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Contact Toxicity, Fumigant Toxicity And Repellence Assessment Of Thymus Vulgaris And Cymbopogon Winterianus Against Blattela Germanica

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ABSTRACT

Background: Natural materials such as plant essential oils provide an exceptional alternative to synthetic pesticides to control pests and decrease the negative impact the environment and human health. The move towards using green products and the continuing need to develop new methods to control and prevent pest infestation is increasing. Pests such as cockroaches have demonstrated a growing resistance against the common synthetic pesticides. However, synthetic pesticides have been shown to have detrimental effects on humans and the environment. Thus, the need to develop safer ways for pest management.

Materials and methods: This study evaluated the effect of Java citronella and red thyme essential oils against the German cockroach, *Blatella germanica*, using three bioassay methods to determine the contact toxicity, fumigant toxicity and repellent activity. The essential oils were diluted in hexane. The dilutions were 5%, 10%, 15%, 30%, and 100% essential oil for all Java citronella and red thyme assays. Aliquots of 2.5 ml were used for every assay to treat the filter papers.

Results. Generally, the results showed a higher insecticidal effect in test groups treated with Java citronella oil than in groups treated with red thyme essential oil.

Conclusion: Java citronella and red thyme have been shown to have an insecticidal effect against the German cockroach, with Java citronella showing more affinity over red thyme as a potential cockroach repellent. This further proves that essential oils can be explored as a safer alternative pesticide.

Keywords: repellence, insecticidal, fumigant toxicity, contact toxicity

INTRODUCTION

Cockroach infestations are prevalent in warm areas, especially in residences without sufficient ventilation, hospitals, restaurants, and businesses with a high ambient temperature and humidity (Sharififard et al., 2016). Conventional insecticides are the most used way for treating cockroach infestations, despite widespread concerns about their potentially harmful side effects (Sharififard et al., 2016). In addition, due to the risk of poisoning, insecticides are prohibited in food preparation facilities, restaurants, storage buildings, and apartments. As the use of chemical insecticides is restricted, there is a greater desire for safer options to combat cockroach infestations (Sharififard et al., 2016). Even though essential oils may not be the perfect replacement for all synthetic pesticides due to their high cost, they have prospects for urban pest control; they have shown to be safer and pose less threat to human health and the environment (Isman, 2020).

In research, several essential oils were found to be equivalent to diethyltoluamide (DEET), a prominent active ingredient in insect repellents that is now advertised as the most efficient mosquito repellent (Sparks et al., 2018). DEET works by stopping insects from activating the I-octen present in human breath and sweat by blocking their olfactory receptor, which they use to find food (Sparks et al., 2018). The use of DEET, on the other hand, has prompted concerns about environmental and human health dangers, particularly in children (Sparks et al., 2018). Essential oils have thus been investigated as alternatives to standard synthetic insecticides due to their repelling properties and low toxicity to the environment, non-target creatures, and people (Pavela and Benelli, 2016). Overuse of synthetic insecticides containing organophosphates to control cockroaches has resulted in major issues that have affected non-target organisms, contaminated land and aquatic habitats, and resulted in insecticide resistance (Soares et al., 2019; Kah et al., 2018) & (Yeguerman et al., 2020).

Insect repellents provide major barriers of personal protection against arthropod-borne infectious illnesses by topical application (Robert and Debboun, 2020). Although essential oils can be used as a mosquito repellent when applied to the skin, they can only be effective at the vapour stage (Burfield and Reekie, 2005) & (Pujiarti, 2017). The German cockroach (Blattella germanica L.) is a widespread synanthropic pest (Yeom et al., 2018). B. germanica is a hazard to human health because it produces asthma-inducing allergens, acts as a mechanical vector for transporting harmful bacteria, and contributes to unsanitary indoor settings (Dietrich et al., 2014; González-Diaz et al., 2019). While pesticides are critical for reducing cockroach populations and improving health outcomes, insecticide resistance has proven to be a persistent cockroach control challenge (Fardisi et al., 2019).

The development of environmentally friendly approaches has been deliberated upon worldwide to replace conventional pesticides, and evidence suggests essential oils could be used for pest control (Campolo et al., 2017). The active compounds in essential oils are biodegradable and have lower negative effects on mammals and the environment (Bedini et al., 2018). Thus, it has been suggested that they could be used on a small scale to treat cockroach infestations in human residences and workplaces (Moretti et al., 2017). The major components of the oils reportedly affect the insect's nutritional physiology and behaviour (Moretti et al., 2017). Furthermore, other studies have reported that essential oils demonstrate lethal and sublethal effects against German cockroaches (Yeom et al., 2018).

However, factors such as great volatility and rapid oxidation affect their biological activity and resolution, reducing their prospect of being used in large-scale applications (Campos et al., 2019). Thus, the aim of this chapter is to test the efficacy of essential oils as potential alternative pesticides against cockroaches.

MATERIALS AND METHODS

Cockroaches

A total of 800 German cockroaches were used for this study. The German cockroach was chosen for this study because it is an urban pest and it is commonly found in human dwellings and food establishments, and it is responsible for transmitting disease-causing microorganisms, a source of nuisance and social stigma where the infestation is present. The cockroaches were sourced from an existing culture at Clinvet Research Facility, where they were bred under conditions that mimic their natural habitat under room temperature (~26 °C) with access to dog food pellets and water ad libitum.

These cockroaches have been cultured for several generations with the frequent infusion of 'wild' ones to maintain genetic heterozygosity and limit laboratory adaptations and inbreeding depression. From the foregoing, it is therefore assumed that the behavioural and physiological responses assessed in this study are a true reflection of free-living wild cockroaches that are not kept under controlled conditions on a standard diet.

Transportation of cockroaches was done in perforated polyethylene containers (Figure 1) to the University of the Free State, where they were kept under controlled conditions (~26 °C; 65% RH) prior to the onset of experiments.

Figure 1. Showing live cockroaches in polyethylene containers used for transportation.

Bioassays: Contact and fumigant toxicity and repellence

Two essential oils, Cymbopogon winterianus (Java citronella) and Thymus zygis (red thyme) were used; the oils were sourced from Thitapoho farm, where they were extracted through the steam distillation method.

Contact Toxicity: The bioassay method developed by WHO (World Health Organization, 1975) was used to determine resistance or susceptibility of the cockroaches to the selected essential oils with some modifications. Briefly, treatment solutions for the bioassay were prepared by dissolving essential oils in hexane as the solvent (v/v) at concentrations of 5%, 10%, 15%, 30% and 100%. Thereafter, aliquots of 2.5 ml for each treatment solution were used to immerse/imbed filter papers before immediately placing them at the base of desiccation jars (27 cm diameter: 28 cm height).

In each jar, five male or female cockroaches were then placed before closing with a lid smeared with petroleum jelly (Vaseline®) to ensure an airtight seal. In all cases, the control group consisted of jars with only the organic solvent hexane on the filter paper. Each jar was regarded as a replication for each treatment, and a total of five replications were done to yield a sample size of 25 cockroaches used for control.

Fumigant Toxicity: Essential oil treatment mixtures were prepared in hexane as the solvent (v/v) at concentrations of 5%, 10%, 15%, 30% and 100%. Thereafter, aliquots of 2.5 ml for each were used to immerse filter papers and immediately place them at the top of desiccation jars at 10 ml solution. Groups of five cockroaches were transferred using a mechanical aspirator into desiccation jars (27 cm diameter: 28 cm height). Care was taken to ensure that filter papers were not soggy (too wet) and dripping such that any cockroach mortality would have been because of fumigant toxicity and not coming in direct contact with the oil.

In each jar, five male or female cockroaches were then placed before closing with a lid smeared with petroleum jelly (Vaseline®) to ensure an airtight seal. In all cases, the control group consisted of jars with only the organic solvent hexane on the filter paper. Each jar was regarded as a replication for each treatment, and a total of five replications were done to yield a sample size of 25 cockroaches used for control. The mortality for both assays was calculated between the moment of exposure to the first 24 hours. Mortality was assessed by the cockroaches laying on their backs and the ability of cockroaches to move when poked with a brush on their stomachs.

Repellency: The methods by Appel, Gehret, and Tanley (2001), Manzoor, Munir, Amdreen, and Naz (2012) and Sharififard, Safdari, Siahpoush, and Kassiri (2016) were used for this study with some modifications. A total of 360 cockroaches were used in this method.

A filter paper was cut into two equal pieces, and one half was treated with Java citronella and red thyme, respectively, using a pipette, and the other half was treated with hexane. Different concentrations of (5%, 10%, 15%, 30% and 100% v/v) oil solutions were used at five replicates per concentration. Each treated filter paper was left for two minutes to dry before the start of the repellence test. It was followed by placing the filter paper at the bottom of the rectangular airtight, transparent box divided in the middle and sealed with parafilm. The filter paper treated with hexane was placed on the right side of the assay box. The five adult German cockroaches were released at each replicate into the centre of each assay box. The distribution of the test subjects was observed at 0, 5, 10, and 15 minutes.

Data Analyses

The effects of essential oil type and concentration on contact toxicity and repellence were compared using Two-Way ANOVA in Statistica 9.0 (Statsoft, Tulsa, OK, USA). In this instance, the type of essential oil and concentration were regarded as the categorical predictors whilst either survival (contact toxicity assays) or the number of cockroaches away from the odour source (repellence assays) were dependent variables. Key assumptions of ANOVAs for homogeneity of variance and normality of data distributions were met following tests using the Hartley-Bartlett test and Shapiro-Wilk test, respectively. Tukey's HSD post-hoc tests were used to identify statistically homogenous groups at $p = 0.05$.

RESULTS AND DISCUSSION

Contact toxicity: Concentrations of 15%, 30% and 100% of Java citronella essential oil caused 80%, 92% and 100% mortality, respectively, after the first 24 hours. The same concentrations of red thyme essential oil resulted in mortality rates of 44%, 52%, and 72% after 24 hours, which differed compared to Java citronella.

Furthermore, a difference was noted at the lowest concentration between the two oils, where Java citronella caused mortality rates of 52% and 64% at 5% and 10% concentrations and red thyme had mortality rates of 20% and 15% at 5% and 10% concentrations. The results demonstrate that Java citronella has higher activity against the German cockroach than red thyme as a possible insecticide. A similar study was done where insecticide properties of essential oils and some of their constituents were tested on the Turkestan cockroach. In this study, cockroaches were shown to avoid the essential oil, further proving that essential oils present insecticide activity against arthropods (Sudip et al., 2017).

Figure 2. Showing dead cockroaches following contact toxicity treatment.

Fumigant toxicity: Fumigation with Java citronella caused mortality rates of 60%, 72% and 96%

and 15%, 30% and 100% compared to red thyme at the same concentrations, but with mortality rates of 40%, 36% and 84% using red thyme essential oil, mortality rates were 44%, 52% and 72%, after 24 hours. Furthermore, a difference was noted at the lowest concentration between the two oils, further proving that Java citronella is more effective as a possible fumigant insecticide against the German cockroach over red thyme. No mortality was observed for the control (hexane) in all replications of both contact toxicity and fumigant toxicity.

Figure 3. Showing dead cockroaches following contact toxicity treatment.

Table 1. Summarized output of a Two-way ANOVAs testing the effects of essential oil type and concentration on the mortality of German cockroaches following direct contact exposure and fumigation using Java citronella and red thyme. Significant treatments are denoted in bold font. The results show that Java citronella has more affinity over Red thyme on contact exposure and fumigant exposure, and can be explored as a safer alternative pesticide, that is less harmful to humans and non-target organisms.

	Source/Effect	**SS**	**DF**	**MS**	**F**	**P**
Contact exposure	Oil type	11760	1	11760	65.3	**<0.001**
	Concentration	47333.3	5	9466.6	52.6	**<0.001**
	Oil type Concentration	2960	5	592	3.3	**0.012**
	Error	8640	48	180		
Fumigation exposure	Oil type	2940	1	2940	13.6	**0.001**
	Concentration	48753.3	5	9750.7	45	**<0.001**

Oil type	Concentration	1340	5	268	1.23	0.306
	Error	10400	48	216.7		

Repellence: The repellent effect of Java citronella and red thyme against the German cockroach is presented in Figure 4. The study revealed an increasing repellent effect on the German cockroaches when Java citronella was used at a lower concentration of 5%. In comparison, the repellence effect started showing against red thyme at concentrations of 15% of the oil solution. An increased repellence was observed in all concentrations with increased exposure time, with Java citronella still showing the most repellent effect against the German cockroach.

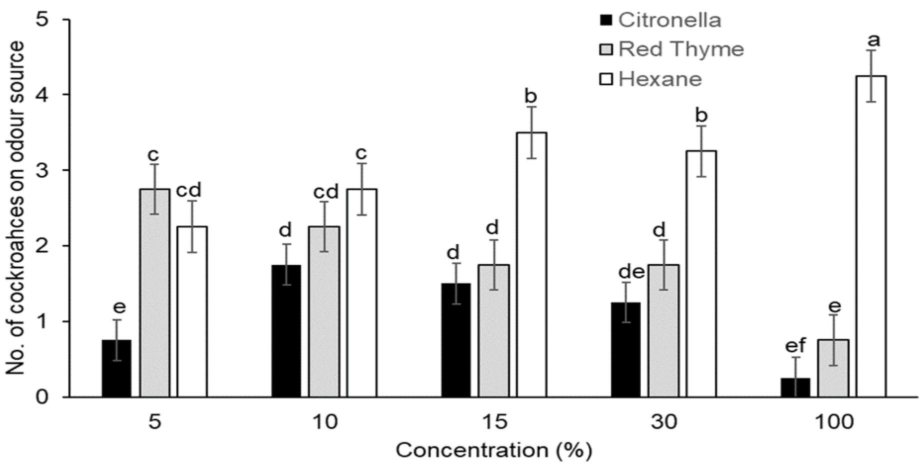

Figure 4. Repellent effect of Java citronella and red thyme against the German cockroach.

Mechanism of action of Java citronella against the German cockroach

Java citronella (Cymbopogon winterianus) shows to be the more effective oil that presents with insecticidal activity; this is because of the active compound in the oil, a-Terpineol. Therefore, it is suggested that α-Terpineol may be a potential agent for developing natural insecticides or fumigants and repellents to control insects. Cockroaches have strong chemoreceptors, consisting of 144 olfactory receptors for smell and 544 gustatory receptors for taste.

As a result, they consume anything they encounter, including non-food items such as cardboard and plants that can poison them and other animals. Therefore, essential oils, due to their pungent aroma, serve as the best alternative to get rid of these pests by attracting cockroaches to them, posing toxicological effects, and potentially killing them, but that is not the case. This is because cockroaches have an enzyme called cytochrome p450s which helps them withstand poisonous chemicals. These genes help the cockroach by detoxifying the poison and keeping it safe.

However, most essential oils, like Java citronella, work as neurotoxins to the cockroach by disrupting the neurotransmitter, and other compounds like tannin are also found in plants, which are inhibitors of enzyme activities that aid digestion in insects (Ajayi et al., 2018). The chemoreceptors in the cockroach are responsible for the attraction of the cockroach to the oil; the toxins work by penetrating the insect's body through its respiratory tract as soon as the oil makes contact with the cockroach’s body (Feroz, 2020). Although cockroaches can detoxify most chemicals, they cannot do the same with Java citronella because the mode of action is different.

CONCLUSION

Because the German cockroach can be found in all areas of a building, including garment lockers, mattresses, furniture, and cupboards, spraying chemical insecticides in these areas might be difficult. Synthetic pesticides are also prohibited in the food industry due to their long-term effects, which can be detrimental to consumers. Because essential oils are less hazardous to humans and leave no toxic residues, they are a safe choice for indoor cockroach control. Considering all of the active chemicals in the oil that are responsible for its efficacy, Java citronella oil demonstrated a significant effect against Blatella germanica at the lowest concentration of 5%, and so can be recommended as a potential repellent.

Investigation on a larger scale and under different field conditions is needed to assess its efficacy and the practical application, and develop favourable formulations of essential oils against cockroaches. However, polymeric nanoparticles’ design is an innovative idea for new bioinsecticides technologies. These technologies include incorporating EOs in a nanoencapsulation to prevent the rapid degradation and evaporation of EO constituents, increasing their toxicity and shelf life and improve the handling.

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CONFLICTS OF INTEREST

The author does not have conflicts of interest to declare.

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