm in diameter and 4 cm deep. One chamber was located

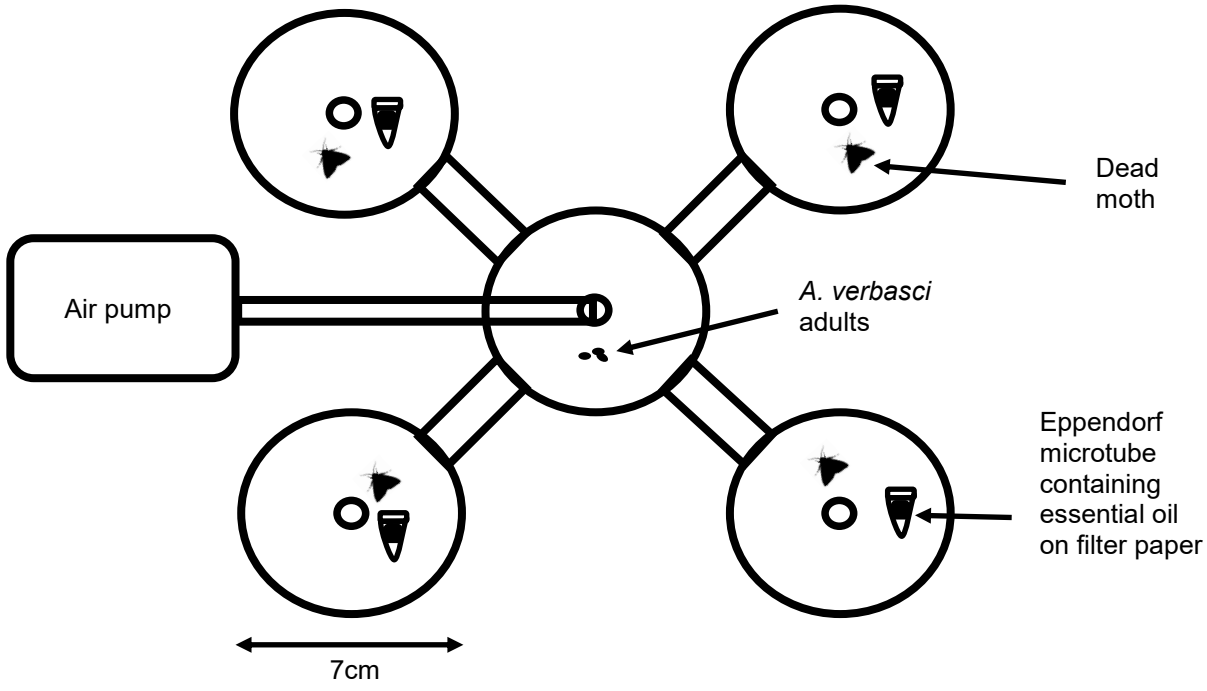

Figure 1. Choice chamber apparatus. See materials and methods for details.

centrally with the remaining four positioned equidistant around it. Each chamber had a removable lid, again made of Perspex, with a 1 cm hole drilled in the centre. The central chamber was connected to each outer chamber by a 2.5 cm length of clear plastic tubing flush with the floor of the chamber. The design allowed a gentle flow of air (approximately 1 litre per minute) to be drawn in through the lids of the outer chambers by attaching a vacuum pump to the lid of the central chamber. This level of suction had been identified as sufficient to prevent chemical saturation in similar choice experiments used to examine the response of the greater grain borer *Prostephanus truncatus* (Horn) (Coleoptera Bostrichidae) (Burkinshaw, 1998) and *Sitophilus oryzae* (L.) (Dent *et al*., 2003) when exposed to both food and pheromones. The flow rate was insufficient to suck any insects out of the chambers or to interfere with their environment. Finally, the chambers were glued to a firm plastic base to make them robust enough for cleaning and repeated use.

Each EO was delivered to the insects by dropping an undiluted volume of 0.05 ml onto a small piece of filter paper within a 1.5 ml Eppendorf tube using a micropipette. A 1 mm diameter hole was made in the top of the Eppendorf tube to allow the EO to diffuse into the chamber. All trials were run at 25 °C in complete darkness.

Experimental design

P a i r e d t e s t s

Each EO was tested against every type of EO in a pairwise manner, making six possible pairwise combinations. Prior to each test, each outer chamber was allocated a number from 1 to 4 at random. An attractive oviposition source (a single dry moth, *Orthosia* spp.) was placed into each chamber. The moths were captured using Robinson moth traps operated at two sites: grid references SU 736713 and SU 316636 during March and April 2019. The first EO type was dropped into an Eppendorf tube, allocated a random number between 1 and 4 and then placed into the outer chamber with the same number. A different EO was similarly placed into an Eppendorf tube with another randomly allocated number between 1 and 4 and placed into the outer chamber with the same number. In one other outer chamber chosen at random, an Eppendorf tube containing 0.05 ml carrier oil (hemp, *Cannabis sativa*) was placed along with a moth. The final outer chamber contained only a moth and an empty Eppendorf tube. All four choice-chamber apparatus were run in parallel with the outer chambers in each randomized independently.

To run the tests, ten randomly picked and unsexed adult *A. verbasci* were dropped into the central chamber of each apparatus. The insects were left to disperse throughout each apparatus for 1 hour and then the whereabouts of each individual was recorded. Any insects remaining in the central chamber or the connecting tubes were not included in the count. Individuals in each of the outer chambers were counted. Every 30 minutes following the first count, the insects in each outer chamber were counted again until the final count 3 hours after the start of the experiment. At the end of each trial, the five counts for each chamber were averaged to produce one value for each chamber for each trial prior to analysis. Each pairwise combination was replicated 4 times. After each trial, all *A. verbasci*, dead moths, and Eppendorf tubes were discarded. Nothing was used for a second time. After each trial, each chamber was thoroughly cleaned using 70% ethanol and left to dry before being used again.

F o u r - w a y t e s t s

Each outer chamber was randomly numbered and supplied with a dead moth. A 0.05 ml aliquot of each of the four EOs was placed individually into Eppendorf tubes using the micropipette. Each tube was assigned at random to one of the outer chambers using the process described for the paired tests. To start each trial, ten randomly chosen and unsexed *A. verbasci* were dropped into the central chamber. Their whereabouts in the choice set up was assessed after 1 hour and the number of insects in each outer chamber was recorded. Every 30 minutes after the first count, the number of insects in each outer chamber were counted again, until the last count 3 hours after the start of the experiment. At the end of each trial, the five counts for each chamber were averaged to produce one value for each chamber for each trial prior to analysis. The experiment was replicated as many times as the remaining insects in good condition would allow to maximize the power of the statistical analysis. There were enough Greek insects remaining for 44 replicates and sufficient Mallorcan insects for 20 replicates. The data were log (n+1) transformed to conform to normality and homoscedasticity. The data were run through a general linear model to account for the unbalanced data set.

Analysis

All statistical tests were carried out using Minitab version 19.1.1.

Results

Paired tests

The insects were very active, moving around throughout the experiment and not settling, which is why the five counts for each trial were averaged. The distributions of values were marginally non-random, so Mann-Whitney tests were deployed. There were no differences in the numbers of insects in the control chambers (empty tube and tube containing carrier oil) for both Greek and Mallorcan insects ($H = 595$, $n_1 = n_2 = 24$, not significant [ns], and $H = 667$, $n_1 = n_2 = 24$, ns, respectively). The data sets were combined for each country to form values for control chambers. There was no difference between control values for Greek and Mallorcan insects ($H = 2319$, $n_1 = n_2 = 48$, ns). There was no difference in counts in chambers containing an EO between insects from Greece and Mallorca ($H = 2360$, $n_1 = n_2 = 48$, ns). The data for insects from Greece and Mallorca were combined. There was a significant difference between the median number of insects in control chambers (median = 2.1) versus chambers containing an EO (median = 1.6) ($H = 10367$, $n_1 = n_2 = 96$, $p = 0.004$), indicating that the EOs were delivering a significant repellent effect overall.

The EOs were tested in pairs to establish the level of repellency offered by each oil when the insects had control chambers (refugia) to wander into. Table 1 shows the median number of insects in each essential oil chamber when tested against all other essential oils. The data in table 1 indicate that eucalyptus and lavender had higher repellency than lemon and clove, and this was the case for both the Greek and Mallorcan insects. Table 1 further shows that lemon had greater repellency than clove for both Greek and Mallorcan insects. The qualitative repellency ranking of the EOs used from the data in table 1 is eucalyptus = lavender > lemon > clove for both Greek and Mallorcan insects.

Four-way tests

The results from the paired tests enabled us to predict the outcome of the test of all four EOs run against each other at the same time. In this test there were no refugia; the insects would distribute themselves based on the relative repellency of the essential oils used. There was no difference in the mean number of insects per outer chamber, confirming that the beetles from Greece and Mallorca were equally distributed away from the central chamber and connecting tubes ($F_{1,248} = 1.33$, ns). There was a significant difference in the numbers of beetles associated with each EO ($F_{3,248} = 10.09$, $p < 0.001$). The interaction between location and EO type was not significant ($F_{3,248} = 2.05$, ns) indicating that the insects were distributed among the oil types in a similar manner for both Greek and Mallorcan specimens.

Figure 2 shows a plot of the distribution of insects among the different EOs for both Greek and Mallorcan insects. Figure 2 illustrates that eucalyptus and lavender EOs were associated with the lowest numbers of insects, whilst the highest numbers were associated with clove EO. The order of repellency yielded by the four-way test was the same as the order from the paired tests.

Discussion

This study demonstrates that the aromatic chemicals in EOs commercially available to the public have a repellent effect on *A. verbasci* and that the EOs vary in their degree of repellency, warranting further investigation into their potential use in museum IPM. With the loss of so many synthetic chemicals (e.g., dichlorvos and naphthalene) as standard components of pest management strategies (Linnie and Keatinge, 2000), having natural alternatives to manage museum pests is very attractive.

The value of EOs has been investigated against other types of pest insects, in particular biting insects such as mosquitoes and some beetle species of stored food products (Ilboudo *et al*., 2010; Caballero-Gallardo *et al*., 2012;

Table 1. Median numbers of adult *A. verbasci* in choice chambers containing a test essential oil when paired against other essential oils for insects from Greece and Mallorca. Lower numbers reflect greater relative repellency.

Test oil	Against	Median Greek	Median Mallorca
Lemon	Clove	1.7	1.1
	Lavender	2.0	1.8
	Eucalyptus	2.2	3.2
Clove	Lemon	2.0	2.3
	Lavender	2.3	2.3
	Eucalyptus	2.9	2.3
Lavender	Lemon	0.9	1.0
	Clove	1.3	0.8
	Eucalyptus	1.2	1.3
Eucalyptus	Lemon	1.0	0.8
	Clove	0.5	1.6
	Lavender	1.0	1.0

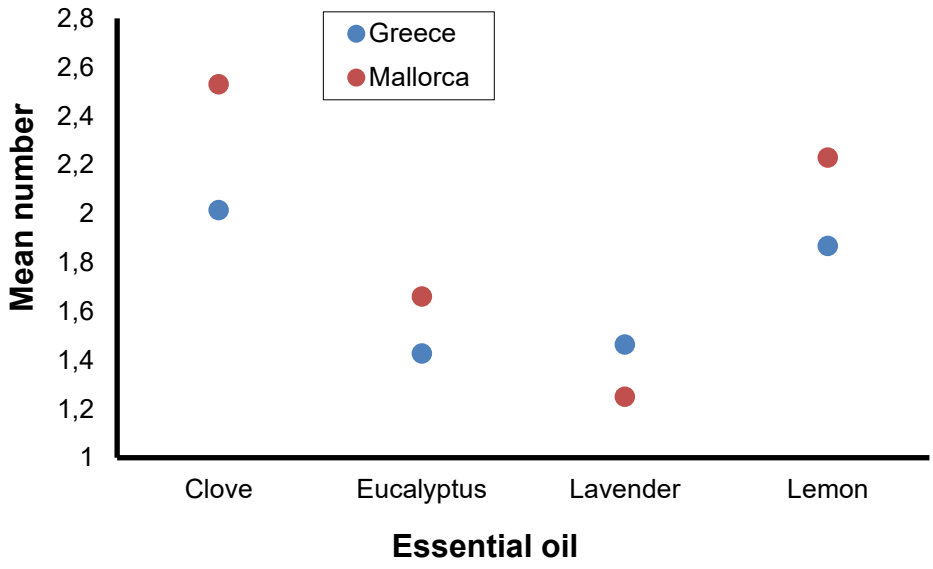

Figure 2. Mean number of *A. verbasci* adults associated with each essential oil in a four-chambered choice test for Greek and Mallorcan insects. Lower numbers indicate greater repellency.

Olivero-Verbel *et al*., 2013; Hernandez-Lambraño *et al*., 2015; Germinara *et al*., 2017; Titouhi *et al*., 2017; Marsin *et al*., 2020). Research has indicated that clove EO can protect against mosquito bites for 2 hours (Trongtokit *et al*., 2005) whilst lemon EO only protects for 30 minutes (Oshagi *et al*., 2003; Amus and Mehlhorn, 2006). Lavender EO offers close to 90% protection against biting for 8 hours (Amer and Mehlhorn, 2006) whereas eucalyptus EO can offer 100% protection against biting for 8 hours (Amer and Mehlhorn, 2006; Barbosa *et al*., 2016). It is notable that eucalyptus and lavender offer much longer lasting protection against mosquito attack that lemon and clove, a pattern reflecting the differences of repellency of these compounds against *A. verbasci* in the present study.

Here we were concerned with EOs that are simple to obtain and could be deployed easily in a museum setting. Given that they are harmless to humans, their use in museum IPM could offer a very safe alternative to synthetic compounds. The next stage would be to look at different oil products, since it is likely that different manufacturing processes produce oils with a varied profile of chemical components as well as concentration required for repellent effect. The findings here are encouraging and additional oils that are similarly easy to obtain, for example geranium (*Geranium* sp.), neem (*A. indica)* and ylang-ylang (*Cananga odorata*) could be tested and all promising candidate oils analysed in terms of repellency, persistence, and fumigant action against *A. verbasci*.

Conclusions

The study has shown that essential oils have a repellent action against *A. verbasci*, a major pest in natural science museums. There is also variation in the repellency among the different essential oils tested, with eucalyptus and lavender being more repellent than lemon and clove. This variation in repellency highlights that further research on this topic with respect to *A. verbasci* should be carried out to establish the essential oils with the most effective action against the pest insect, and which might have the potential to form a component of IPM.

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Authors' addresses: Graham J. HOLLOWAY (corresponding author: g.j.holloway@reading.ac.uk), Kayleigh PEACE, Amanda CALLAGHAN, School of Biological Sciences, HLS Building, Whiteknights, University of Reading, Reading RG6 6EX, UK; Dimitrios E. BAKALOUDIS, Aristotle University of Thessaloniki, School of Forestry and Natural Environment, PO Box 241, University Campus, 541 24 Thessaloniki, Greece; Ivan CAÑADA LUNA, 44 General Riera Street, Palma, 07003 Mallorca, Spain.

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