Toxicity and Physiological Effects of Essential Oil Components against the German Cockroach, *Blattella germanica* (L.) (Ectobiidae)

by

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Abstract

The overarching goal of this research is to extend the scholarship on and provide new evidence that rationalizes the inclusion of low toxicity, plant-based essential oils (EOs), and their components (EOCs) into integrated pest management systems (IPM) for insect pests such as the intractable urban insect, the German cockroach, *Blattella germanica* (L.). In Chapter One, I attempted to disentangle the myths surrounding the origin of *B. germanica*, provided a commentary on their co-evolution with humans, reviewed associated public health concerns, and provided genetic insights into *B. germanica* resistance to conventional insecticides. Additionally, I suggested that EOCs could be used to either delay or circumvent insecticide resistance or even suppress *B. germanica* cockroaches. As a corollary, in Chapter Two, I provided a synthesis on EOs and EOCs employed in the management of urban insects. Notably, I summarized key data on the use of EOs, EOCs, and commercially available EO formulations against ants, bed bugs, cockroaches, fleas, head lice, stored product moths, silverfish, and termites. This synthesis highlights insecticidal activities of EOCs against a broad range of urban insect pests, which I selected for my subsequent investigations against *B. germanica* in later chapters. Further, I discussed knowledge gaps, conundrums, and offered probable insights into how laboratory/field-based investigations of EOs/EOCs should be approached if eventual integration into urban insect management is sought. In Chapter Three, I evaluated the toxicity profiles of eight EOCs against susceptible and multi-resistant *B. germanica* cockroaches. The results demonstrated that limonene (aliphatic), carvacrol, eugenol, and tropolone (aromatic) were the most toxic against *B. germanica*. Structural-activity relationship revealed that vapor pressure and molecular weight are important metrics of EOCs that moderate toxicity. The higher the molecular weight and vapor pressure of an EOC, the more toxic it is likely to be against *B. germanica*. The use of EOCs in

controlling urban and structural pests is limited because of their high volatility. Consequently, in Chapter Four, I explored superabsorbent polymer (SAP) gels as a carrier to deliver selected EOCs in a bid to prolong their insecticidal activity against *B. germanica*. The results provided several new insights on how these EOCs can suppress the reproductive fitness of *B. germanica*, including reductions in (i) female reproductive period (ii) oothecal hatchability, and (iii) fecundity. The findings highlight the potential use of SAP gels to prolong the bioavailability of EOCs, thereby solving the problem of high volatility and achieving extended insecticidal effects against targeted pests. The use of SAP carriers also demonstrates the potential of incorporating EOCs as baits. In Chapter Five, I investigated the physiological effects of limonene, carvacrol, and β-thujaplicin on the DGC of insecticide-susceptible and multi-resistant *B. germanica*. Two observations were noteworthy: (1) the EOCs resulted in *B. germanica* abandonment of DGC, and (2) increased respiratory water loss following treatment with an EOC. In toto, the cost of this DGC loss substantiates previous findings and my meta-analytic conclusion: DGC serves to reduce respiratory water loss in insects. Importantly, it suggests that EOCs probably achieve kill via lethal desiccation. In Chapter Six, I conducted a meta-analysis to resolve the controversies surrounding the hypotheses postulated to explain why insects shut their spiracles periodically. This included data from 46 insect species in 24 families across nine orders. Insects breathe with the aid of thin capillary tubes that open out to the exterior of their body as spiracles. These spiracles are often modulated in a rhythmic gas pattern known as the discontinuous gas exchange cycle (DGC). Several explanations have been put forward to rationalize this process, but two controversial ones gain the most support: the rhythmic pattern is to (1) reduce water loss or (2) facilitate gaseous exchange in environments with high carbon dioxide and low oxygen. The meta-data supports that DGC reduces water loss in insects. However, DGC does not facilitate gaseous exchange in a high carbon dioxide or low oxygen environment. This investigation is the first meta-analytic attempt to resolve the controversies surrounding the merit of adaptive hypotheses in insects. In Chapter Seven, I summarized the major findings of my dissertation research. As existing cockroach control strategies are not always sufficient, future studies should seek to investigate formulations that could be used to deliver EOs and EOCs in field-based studies. In addition, possible synergistic combinations of EOs/EOCs with currently used conventional insecticides is an under-researched area.

Taken together, the outcomes of my research contribute to the pest management industry by providing alternatives to synthetic insecticides, delaying resistance development, creating environmentally conscious pest management tools, providing solutions for public health pests, and creating affordable options.

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Dedication

This milestone is dedicated to my late mother, Ms. Oluwafiropo Abiona. Dear mother, your soul continues to kindle a light even in a world filled with darkness.

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List of Abbreviations

EOs	Essential oils
EOCs	Essential oils components
IPM	Integrated pest management
AI	Active ingredient
LD	Lethal dose
LC	Lethal concentration
LT	Lethal time
PBO	Piperonyl butoxide
SEM	Standard error of mean
SAP	Superabsorbent polymer
DGC	Discontinuous gas exchange
CO_2	Carbondioxide
O_2	Oxygen
RWL	Respiratory water loss
CWL	Cuticular water loss
UNE	Unknown mode of action

Chapter 1

Blattella germanica (L.): The Myth, The Insect, and The Public Health Menace

1.1 Where do *Blatella germanica* originate from?

Throughout nature, science has been plagued with numerous misnomers. One of such is in the common name German cockroach, *Blattella germanica* (L.), that deceptively suggests origination from Germany. From its scientific name, it is clear that the currently designated specimen was typed by the Swedish scientist, Carl "L" for Linnaeus (1707 – 1778). Interestingly, it is commonplace in the 17th century to assign names to species based on the source of their specimen (Schal, 2011). In fact, *Blattella germanica* was never the senior homonym (i.e., the first name assigned to this cockroach species), *Blattella transfuga* assigned by Brünnich in 1763 was. Yet, the commission on zoological nomenclature simply decided to use Linnaeus' nomenclature instead of that of Brunnich and thereafter put his name in parentheses i.e., "(L)" to evince this change. So, we know the German cockroaches were not "manufactured" by the Germans. If anything, cockroaches, including the German cockroach, predate human existence. Indeed, the carboniferous period of the late Paleozoic era is repleted with cockroach fossils such that it is jokingly dubbed the "Age of cockroaches" (Grandcolas 1998). As a result, one can reasonably conclude that cockroaches have been around for about 300 million years before the first humans appeared on the geological time scale. But as one supercontinent broke apart, cockroaches probably found a way to move across continents. For example, the ancient supercontinent, Gondwana, split into landmasses recognized today as Africa, South America, Australia, the Indian subcontinent (probably southeast Asia), and the Arabian Peninsula (Ali and Krause 2011). Of course, such migration was undoubtedly accelerated by humans. This is probably why numerous authors point to the split landmasses (i.e., Africa, the Indian subcontinent, and the Arabian Peninsula) as the likely origin of *B. germanica* (Roth 1985, Schweid 1999). The clearest road map of where *B. germanica* originated from and how they got into America is probably from the Purple people (i.e., Phoenicians). Through their maritime trading culture, Phoenicians spread *B. germanica* from Phoenicia (present-day Lebanon) along the Arabian Peninsula, in Greek vessels, to Byzantium (present-day Istanbul). Istanbul is bordered by the Black Sea, Asia Minor, and Russia (Schweid 1999, Schal 2011). It was first hypothesized that from Russia, *B. germanica* made its way to western Europe and the Americas (Schweid 1999). However, this hypothesis has been questioned (Roth 1997, Tang et al. 2019). This is because *B. germanica* from Asia are more morphologically similar to those currently found in Africa (Princis 1950, Roth 1997). Neither hypothesis is definitive nor “bulletproof” since there was no clear transport route from Asia to Eastern Europe at the time (Tang et al. 2019).

1.2 *B. germanica* co-evolution with man – a prequel to public health concerns

While the origin of *B. germanica* is a topic of debate, what isn’t is their co-evolution with mankind. In fact, no *B. germanica* populations have been discovered in locations devoid of man-made structures. The allure to a man-made structure is straightforward, *B. germanica* prefers a warm environment (28–32 ºC). The co-evolution of *B. germanica* with humans is particularly interesting. It is a type of relationship where *B. germanica* “need” humans to survive and must, in addition, shaped its biology to survive despite humans. For example, cockroaches of many kinds are active during the day (i.e., diurnal). Thus, *B. germanica* preference for nighttime activity (i.e., nocturnal) can be assumed to be an avoidance behavior to avert being seen by humans. This is probably why the Romans referred to them as "*lucifaga"* for their habit of avoiding and fleeing from light (Schal 2011). Besides, to survive humans and other environmental variables, *B. germanica* has a high lifetime reproductive output. An adult female typically produces 5–8 egg cases (i.e., oothecae) per lifetime (Bell et al. 2007, Oladipupo et al. 2020a). Each ootheca houses 30-40 eggs (Bell et al. 2007). Hence, a female can produce 150–320 young ones (i.e., nymphs). This high nymphal output may be necessary to keep the *B. germanica* life cycle in motion despite human interference. Furthermore, both *B. germanica* (found indoor) and its closely related sister, *B. asinahi* Mizukubo (found outdoors) have wings. So close is the relationship between both species that an untrained eye cannot tell one apart from the other. Yet, *B. germanica* does not fly while its sister, just outside, does. The loss of flight in *B. germanica* is arguably a necessary token that must be paid lest they evoke the wrath of humans by flying around in homes– a trait that allows it to survive despite humans. Moreover, *B. germanica* like other cockroach species is dorsoventrally flattened. This body architecture allows it to interject between cracks and crevices swiftly and conveniently in homes further limiting exposure/evading humans. Finally, humans eat plants and animals (i.e., omnivorous), *B. germanica* eats everything a human will. Consequently, *B. germanica* seeks kitchen counters, sinks, tabletops, water faucets, and food storage closets to fulfill their daily ration of food and water.

Unfortunately, *B. germanica* won't stop at eating only what humans eat. They take whatever they can get, wherever they can get it. For example, they can also feed on animal wastes (detritivorous), decaying organic matter (saprophagous), and feces (coprophagy) (Schal et al. 1984, Nalepa et al. 2001). It is these diverse feeding strategies that implicate *B. germanica* as a significant public health menace. They have capacity to not only serve as a vector from the various substrates they source their food from, but also likely to be a reservoir host keeping the cycle of noxious and pathogenic organisms alive. For example, *B. germanica*, collected from swine feces on swine farms, can share the same pathogens as those present in the swine (Ahmad et al. 2011). *Blattella germanica* has the propensity to regurgitate fluid from their mouth while feeding (Buczkowski and Schal 2001), and farm animals can pick up *B. germanica* as food (Ahmad et al. 2011). Even if the swine were treated for *Enterococcus feacalis*, *B. germanica* would have serve two roles: first, as a vehicle to transmit these pathogens from one organism to another (i.e., a vector) and as a "storage tank" (i.e., reservoir host) to keep the cycle of such pathogen in place. Besides, the gut of *B. germanica* houses a plethora of pathogenic bacteria (Carrasco and Pérez-Cobas 2014, Pérez-Cobas et al. 2015). The consequence of these in homes is that *B. germanica* can disseminate human pathogens and contaminate food and surfaces (Zurek and Gorham 2008). Similar observations have been made in hospitals where *B. germanica* was implicated in transmitting nosocomial infections (Pai et al. 2004, Elgderi et al. 2006, Donkor 2019). The most dominant bacteria housed by *B. germanica* include *Escherichia*, *Klebsiella*, *Enterobacter*, *Streptococcus*, *Salmonella*, *Shigella*, and *Staphylococcus* (Pai et al. 2004, Elgderi et al. 2006, Solomon et al. 2016, Chehelgerdi and Ranjbar 2021, Mpuchane et al. 2006). What is found in the gut of *B. germanica* reflects what they forage on (Pérez-Cobas et al. 2015). The accidental entry of *B. germanica* into human body has been reported (Bressler and Shelton 1993, Kroukamp and Londt 2006). For example, a man in New Zealand thought his ear was blocked from water only to find that the blockage was a cockroach (Gilbert 2022). In the US, there was a similar case in Florida (Jones 2018., Phillips 2018). It has also been found lodged inside the human intestine via colonoscopy examination (Seriously Science 2015).

Blattella germanica presence in homes can also be a source of embarrassment to the homeowners.

Perhaps, the most important public health concern of *B. germanica* is their trigger of asthma in sensitive individuals. This is a result of a combination of their attar, dry feces, exoskeleton, and exuviae. An attar constitutes the fluid from their abdominal scent gland, the brown fluid they regurgitate from their mouth while feeding, and their excrement (Schal 2011). Asthma is an inflammatory condition in which the lining of the bronchia of the lungs undergoes reversible constriction (Lemanske 2000). Asthmatic patients may suffer from episodes of breathlessness, mucus cough, wheeze cough, and a hyper-response to a variety of environmental triggers (Gore and Schal 2007). It is estimated that 32 million people in the US suffer from asthma (Dey and Bloom 2005). In 1998, the cost associated with the treatment of asthmatic patients was about $13 billion (Weiss and Sullivan 2001). Adjusting for inflation (accumulated inflation rate of 71.04%), puts it at ~$22 billion today. Unsurprisingly, asthma is the most frequent cause of hospitalization among children (Rabito et al. 2011, Do et al. 2016). This is because children typically spend more time indoors, play with pets that would have otherwise picked up attar from *B. germanica* runways. The relationship between *B. germanica* as triggers and asthma is complex because there are other factors involved in the pathogenesis of asthma (Toskala and Kennedy 2015). Two components, Bla g 1 and Bla g 2, isolated from gut and feces of *B. germanica*, respectively, have been found in 63% of homes in high-rise apartments, older homes, and homes of low-income households (Cohn et al. 2006, Pomés et al. 2017). Twenty-six of the US population is sensitive to *B. germanica* allergen by skin test (Arbes Jr. et al. 2005). The prolonged exposure to *B. germanica* has been cited as a recurring trigger of asthma especially in multi-unit apartments (Krieger et al. 2010, Rabito et al. 2011). Prolonged exposure would be further compounded by today's housing design, movement restrictions imposed by COVID-19, would ensure that more time is spent indoors ultimately leading to prolonged exposure to *B. germanica* attar. So, the question remains, how do we stop all of these?

1.3 Overview of interventions for *B. germanica* control

Like every pest control tactic, the primary line of defense against these public health concerns is to minimize *B. germanica* infestation. Consequently, the goal of an effective pest control tactic would be to put a barrier between humans and the pest. In a home sparsely infested with *B. germanica*, the understanding of why the insect seeks refuge in a home in the first place: for food, water, and shelter, could be exploited for control. This can be done by eradicating all possible sources of food and practicing protocols to quickly deal with them. In an unifested home, the approach would be to anticipate all possible scenarios of *B. germanica* introduction and mitigating possible establishment of *B. germanica*. Taken together, these measures could frustrate the introduction and establishment of *B. germanica* in homes. Homeowners can also purchase their pesticides and try to control this pest on their own. On a larger scale, *B. germanica* is commonly managed by seeking the help of a pest management service. Pest management companies rely heavily on the use of insecticides, including gel bait insecticides to manage *B. germanica*. However, this approach has been largely ineffective and inefficient for several reasons: (i) it lacks prior monitoring, (ii) it is wasteful and uneconomical as it provides treatment to units with no *B. germanica* infestation, (iii), it is not enough – provides too little treatment where much is required, and most importantly (iv) the development of insecticide resistance.

1.4 Where are *B. germanica* so resistant to insecticides?

The development of insecticide resistance is not new. Shortly after pest control started in the US in 1840, Axel Melander reported the first case of insecticide resistance – San Jose scale resistance to lime-sulfur in 1914 (Melander 1914). Today, *B. germanica* has developed both cross-resistance and multiple resistance to synthetic insecticides. For example, *B. germanica* has developed resistance against insecticides that act on sodium channel modulators (e.g., λ-cyhalothrin and permethrin), GABA-gated chloride channel blockers (e.g., fipronil) glutamate-gated chloride channel allosteric modulators (e.g., abamectin), Acetylcholinesterase (AChE) inhibitors (e.g. propoxur and chlorpyrifos), nicotinic acetylcholine receptor (nAChR) competitive modulators (e.g., thiamethoxam and imidacloprid), voltage-dependent sodium channel blockers (e.g., indoxacarb), and non-specific inhibitors (e.g., boric acid) (Limoee et al. 2007, Chai and Lee 2010b, Gondhalekar et al. 2013, Wu and Appel 2017a, Fardisi et al. 2019, Hou et al. 2021). Additionally, baits often touted as effective at suppressing *B. germanica* populations (Rabito et al. 2017) can no longer be relied upon (Wang et al. 2004, Gondhalekar et al. 2013, Wada-Katsumata et al. 2013). So, the question is why is *B. germanica* successful at developing resistance?

While physiological mechanisms such as increased sequestration and excretion (Valles et al. 1996), target site insensitivity (Liu et al. 2000), decrease in the rate of cuticular penetration (Wei et al. 2001), and bait aversion (Wada-Katsumata et al. 2013) are often used to explain the mechanisms via which *B. germanica* metabolize or avert synthetic insecticides, transcriptome explanations exist (Wei et al. 2001, Silva et al. 2012, Harrison et al. 2018). Transcriptomics offers genetic insights into the functional genes related to important physiological functions. *B. germanica* can metabolize insecticide with different active ingredients (AI). For example, 258 genes are designed for detoxification while 16 genes mediate target-site insensitivity (Zhou et al. 2014). One hundred and sixty three of the 258 detoxification genes are targeted for cytochrome P450s, 64 for glutathione s-transferases (GSTs), 12 for carboxylesterase, 19 for ATP-binding cassettes. For comparison, *Aedes aegypti* (L.) can show up to 372-fold resistance (Sumra et al. 2021) and this is only mediated by 178 cytochrome P450s (Silva et al. 2012). Also, *B. germanica* can eat anything. This digestive ability is mediated by 274 genes (Harrison et al. 2018). Yet, a combined 274 genes are designed for detoxification and target site insensitivity in *B. germanica*. In toto, transcriptomic investigations have revealed how *B. germanica* can metabolize a broad range of insecticides into less-harmful compounds using cytochrome P450s (Silva et al. 2012), hydrolyze carboxylic esters in organophosphates and pyrethroid insecticides using carboxylesterases (Hemingway and Karunaratne 1998), and convert/conjugate DDTs and organophosphates into water-soluble conjugates that can be readily excreted/sequestered using GSTs (Enayati et al. 2005). Interestingly, a GST enzyme, Bla g 5 is also an asthma trigger (Arruda et al. 1997). So, *B. germanica* is not only a resistant nuisance pest but also possesses' allergens of public health concerns.

As existing *B. germanica* management strategies are not always sufficient (Pai et al. 2005, Fardisi et al. 2019, Hou et al. 2021), and must either be bolstered by other techniques (Perry and Choe 2020, Hamilton et al. 2021) or replaced by new methods (Gaire, Scharf, et al. 2020, Oladipupo et al. 2020a, 2020b), there is a desire for new alternatives. As with most insecticide resistance management programs, the concepts of integrated pest management (IPM) are often encouraged (Gordon 2020, Ko 2021). However, IPM presents unique challenges. The decision to judicious use of insecticide in an IPM program depends on the economic threshold. The economic threshold is the population density at which control treatment will provide an economic return (Gordon 2020). In other words, it is the tolerable level of pest infestation. This is a metric that would differ from one case to another. And thus, it would be problematic to establish a concencus on economic threshold for cockroach control. In other words, IPM precepts could be difficult to implement for urban insects such as *B. germanica*. However, lessons from plant-insect interactions, such as induced defenses used by plants (via phenolics and terpenoids) to ward off insect attack (Berenbaum et al. 1986, Li et al. 2002), show plant secondary metabolites also known as essential oils (EOs) can be exploited for insect control. Additionally, EOs are relatively safe, inexpensive, and many are exempt from Environmental Protection Agency screening (Koul et al. 2008) to facilitate laboratory investigations and product development. Thus, the shift from the use of synthetic insecticides to EOs would achieve three goals: (i) reduction in synthetic insecticide application, (ii) delay of insecticide resistance development, and (iii) *B. germanica* population reduction.

1.5 Study Objectives

The objectives of this study were to investigate the toxicity and physiological effects of EOs against *B. germanica*. Specifically, I:

(a) Reviewed EOs and essential oil components (EOCs) used in urban pest management. This review:

  i. Systematically presents knowledge on EOs, EOCs, and commercially available EO formulations employed in the management of urban insects.

  ii. Highlights each urban insect pest, we presented the range of insecticidal effects of these natural products and outlined laboratory and field evidence.

    iii. Discusses the gaps in knowledge and possible prospects for EOs in urban insect management.

(b) Determined the topical toxicity of aliphatic and aromatic essential oil components (EOCs) against insecticide-susceptible and insecticide-resistant *B. germanica*. The goal was to:

    i. Evaluate toxicity profiles of susceptible and resistant German cockroach strains to different EOC classes.
    ii. Investigate the role of the synergist PBO in increasing EOCs toxicity against cockroach strains.
    iii. Can “insecticide resistance” be documented for EOCs?

(c) Evaluated the propensity of sublethal dose of essential oil components in superabsorbent polymer (SAP) gel to modify biological parameters of *B. germanica*. The goal was to:

    i. Investigate the ability of SAP gels to deliver essential oil components.
    ii. Understand how these EOCs shape the biological parameters of *B. germanica.*

(d) Measured the disruption and recovery characteristics of the discontinuous gas exchange cycle of insecticide-susceptible and resistant *B. germanica* strains by essential oil components. The goal was to:

    i. Investigate the influence of limonene, carvacrol, and β-thujaplicin on discontinuous gas exchange (DGC) characteristics of *B. germanica* strains.
    ii. Understand how these EOCs shape water conservation and metabolic rate of *B. germanica.*

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Chapter 2

Essential Oils in Urban Insect Management – a Review

2.1 Abstract

The allures of city life have culminated in the rise of urban populations resulting in social issues such as overcrowding, poor housing, inadequate sanitation, and solid waste disposal. These conditions have accelerated the degree of synanthropic of certain urban insect pests such as ants, bed bugs, cockroaches, fleas, head lice, silverfish, stored product moths, and termites. Globally, the public health significance of these urban insect pests is enormous, ranging from billions of dollars to loss of lives. Most chemical insecticides no longer provide the anticipated level of control, and significant insecticide resistance has been reported. Therefore, there has been a spike in interest for alternatives to conventional insecticides. Among them, natural products from plants such as essential oils (EOs) and essential oil components (EOCs) have enjoyed the most attention owing to widespread reports of efficacy and toxicity even against insecticide-resistant urban insects. Yet, there is no comprehensive synthesis on the extent and impact of the management of urban insects using EOs or EOCs. Such a review is highly relevant since it provides a means to assess the extent of progress made, shortfalls, limitations, and prospects. More so, we hope it can be used to make informed decisions and develop relevant policies reliably. We present the ranges of insecticidal effects of EOs, EOCs, and commercially available EO-based products from laboratory and field studies. Finally, we discuss the gaps in our knowledge and prospects for the sustainable use of EOs.

2.2 Introduction

As Thomas Edison illuminated the dark corners of the world with the electric light bulb and the internet allowed real-time communications beyond the range of a single human voice, the industrial revolution rescued the pre-industrial world from its economic upheaval. However, the industrial revolution has resulted in many consequential problems. Many pre-industrial communities were, mildly put, hunter-gatherers who lived simply within a natural and rustic environment (Svizzero and Tisdell, 2016). The infrastructure and demand imposed by the industrial revolution transformed dense forests and landmass to accommodate urbanization. These (urban) transformations have triggered a profound rural to urban migration as rural inhabitants move to enjoy the allures of urban communities. While urban communities typically boast superior health care facilities, education, and convenient transportation, the burden of unchecked migration often overwhelms these infrastructures (Mitlin and Satterthwaite, 2013). Thus, overcrowding, poor housing, inadequate sanitation, and solid waste disposal, and unsafe drinking water become common in rapidly urbanizing areas.

Within the context of urban entomology, the inter-mixing of humans between urban communities, ease of transportation, “erasure” of geographical borders due to greater mobility, and cultivation of landmass to accommodate increasing urban population expansion may have amplified the proliferation and degree of synanthropy of certain urban insect pests. Even worse, decaying and unhygienic conditions that are common in many urban cities may have helped to establish the “housekeeping” triumvirate viz. – food, shelter, and water – central to the basic survival of urban insect pests. Additionally, the aging and decay of “old” infrastructures such as sewage systems, roads, bridges, and even buildings lead to decay and more harborages for these urban insects. Notably, ants (Hymenoptera), bed bugs (Hemiptera), cockroaches (Blattodea), fleas (Siphonaptera), head lice (Phthiraptera), silverfish (Zygentoma), stored product moths (Lepidoptera), and termites (Isoptera) benefit immensely from the new opportunities created by urbanization. For example, sewage systems and septic tanks are a consequence of urbanization, which paradoxically, serves as a reservoir for certain cockroaches to breed and thrive. While termites construct exploratory tunnels to gain access to cellulose in wooden structures that are typical of modern-day homes, ants also often construct interconnected nests in response to a food source (Su, 2002; Siramon et al., 2009; Potter, 2011). Silverfish and stored products pests (e.g., moths) thrive in homes in response to the presence of books, organic fibers (wool), skins (leather), warmer conditions, and stored products (e.g., grains, flours, and nuts) (Kuo et al., 2007; Maedeh et al., 2012; Bergmann and Raupp 2014; Jesser et al., 2020).

Globally, urban insect pests' financial and public health significance is enormous. In the US alone, termites are responsible for an estimated annual loss of up to $40 billion (Su 2002, Potter et al. 2010). As they scramble through sewage, garbage, and kitchen drains, peridomestic cockroaches can mechanically vector harmful pathogens and asthma-triggering allergens (Brenner 1995, Stout et al. 2009). Meanwhile, the scourge of bed bugs leaves undesirable infections, irritations, and severe stress on homeowners and tenants (Shum et al. 2012). Ants seek buildings with wall voids, landscape timbers, wooden porches, fences, lawns, or gardens to nest (Oi and Vail 2011). The ectoparasitic fleas and lice feed on the blood of humans and pets, causing irritation and transmitting disease-causing agents capable of decimating the human population (Gage and Kosoy 2005, Speare et al. 2006). Moths and silverfish are fabric and museum pests that cause up to $1 billion of damage per year to clothes, furnishings, books, and animal materials in the United States (Cox and Pinniger 2007).

These urban insect pests are usually managed with synthetic insecticides (Wang, Singh, et al. 2016, Rabito et al. 2017, Wang, Zhao, et al. 2020). While the argument about the current efficacy of synthetic insecticides is equivocal, what is clear is the genuine concerns associated with their usage. Examples of such problems include increasing insecticide resistance, increasing legislation against use, effects on non-target pests, and environmental contamination (Zhu et al. 2016, Wu and Appel 2017, Fardisi et al. 2019). There has been a paradigm shift in the attitude of homeowners about insecticides and increasing interest in the use of alternatives such as natural products (Koul et al. 2008).

The use of natural products to manage insect pests is not new. The Chinese used natural products such as chalk, wood ash, and botanicals in 1200 BC for fumigation and seed coating (Flin et al. 1981). Pulverized chrysanthemum flowers were used to manage head lice in the Achaemenid empire, present-day Iran (486 – 465 BC) (Addor 1995). The German pharmacist, Friedrich Sertürner, conducted the first extraction from a plant – isolating morphine from *Papaver somniferum* L. in 1803 (Patwardhan et al. 2004). Since then, there have been over 30,000 articles on natural product research globally. In the US, research scientists and companies leverage the registration exemption of section 25b of the US Environmental Protection Agency (EPA) to continue laboratory bioassays to provide empirical information on the insecticidal profiles of natural products against urban insects. The registration exemption waives expensive toxicology studies and accelerates potential discovery and marketing for natural products.

Consequently, essential oils (EOs) have witnessed deserving attention due to the widespread efficacy reports even to insecticide-resistant urban insect pests (Albuquerque et al. 2013, Gaire et al. 2020, Oladipupo et al. 2019, 2020a, 2020b, Lee and Rust 2021). Yet, there is no synthesis on the extent and the impact of the management of urban insects using essential oils or essential oil components (EOCs) or commercially available EO formulations. Such a review is of high importance since it provides a measure for the objective evaluation of the range of their effectiveness. Such information can be used to reliably make informed decisions and policies regarding widely reported efficacies and assess shortfalls, limitations, and prospects.

Therefore, this review attempts to systematically present knowledge on EOs, EOCs, and commercially available EO formulations employed in the management of urban insects. Based on the intersection of the most common and often encountered insect pests, control efforts, and homeowners’ frustrations, the emphasis was placed on ants, bed bugs, cockroaches, fleas, head lice, silverfish, stored product moths, and termites. Furthermore, highlighting each urban insect pest, we presented the range of insecticidal effects of these natural products and outlined laboratory and field evidence. Finally, we discussed the gaps in knowledge and possible prospects for EOs in urban insect management.

2.3 Natural products, plants extract, and essential oils

2.3.1 *Natural products*

As defined by Asolkar et al. (2013), a natural product refers to any naturally occurring organic compounds that do not appear to participate directly in the growth and development of the source organism. Natural products can be derived from plants, animals, and inorganics. Natural products from plants are termed secondary metabolites and roughly categorized into terpenoids, phenolic compounds, and alkaloids (Agostini-Costa et al. 2012). Plants utilize these metabolites for communication and defense to limit insect-feeding damage (Aljbory and Chen 2018).

2.3.2 Plant extracts, essential oils, and essential oil components

The extraction method (from plants) determines if the product is a plant extract or essential oil. If obtained via solvent extraction, it is termed plant extract, whereas it is termed an essential oil if distilled or expressed. The term “essential oil” was coined by a Swiss physician, Theophrastus von Hohenheim, popularly known as Paracelsus, in an attempt to isolate the “Quinta essentia” of certain herbal drugs in ca. 1523 (Guenther 1950). Essential oils are a mixture of many components whose composition is determined by the plant family, plant part, expressed method, edaphic factors, and other environmental conditions (Isman and Paluch 2011). In other words, essential oils are concentrated hydrophobic/hydrophilic liquids containing aromatic/aliphatic volatile compounds from plants. Usually, the components (or constituents) of an EO (hereafter referred to as EOC) are identified by gas chromatography coupled with mass spectrometry (GC-MS). From the available literature on the use of natural products to manage urban pests, it appears steam distillation is the most preferred method of EO extraction, thereby implying that the components are heat stable.

These individual essential oil components (EOCs) can be classified based on (1) the number of isoprene units (i.e., five carbon atoms with double bonds), (2) functional groups, and (3) the molecular structure of terpenes (Fig. 1) (Buckle 2015, Perveen 2018). Based on isoprene units, there are hemiterpenes (1 isoprene unit), monoterpenes (2 isoprene units), sesquiterpenes (3 isoprene units), and up to tetraterpene (8 isoprene units) (Fig. 1a). The functional groups include aldehydes, ketones, alcohol esters, phenols, and ethers (Fig. 1b). The molecular structure of the terpenes can be cyclic (one ring; e.g., D-Limonene), bicyclic (two rings; e.g., zingiberene), tricyclic (three rings), or acyclic (linear and has no ring; e.g., isoprene) (Fig.1) (Buckle 2015, Perveen 2018).

Application methods routinely employed to deliver EOs and EOCs in the laboratory to their target urban insect pest is diverse (Philips and Appel 2010, Gaire et al. 2017, Wu and Appel 2018, Oladipupo et al. 2020). Common examples include fumigant, contact/topical, repellency, and continuous exposure. Briefly, topical applications involve delivering a known concentration of a toxicant to a defined area of the insect body. While concentrations routinely employed for topical applications are expected to achieve intended effects within a short time (mostly 24 – 48 h), the continuous application involves providing a concentration that the insect is continuously exposed to for an extended period. Fumigation involves the delivery of the toxicant through vapor while preventing physical contact with the toxicant. Contact is the opposite as it involves direct contact with the toxicant. Repellency combines contact and fumigation effects with avoidance behavior. The toxicant is placed within the experimental arena, and the insects' behavior is measured.

Nevertheless, the application method employed depends on practicality (i.e., convenience) and the probit metric of evaluation sought. As a gold standard, the probit metric 50 (i.e., P_{50}) evaluates the dose in milligram (LD_{50}), concentration in milligram/liter (LC_{50}), or time in minutes (LT_{50}) of a toxicant per body weight (kg) that would kill 50% of the population of interest. When the intent is to knock down or to inhibit egg-laying/hatch, KT_{50} and hatch inhibition are used, respectively.

2.4 Management of urban insect pests using natural products

2.4.1 Ants

In urban environments, stinging ants such as the red imported fire ant, *Solenopsis invicta* Buren, and its hybrid, *Solenopsis invicta* x *ritcheri*, are often found outdoors in parts of Asia and North and South America. Indoors, stingless ants such as the black garden ant, *Lasius niger* (L.), and the Argentine ant, *Linepithema humile* (Mayr), are frequently encountered. The tawny crazy ant, *Nylanderia fulva* (Mayr), a stingless species, can be found indoors and outdoors. These ant species colonize gardens, lawns, compost piles, pavement cracks, and schoolyards in southeastern US and Brazil (Collins and Scheffrahn 2001, Cheng et al. 2008, Albuquerque et al. 2013, Fu et al. 2015, de Oliveira et al. 2020). These ant species constitute a nuisance by building colonies in electrical circuits within utility boxes. Fire ants and their hybrids sting can cause white pustules on homeowners or residents and laborers, thereby reducing labor in agriculture fields (Collins and Scheffrahn 2001). Some species display aggressive nature (e.g., fire ants), cryptic behavior (e.g., black garden ants), high reproductive rate, mound relocation (especially with fire ants), and polygyny (i.e., multiple queen colonies) that readily frustrates control efforts (Appel et al. 2004, Fu et al. 2015, de Oliveira et al. 2020).

About six plant families have been explored for their insecticidal effects against urban ant species (Table 1). Most of these experiments were conducted on ants via a fumigant toxicity test. Many ants exist in colonies underground, and a fumigant bioassay is a close simulation that reflects such an environment. The insecticidal activity of each EO, the plant details, bioassay type, and range of toxicity reported are compiled in Table 1. However, no direct comparison can be made due to the wide range of bioassay methods, ant species, and insecticidal endpoints reported. Mint oil (Lamiaceae) resulted in a 49 – 100% repellency at 147.8 mg cm^{-2} against *S.*

invicta workers (Appel et al. 2004). The fumigation activity of *Varronia currasavica* Jacq. EO (Cordiaceae) was substantial (LC_{50} range: 0.7 – 1.3 µL L^{-1}) against *D. thoracius* than other EOs against *S. invicta* (de Oliveira et al. 2020). In general, *S. invicta* minor workers (LC_{50}: 1.7 µg mol^{-1}) were more affected than major workers LC_{50}: 1.7 µg mol^{-1}) in a series of fumigation toxicity tests exploring *Cinnamomum camphora* EOs (Zhang et al. 2014, Fu et al. 2015). The knockdown time (LT_{50}) was least (21.2 min) for *C. osmophloeum* EO against *S. invicta* in a closed fumigation experiment (Cheng et al. 2008). For both *Cinnamonum spp.* (Lauraceae), the most abundant EOC isolated, had a comparable level of control against *S. invicta* as *Cinnamonum* EO (Table 1). In another study, Addesso et al. (2017) observed that *Cuppressus nootkanensis* D.Don EO (Cuppressaceae) suppressed *S. invicta* x *ritcheri* digging behavior by 50%. In contact tests, EOs distilled from aerial parts of *Piper aduncum* L. had remarkable activity (LD_{50}: 114.4 mg L^{-1}) compared to other *Piper spp.* (LD_{50} range: 207.8 – 571.1 mg L^{-1}) (Souto et al. 2012). In a behavioral experiment using electroantennographic techniques, *Eucalyptus maculata* Hook EO caused greater depolarization to *Atta sexdens rubropilosa* Forel. A response profile such as this suggests characteristic repellent properties (Batista-Pereira et al. 2006). GC-MS analysis identified α-pinene (39.4%) as the major component of the EO extract.

The above reports show that one way to affect urban ant pests is through fumigation using EOCs. This suggests that EOs vapors gain access into ants’ bodies via the spiracles (i.e., route of entry is inhalation) to exert their effect and elicit olfactory responses in ants. But such an application might have limited application towards ants that colonize above-ground galleries and open air. This is because contact is the primary delivery approach for ants. After all, in practice, the treatment application is sprayed over the ground. Even so, based on the information provided in Table 1, lower concentrations are required for fumigant toxicity. EOs from Cordiaceae appear to possess the most potent insecticidal activity against ants. Perhaps the injection of EOs/EOCs into ant mounds or even wall voids would be similar enough to act as a fumigant.

2.4.2 Bed bugs

Two species of bed bugs are important ectoparasites of people and occasionally other animals; the common bed bug, *Cimex lectularius* L., and the tropical bed bug, *C. hemipterus* F. (Liu et al. 2014). Both species rely on blood meals for growth, development, and reproduction, and thus have become synanthropic with humans (Lai et al. 2016). In homes, these nocturnal insects feed multiple times on sleeping humans. Bed bugs' blood-feeding behavior on humans may cause allergic reactions. However, they are not known to transmit pathogenic organisms to humans yet. Recently, there have been reports of a resurgence of both species, presumably owing to reduced use of spray insecticides indoors, increased global travel, and increased incidences of insecticide resistance (Doggett et al. 2014, Politi et al. 2017). The available literature on EOs, EOCs, and commercially available EOs explored for insecticidal effects against bed bugs are summarized in Table 2.

Notably, EOs from the families Asteraceae, Lamiaceae, and Schisandraceae were investigated against adult *C. lectularius* using treated surfaces, impregnated paper disk tests, and topical application (Table 2). Sharififard et al. (2018) reported an EC_{50} (i.e., effective concentration required to cause 50% repellency) of 4.5 mg cm^{-2} with EO from *Oreganum vulgare* L. leaf against *C. lectularius* after 24 h in a treated surface bioassay and 100% repellency after 3 h when 10% of the EO was used. Politi et al. (2017) reported an LC_{50} of 0.17 mg ml^{-1} with EO of *Tagetes patula* L. in an impregnated filter-paper test. The most dominant compound in the extract of Asteraceae and Lamiaceae plants was terpineol. In topical (LD_{50}; 27.5 – 560 µg mg^{-1}) and fumigant (LC_{50}; 20.5 – 1474.6 mg L^{-1}) bioassays, Gaire et al. (2019) demonstrated the efficacy of several essential oil components used to control bed bugs. In synergistic mixtures, EOCs certainly tend to achieve an even better result with lower concentrations. Gaire et al. (2020) reported that a mixture of carvacrol, thymol, and eugenol was much more effective (LD_{50}; 19 µg mg^{-1}) against *C. lectularius* in topical application studies than if administered singly (carvacrol = 27.5 µg mg^{-1}, thymol = 32.5 µg mg^{-1}, and eugenol = 52 µg mg^{-1}). Commercially available EO blends are also more effective than when individual EOCs are applied singly. For example, rosemary, peppermint, thyme, and Cinnamon EO blends, respectively, were more effective than their respective components against *C. lectularius* in a fumigation test (Feldlaufer and Ulrich 2015). Despite the variety of bioassay designs, one constant was that only adult *C. lectularius* were investigated for their susceptibility to EOs and EOCs (Table 2). This is surprising as there are generally more nymphs in a population than adults (Liu et al. 2014). One might argue that the behavior of adult females to lay eggs in secluded places is the rationale for adopting a more practical approach such as fumigation. Practically, this would involve sealing up such an environment. Thereby making EOs and EOCs worthy candidates for further exploration for the management of this species.

2.4.3 Cockroaches

The German cockroach, *Blattella germanica* (L.), and the American cockroach, *Periplaneta americana* (L.), are common indoor cockroach species. These species have become the most important pests in the urban environment. The Department of Housing and Urban Development (HUD) estimates that at least 60% of low-cost housing units have significant levels of cockroach allergens (Stout et al. 2009). During the day, cockroaches prefer dark environments/conditions and are thus restricted to areas such as around kitchen pipes, sinks, cupboards, sewer pipes, and other dark voids and crevices in homes. These pests come out to feed on leftovers in kitchen sinks and garbage at night. Such behavior makes cockroaches an efficient mechanical vector of pathogens and a source of allergens (Togias et al. 2010, Fakoorziba et al. 2014, Menasria et al. 2014). In response to the call for research on sustainable alternatives, some plant EOs, and their components have been investigated for their insecticidal effects against *B. germanica* and *P. americana* (Table 3). Studies conducted using natural products and essential oils against *Blattella germanica* are also summarized in Lee and Rust (2021).

Wagan et al. (2017) reported 49% and 55% repellency by *Piper nigrum* L. EO delivered at 31.5µg cm^{-2} (Piperaceae) against *B. germanica* nymphs and adults, respectively after 12 h. Similar effects against *B. germanica* nymphs and adults were observed when Lamiaceae EOs were used (Peterson et al. 2002). Interestingly, the most abundant component from the Lamiaceae plant (Z, E-nepetalactone from *Nepeta cataria* L.) achieved a higher level of repellency (68.2%) than the EO (55%) (Peterson et al. 2002). Eugenol appears to be more repellent to *B. germanica* (85%) than to *P. americana* (77.1%) (Ngoh et al. 1998, Neupane et al. 2019). Eucalyptus oil is a poor repellent (27.7%) of the brown-banded cockroach, *Supella longipalpa* Fabricius while rosemary, oregano, and yarrow oils are better repellents (86.7 – 96.5%) (Sharififard et al. 2016). The use of repellents for cockroach management is likely to be problematic. At worst, a repellent would disperse the cockroaches throughout a house or apartment. A better approach would be to seal off the intended treatment area (i.e., cockroach-proof an area) before applying such repellent.

In fumigant toxicity studies, Zhu et al. (2012) reported an LC_{50} of 4.1 mg L^{-1} by *Chenopodium ambrosoides* L. (Chenopodiaceae). *Thymus persicus* EO (Lamiaceae) had an LC_{50} of 28.8 µl L^{-1} against *B. germanica* while *Eucalyptus camaldulensis* Dehn. EO (Myrtaceae) LC_{50} was 21.8 µl L^{-1} (Rezaei et al. 2019). Some commercially available EOs were also explored for their fumigant toxicity against *B. germanica* nymphs. The results suggest that these oils may not be effective fumigants. For example, Gaire et al. (2017) reported the range of toxicity (LD_{50}) of Red thyme, Clove bud, and Java citronella oils to be 160.5 – 746.7 mg L^{-1} against *B. germanica* nymphs. However, EOCs were much more effective as fumigants against *B. germanica* than some commercially available EOs. For example, α-pinene had an LC_{50} of 11.8 mg L^{-1} against adult males and 26.1 mg L^{-1} against adult females, while limonene achieved 13 mg L^{-1} and 15.3 mg L^{-1} against adult males and females, respectively (Phillips and Appel 2010a). Similalry, Zhu et al. (2012) reported a better fumigant effect (LC_{50}; 2.1 mgL^{-1} and 0.6 mgL^{-1}) by another EOC (isoascaridole and ascaridole) against *B. germanica*. In homes, cockroaches live in crevices, holes or occupy spots beyond the reach of humans. In this situation, fumigation might be appropriate by taping and sealing before application.

American cockroaches are much larger than German cockroaches. Thus, a greater concentration or volume of EO is required to achieve the same effect. For example, in topical toxicity studies, Appel et al. (2001) demonstrated that 32% more mint oil is needed to achieve the same effect (LD_{50}) against *P. americana* than for *B. germanica*. Philips et al. (2010) reported that males are generally more susceptible to insecticidal effects of EOCs than females. Females require larger doses of EOs to achieve similar effects as males. This is because females are larger than males and have more fat in which the EO/EOC dissolves.

2.4.4 Fleas

The human flea, *Pulex irritans* L., oriental rat flea, *Xenopsylla cheopsis* Rothchild, and the cat flea, *Ctenocephalides felis* (Bouché) are commonly found in homes due to the association of people with pets (such as cats and dogs) and rats (de Avelar et al. 2011, Batista et al. 2016). These ectoparasites can transmit or serve as an intermediate host of a broad spectrum of pathogens that causes diseases to humans and pets such as plague, murine typhus, tapeworm, and flea allergic dermatitis (Su et al. 2014, Rust, 2017). Also, they can be a source of discomfort to pets and homeowners (de Avelar et al. 2011, Batista et al. 2016). The constant interaction between people and pets is arguably why larvae/pupae (such as cat flea) can be found in beddings, furniture, and carpets.

Given the safety concerns about current insecticides used to manage fleas, a series of plant essential oil and their components were examined for flea management (Table 4). Extract of leaves of *Cinnamomum osmophloeum* Kaneh (Lauraceae) and *Plectranthus amboinicus* (Lour) (Lamiaceae) and *Taiwania cryptomeriodes* Hayata (Cupressaceae) were repellent (68.6 – 97.7%) against *C. felis* (Su et al. 2014). Similarly, Barbosa dos Santos et al. (2020) reported ovicidal, larvicidal, and adulticidal effects of Lamiaceae, Poaceae, and Zingiberaceae EOs against *C. felis* in impregnated filter-paper tests. Notably, the susceptibility of *C. felis* to the EOs was larvae > egg > adult. In contact toxicity tests, Dolan et al. (2007) reported that the median lethal concentration (LC_{50}) of EOs obtained from the heartwood and woodshavings of Cuppressaceae plants ranged from 0.24 – 1.21 mg ml^{-1}. At 1.6 mg cm^{-2}, *Myrtus communis* EO (Myrtaceae) had an effective dose (ED_{50}) of 229 µg cm^{-2} against *P. irritans* (Ghavami et al. 2017).

Similar to EOs, EOCs were quite repellent to fleas. Su et al. (2014) reported that the major components (*trans*-cinnamaldehyde and thymol) identified from *C. osmophloeum* and *P.*

amboinicus EOs had comparative effects (repellency of 90 – 97%) against *C. felis* as with the EOs. In contact tests, the LD_{50} required to kill 50% of *X. cheopsis* ranged from 0.003 – 0.04 (wt: vol) for carvacrol, valencene, nootkatene, and nootkatone (Panella et al. 2005). Collectively, the insecticidal effects of EOs and EOCs against fleas suggest their potential use, particularly since the safety of conventional insecticides is a source of concern. Children may frequently contact treated surfaces such as floors and carpets or play with treated household pets (de Avelar et al. 2011, Batista et al. 2016).

2.4.5 Headlice

The human head louse, *Pediculus humanus capitis* De Geer, is an urban insect pest commonly associated with school-aged children (Toloza, Zygadlo, et al. 2010). Its life cycle is completed entirely on the host, and its infection can cause scalp irritation, pruritus, social disruption, sleep loss, nausea, loss of school time, and introduce secondary bacterial infection from wounds made from scratching (Yang et al. 2004, Koch et al. 2016). The insecticidal effects of EOs and EOCs against *P. humanus capitis* have been widely investigated and are summarized in Table 5. Notably, plants from Myrtaceae were examined for their ovicidal (Di Campli, Di Bartolomeo, Pizzi et al. 2012), contact (Bagavan et al. 2011, Yones et al. 2016, Candy et al. 2018), and fumigant (Toloza et al. 2006, 2010a, 2010b) effects against *P. humanus capitis*. The range of the median knockdown time (KD_{50}) of the Myrtacae EOs against adult *P. humanus capitis* in contact (1 – 8%) and fumigant (0.25 – 1.75 mg cm^{-2}) bioassays was 10 – 43.2 min and 1.2 min – 73.4 min, respectively. The most abundant component of the Myrtaceae plant was 1,8-cineole which alone had a KD_{50} of 11.10 min against *P. humanus capitis* in a fumigant bioassay. EOs from Apiaceae, Asteraceae, Cucurbitaceae, Lamiaceae, Lauraceae, and Verbenaceae also had impressive adulticidal effects against *P. humanus capitis.* EO from *Aloysia citrodora* Paláu (Verbenecae) leaf had a KD_{50} of 3.02 min against *P. humanus capitis* in a fumigation bioassay (Toloza, Zygadlo, et al. 2010). Yones et al. (2016) reported a KD_{50} of 11.4 min from EO from the bark of *Cinnamomum aromaticum* (Lauraceae) against *P. humanus capitis*.

Beyond adulticidal effects, EOs also demonstrated ovicidal effects against headlice. Yones et al. (2016) reported a 97% hatching inhibition against *P. humanus capitis* eggs at 0.25 mg cm^{-2} of *Mentha spicata* L. Di Campli et al. (2012) observed that 25% of the EO from the leaf of *Melaleuca alternifolia* (Maiden & Betche) (Myrtaceae) produced 50% abortive eggs four days after treatment. These results from EO-fumigation experiments in open containers imply that *P. humanus capitis* infestations could be managed via fumigation. This can be done with a shampoo or a combination of shampoo with a plastic headcover.

2.4.6 Silverfish and Brown marmorated stink bug

The common silverfish (*Lepisma saccahrina* L.) is a domestic indoor pest that inhabits homes due to food or warmer conditions. The brown marmorated stink bug (*Halyomorpha halys* Stål) only seeks over-wintering shelters indoors; populations rarely establish indoor. Only a few studies have explored using EOs and EOCs to manage these urban insects (Table 6).

The most abundant activities of an EO, against *L. saccharina*. Kuo et al. (2007) reported 100% mortality of *L. saccharina* after 2 hours of exposure to *Chamaecyparis formosensis* Matsum Cuppressaceae EO obtained from wood chips. The commercially available products, methyl benzoate and EcoSmart neem oil, were investigated for their insecticidal effects against *H. halys* (Bergmann and Raupp 2014, Feng and Zhang 2017). Methyl benzoate had impressive ovicidal (LC_{50}; 0.02 mg cm^{-3}) and nymphcidal (LC_{50}; 1.01 – 2.39 µLvial^{-1}) effects while neem oil resulted in 15% mortality against *H. halys* in 48 h post-exposure in topical application experiments (Bergmann and Raupp, 2014; Feng and Zhang, 2017).

2.4.7 Stored product moths

Globally, the Mediterranean flour moth, *Ephestia kuehniella* (Zell.), and the Indianmeal moth, *Plodia interpunctella* (Hübner), are important stored-product insect pests that cause significant damage to nuts, grains, and processed foods (Maedeh et al. 2012, Jesser et al. 2020). Usually, the infestation of both species is controlled using fumigants such as methyl bromide or phosphine (Maedeh et al. 2012, Maroufpoor et al. 2016). However, the associated detrimental effects of these gases including residues and effects on the ozone layer have stimulated the research for alternatives (Maedeh et al. 2012, Pandir and Bas, 2016).

As expected, fumigant bioassays were the most preferred for the investigation of insecticidal properties of EOs and EOCs against both species (Table 7). Fumigation, especially within the context of stored-product management, offers a superior economic advantage and is most practical over other exposure methods. Among the plant families explored, EOs from Lamiaceae were dominant (Ayvaz et al. 2009, Maedeh et al. 2011, Mahmoudvand et al. 2011, Eliopoulos et al. 2015, Pandir and Bas 2016, Jesser et al. 2017). In general, the genera *Mentha* and *Ocimum* had menthol and linalool as the most abundant EOC, respectively. Eliopoulos et al. (2015) reported the effects (i.e., LD_{50}) of *Ocimum basilicum* L. EO to include ovicidal (776 µl L^{-1}), larvicidal (2096 µl L^{-1}), pupacidal (1567 µl L^{-1}), and adulticidal activities (1.4 µl L^{-1}) against *E. kuehniella* in a fumigant toxicity bioassay. *O. basilicum* had similar effects against *P. interpunctella* eggs (779.2 µl L^{-1}), larvae (2036 µl L^{-1}), pupae (1799 µl L^{-1}), and adults (1.2 µl L^{-}

^{1}) (Eliopoulos et al. 2015). In fumigant toxicity bioassays, *Zingiber officinale* Roscoe EO (Zingiberaceae) was four times more toxic to the larvae of *P. interpunctella* (LC_{50}; 69.1 µl L^{-1}) than to *E. kuehniella (*259 µl L^{-1}). However, the larvicidal effects were similar in contact toxicity bioassays (Maedeh et al. 2012). Similar adulticidal effects were observed between both species when *Coriandrum sativum* L. (Apiaceae) EOs were tested (Maroufpoor et al. 2016). These results indicate that larvae of these moths are the hardest to kill, followed by pupae. Unlike conventional interventions such as methyl bromide and phosphine [typically tested at 10-476.5 mg/L against same species (Small, 2007)], EOs and EOCs would probably leave no residue and after-effects on treated stored products post-period of application.

Few commercial EO products have been investigated for *P. interpunctella* management. In a fumigant toxicity bioassay, Park and Lee (2018) reported a range of toxicity (LC_{50}) of cyclohexenone compounds against *P. interpunctella* larvae (2.5 – 3.0 µg cm^{-3}) and adults (3.6 – 4.2 µg cm^{-3}). Seudenone had an LC_{50} of 3.0 µg cm^{-3} and 4.4 µg cm^{-3} against *P. interpunctella* larvae and adults, respectively (Park and Lee 2018). These results demonstrate that these EOCs are more toxic to larvae than to adults of *P. interpunctella*.

2.4.8 Termites

Throughout history, no structural insect pest commands more attention than termites. Notably, the subterranean termites in the genera *Coptotermes* and *Reticulitermes* construct shelter tubes that link soil with wooden structures while the drywood termites (e.g., *Cryptotermes* and *Incisitermes*) live in wood without contact with the soil (Su and Schelfrahn, 1990; Siramon et al., 2009). Together both groups cause billions of dollars worth of damage to wooden structures and incur huge expenditures for control efforts and repair of damage (Su and Schelfrahn 1990, Potter 2011, Su 2002).

In concert with the objectives of the Environmental Protection Agency (EPA 2000), some plant EOs and EOCs were explored for their antitermitic effects; and this information is summarized in Table 8. In a no-choice test, Elango et al. (2012) reported toxicity (LD_{50}) in the range of 253 – 409 ppm against *C. formosanus* from EOs obtained from Acanthaceae, Aristolochiaceae, Compositae, Fabaceae, Moraceae, Papaveraceae, and Solanopceae plants. The topical toxicity (LD_{50}) and contact toxicity (LC_{50}) of *Nepta cataria* L. EO (Lamiaceae) against *R. flavipes* were 8200 µg g^{-1} and 44.4 µg cm^{-2}, respectively (Peterson and Ems-Wilson 2003). The use of impregnated filter paper to deliver the EO required only about 3% of *Eucalyptus spp.* EOs (Myrtaceae) kill 50% of *C. gestroi* Wasmann workers (Mikola et al. 2017).

EOCs also possess antifeedant, contact, fumigant, and repellent activities against subterranean and drywood termites (Table 8). Beyond toxic effects, the EO from the leaf of *Lantana camara* L. (Verbenaceae) exhibited a 78% antifeedant effect against *R. flavipes* (Yuan and Hu 2011), while the repellent effects of the growing plant were greater against *C. formasanus* than *R. flavipes* (Ding and Hu 2010). For the drywood termite, *C. brevis*, the antifeedant index of *Citrus latifolia* Tanaka (Rutaceae) was 100% at 100 mg cm^{-3} of the oil (Sbeghen-Loss et al. 2011).

2.5 General synthesis, Knowledge gaps, and Conclusions

2.5.1 General synthesis

Based on the presented literature, it has become increasingly clear that plant metabolites, known as EOs, and their components (i.e., EOCs) exhibit toxicity and repellency to insects and are much safer (according to EPA 24b list) than conventional insecticides. In truth, there is none human/rat toxicity data to demonstrate the safety of EOs/EOCs. Effects include ovicidal, larvicidal, and adulticidal toxicity against urban insect pests in primarily laboratory and a few field studies. These plant-based products may represent a sustainable approach for urban insect pests management.

In general, our synthesis suggests that EOs from several specific plant families have promising effects on urban pests. This includes Myrtaceae, Lamiaceae, Lauraceae, Zingiberaceae, and Asteraceae (in descending order of effectiveness). EOCs such as eugenol, carvacrol, *trans*-cinnamaldehyde, and thymol are consistently more toxic than other tested EOCs to urban insect pests. Consistent with the physical properties hypothesis (Philips et al. 2010, Yeom et al. 2015, Oladipupo et al. 2020a), it appears that EOCs with a log P value within the range of 1.9 – 3.3, the boiling point of 233 – 254 °C, the vapor pressure of 0.010 – 0.030 mm Hg, the solubility of 0.96 – 2.98 g/liter, and molecular weight of 132.2 – 164.2 g/mol are the most toxic. These aforementioned plant families and EOCs had specific patterns of toxicity based on the insect order and method of exposure. For Hymenoptera (i.e., Formicidae/ants), fumigation was the most used to test ants in laboratory studies. This approach could be extended to field studies by dissolving EOs/EOCs in a micelle or via encapsulation or simply injecting them into the ground. For Hemipterans (specifically, Cimicidae/bed bugs), fumigant assays appear to deliver the most devastating effects.

Fumigation required about 1000-fold less than the concentration needed for topical protocols to achieve killing. For Siphonapterans, EOs are effective as contact insecticides against all life stages. This conclusion makes sense only for adult cat fleas. Since other life stages and adults of other species live in the environment and just visit humans for blood meals, the realization that EOs could potentially work as contact insecticides is promising. Phthirapterans (i.e., Pediculidae) have been well managed by fumigation. A practical way of extending fumigation to field studies on humans would be to cover the scalp with a plastic hairnet or shower cap upon application. EO-fumigation works better on killing lepidopteran adults than larvae. Isoptera (i.e., termites) are probably better managed using antifeedant, contact, and fumigant bioassays. The synthesis of the data on the management of Blattodea (i.e., cockroaches) demonstrates that females are much more difficult to control than males. This is due to their larger body size and fat composition. Females require larger doses of EOs to achieve similar effects as males. Importantly, cockroaches can be killed using either a contact or fumigant approach. Both approaches have their advantages. In homes, cockroaches live in crevices, holes, or occupy spots beyond the reach of humans. In this situation, a fumigant is desired if the application can be made, and such holes are sealed off. In other cases, cockroaches crawl up in walls and other places not welcomed in homes. Thus, EOs can be utilized as contact insecticides. Hence, it is imperative to seek EOCs with high vapor pressure and low boiling point. To move the field of EO research forward, rather than investigating or revealing new EOs/EOCs with insecticidal actions, future studies should focus on consolidating past studies. For example, the understanding of how these urban pests perceive EOs is limited. Thus, there is a dire need for future scientists to characterize the antennal and other sensory responses of these urban pests to

EOs. Such knowledge could help predict the behavior of urban insect pests to EOs in field studies.

2.5.2 Knowledge gaps and conundrums

2.5.2.1 Unknown mode of action

Given the public outcry and sensitization against the widespread indiscriminate use of synthetic insecticides, the time is now right for plant-based products to take center stage. Unfortunately, despite the extensive research done on EOs worldwide, there are still fundamental questions that remain. For example, the mode of actions of most, if not all, EOs and their components are poorly understood. Only a few studies have attempted to describe the mode of action (MoA) of some EOs (Yeom et al. 2015, Gaire et al. 2021). To date, the most direct point of reference is the works of Enan (2005a, 2005b) which, interestingly, were done to identify the binding sites, and not MoA of thymol, carvacrol, α-terpineol, and L-carvone. This work is exemplary because it focuses on the molecular basis of essential oil specificity by investigating the binding sites of specific essential oils on *D. melanogaster* and *P. americana*. The authors implicate tyramine and octopamine ligands explaining why EOs and EOCs trigger neurotoxic-like effects. Such an approach expands our understanding of the potential novel sites of EOs/EOCs. But there is still the need to identify these oils' primary site of action. Evidence suggests that EOs inhibit acetylcholine esterase and cytochrome P450 enzymes (Yeom et al. 2015, Gaire et al. 2021). This suggests a broad spectrum of activity. Future studies should invest in determining MoA of EOCs (such as eugenol, carvacrol, limonene, thymol) that are widely reported as toxic against a range of urban insect pests. Such information, when generated, would be central to the optimization and commercialization of EOs.

2.5.2.2 Response of urban insect's sensory system to EOs and EOCs?

Relatively little is known about these urban insect pests' chemical communication and olfactory mechanisms to EOs. Neither is there an understanding of the interactions between EOCs and the sensory system of urban insect pests. Behavioral studies do not provide convincing results. For example, out of six EOCs investigated, only thymol elucidated significant avoidance behavior in Turkestan cockroaches (Gaire et al. 2017). Interestingly this list included eugenol and trans-cinnamaldehyde, two out of the four EOCs associated with toxicity against a broad range of urban insect pests. Two out of 12 EOs evaluated against *B. germanica* showed repellency in the range of 76.6 to 88.5% (Huang et al. 2020). Thus, electroantennogram studies that document the perception of urban pests to EOs and EOCs are required.

Additionally, identifying odorant receptors can provide valuable information on the chemical ecology of these insects that can be exploited to develop efficient control agents. For example, Pelletier et al. (2015) identified a sensitive receptor, PhumOR2, in *Pediculus spp.* involved in the avoidance of specific chemical cues. PhumOR2 is an odorant receptor that mediates repellency to the hydroxyl functional group EOCs such as thymol, carvacrol, and eugenol. Such a finding demonstrates that understanding the interactions between EOs/EOCs and the insect sensory system could improve our comprehension of the mode of action.

2.5.2.3 The cost-effectiveness and economic viability of EOs and their components

For successful commercialization and adoption, the documentation of EO efficacy against target pests documented in many publications is not enough. It is not enough for an EO to only be toxic (i.e., possess insecticidal effects); it must also be economically viable. On average, the cost per ounce for an EO is approximately four times that of a synthetic insecticide (Oladipupo, personal observation). Hence, much is required to optimize the economic viability and cost competitiveness of EOs against currently used synthetic insecticides.

2.5.2.4 Relationship between bioactivity and Physical properties of EOs and their components

The physical properties of EOCs such as molecular weight (g/mol), log P, solubility, and vapor pressure (kPa) must be considered. For example, log P is a measure of the lipophilicity of an EOC. EOCs with a log $P > 0$ are hydrophobic, while log $P < 0$ are hydrophilic. The vapor pressure (vp) is a property of a liquid-based on the strength of its intermolecular forces. Thus, a high intermolecular force would indicate such EOC has high vapor pressure and a high boiling point (bp). EOCs with such characteristics are likely to be more volatile and persist less. This could be good, bad, or both depending on the context.

2.5.2.5 High volatility of EOs and their components

One recurring challenge of EOs and EOCs is the issue of high volatility resulting in rapid evaporation, faster than desired. An obvious way to tackle this would be to develop formulations that can deliver and retain EOs and EOCs without interfering with bioactivity. This ensures a slow release to provide an effective dose against targeted pests. Oladipupo et al. (2020b) provided an example of this by employing super absorbent polymer gels to prolong the bioavailability of EOCs to *B. germanica*. They reported significant impairment of reproductive parameters of *B. germanica*.

Similarly, Lucia et al. (2017) employed novel poloxamer shells to improve the dispersion of EOCs in water and found them to be physicochemically stable while delivering impressive pediculicidal activity against *P. humanus capitis*. Jesser et al. (2020) observed that EOs loaded in polymeric nanoparticles could withstand temperature variation while increasing contact toxicity to *P. interpunctella*. Song et al. (2018) microencapsulated EOs in a film and reported a prolonged release rate. The encapsulation of EOs in insect-proof halloysite nanotubes provided two folds effects: (1) an efficient barrier to insect infestation and (2) controlled release of the EO over an extended period (Kim et al. 2016). In short, the exploration of materials that can deliver and retain EOs and EOCs,without interfering with bioactivity should be sought.

2.5.2.6 Lack of theoretical framework and hypotheses in studies

Given the approach of most studies, one can not but wonder, “Does the goal of an EOC toxicity study necessarily need to be to replace synthetic insecticides?” This might be the goal of some, but certainly not all. Interestingly, one of the fundamental reasons that stimulated the search/research for alternatives to currently used synthetic insecticides is the development of insecticide resistance and the realization that EOs could be sustainable alternatives. Resistance typically occurs due to increased application pressure. Hence, it follows that with EOs and

EOCs, it might be a question of when. It may be relevant to integrate the exploration of EOs and EOCs with synergistic studies. This may be necessary to slow down the heritable change in the sensitivity of urban insects to EOs. For example, the repeated exposure of *Myzuz persicae* Sulzer to azadirachtin culminated in the development of resistance (Feng and Isman 1995). And there is no reason not to expect otherwise with any other toxicant. Hence, it might be relevant to approach EO research cautiously and explore possible synergistic combinations, additives, and mixtures that hold promising results. An example of such would be those formulated by Tak and Isman (2017). The study explored a 1:1 (w:w) binary combination of carvacrol, trans-cinnamaldehyde, α-terpineol, and thymol against non-insect arthropods and reported that thymol had the most synergistic interaction. The synergist, piperonyl butoxide, was used to synergize EOCs such as carvacrol, limonene, eugenol, and thymol, against the German cockroach (Oladipupo et al. 2020a). Additionally, essential oils could be used to synergize under non-chemical control methods. For example, Perry and Choe (2020) described the potentiality of using essential oils to improve the efficacy of heat treatments against drywood termites.

2.5.2.7 Inherent variability of essential oils data

There is little or no information among the relative potencies of essential oils vis-à-vis plant family or EOCs. It is possible that regardless of where the plant is cultivated, specific components would be abundant. For example, eugenol is routinely associated with the genus *Syzygium*, *trans*-cinnamaldehyde with *Cinnamonum*, and thymol with Thymus (Cheng et al. 2008, Kim et al. 2016, Yones et al. 2016, Lambert et al. 2020). Yet there are substantial variations in compositions of EOCs within other genera like *Cuppresus* and *Piper* (Marsaro et al. 2004, Kuo et al. 2007, Souto et al. 2012, Xie et al. 2013, Wagan et al. 2017). Interestingly, the relative abundance of an EOC in a plant does not necessarily correlate with bioactivity. For example, the most abundant EOC of *R. officinalis*, 1,8-cineole, had lower toxicity when compared to *R. officinalis* EO blend (Miresmailli et al. 2006). Even more intriguing is the inherent variation of the components of an EO across plant families and seasonal variability in EOC in the same plants. Hence, estimating the structural-activity relationship could be a tool to assess, compare, and optimize insecticidal effects across groups. In other words, there should be less emphasis on screening a given plant (i.e., EO or plant extract) for its insecticidal activity to assess the relative potencies of established EOCs. At best, the former generates data that answers the question of "who", "what", "when" and "where", while the latter is certain to provide knowledge (i.e., "how") and mechanism (i.e., "why") that is central to the commercialization of EOs.

Even more concerning is the variabilities across studies. The non-uniformity of bioassays across studies precludes any straightforward comparison and makes it difficult to distill results across studies. Such variabilities make it challenging to compare studies directly. In the interim, a meta-analysis could help overcome such a hurdle. In short, there is a forest of publications describing the potential effects of EOs and EOCs against urban pests and a desert of relevant data from these studies. This is why we plea to authors to include relevant data that can be used. For example, many authors cite mortality and do not report the dose. Their results are therefore of little use. The way forward is to include the concentration of a toxicant (EOC) per body weight in topical or mg L^{-1} in fumigant bioassays. For contact bioassays, accurate information on the type of surface, species, sex, age, stage, insect mass, and EOC formulation should be provided.

2.5.2.8 Disconnect between laboratory and field studies

The contrast between laboratory and field efficacy is arguably one of the major impediments to the commercialization of EOs (Benelli et al. 2016, Isman 2020). First, the design of laboratory bioassays is not reflective enough of field conditions. For example, topical application of cockroaches or bed bugs can not be repeated in the field. The delivery of EOs through superabsorbent polymer gels or nanoformulations against cockroaches might not work in homes due to other sources of food and water that would distract cockroaches unless attractants are used. Second, the rapid biodegradation/volatilization of EOs, paradoxically, make them unusable in the field. Thus, frequent reapplication or specialized (and expensive) formulations may be required to achieve a satisfactory level of control. Economically, this is disadvantageous.

Further, the variability in response to a given EO or EOC is worrisome. Essentially, the "all or none" response to almost "no dose-response". These contrasts create a vacuum for the investigation and design of formulations that increases the persistence and stabilizations of EOs without interrupting bioactivity.

In toto, a recurring theme during the review process is the lack of working hypotheses and prior predictions guiding most investigations. This is exemplified by the general conclusions: "we found another plant that works!" Interestingly, such a revelation is not as exciting as it seems – at least not on paper. There is the need to fit objectives into a theoretical framework before embarking on the study. Authors should realistically ask, "What data do I need to provide" to advance the scholarship on essential oils or "Does my study address broader impacts? Clear methodology detailing approaches might be more useful to everyone. If everyone uses at least one common methodology, we would have a better basis to compare the performance of EOs and EOCs. Additionally, we advocate the release of EOs/EOCs in laboratory-based bioassays in a manner closest to potential field applications. EOs/EOCs would likely be released as fogs/mists in homes to manage urban insect pests. Thus, subsequent laboratory experimental designs should consider the release of EOs/EOCs as ultra-low volume sprays. Such designs would address questions such as (1) Can sufficient concentrations of EOs or EOCs be confined in a volume of air long enough to kill? (2) Are EO or EOC "vapors" repellent? and (3) If so, then would we need to confine pests in a sealed volume of air to prevent escape? Besides, available data implicates aromatic compounds with a benzene ring as the most toxic EOs/EOCs. Why not focus on aromatic compounds rather than showing "we found aliphatic compounds that are toxic too just that they are not as toxic as aromatic EOs". In short, to truly advance the scholarship on essential oils, authors should be more focused on works that advance contribution to knowledge.

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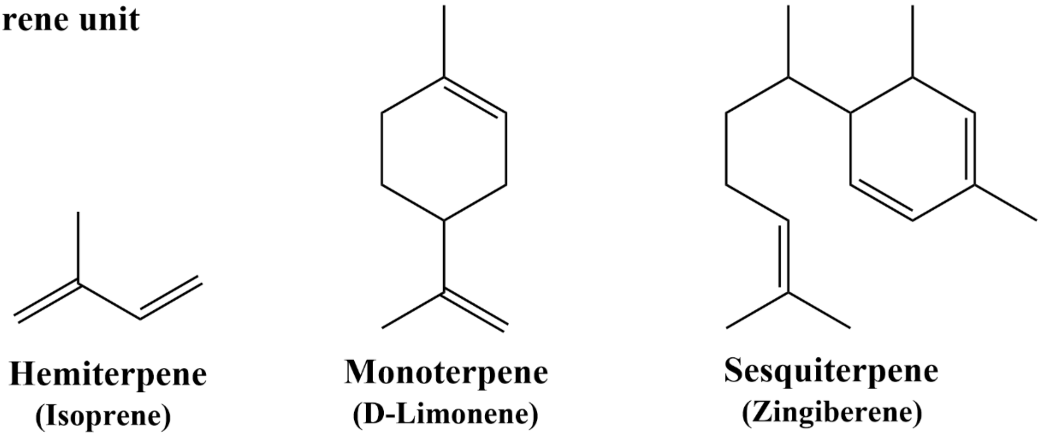
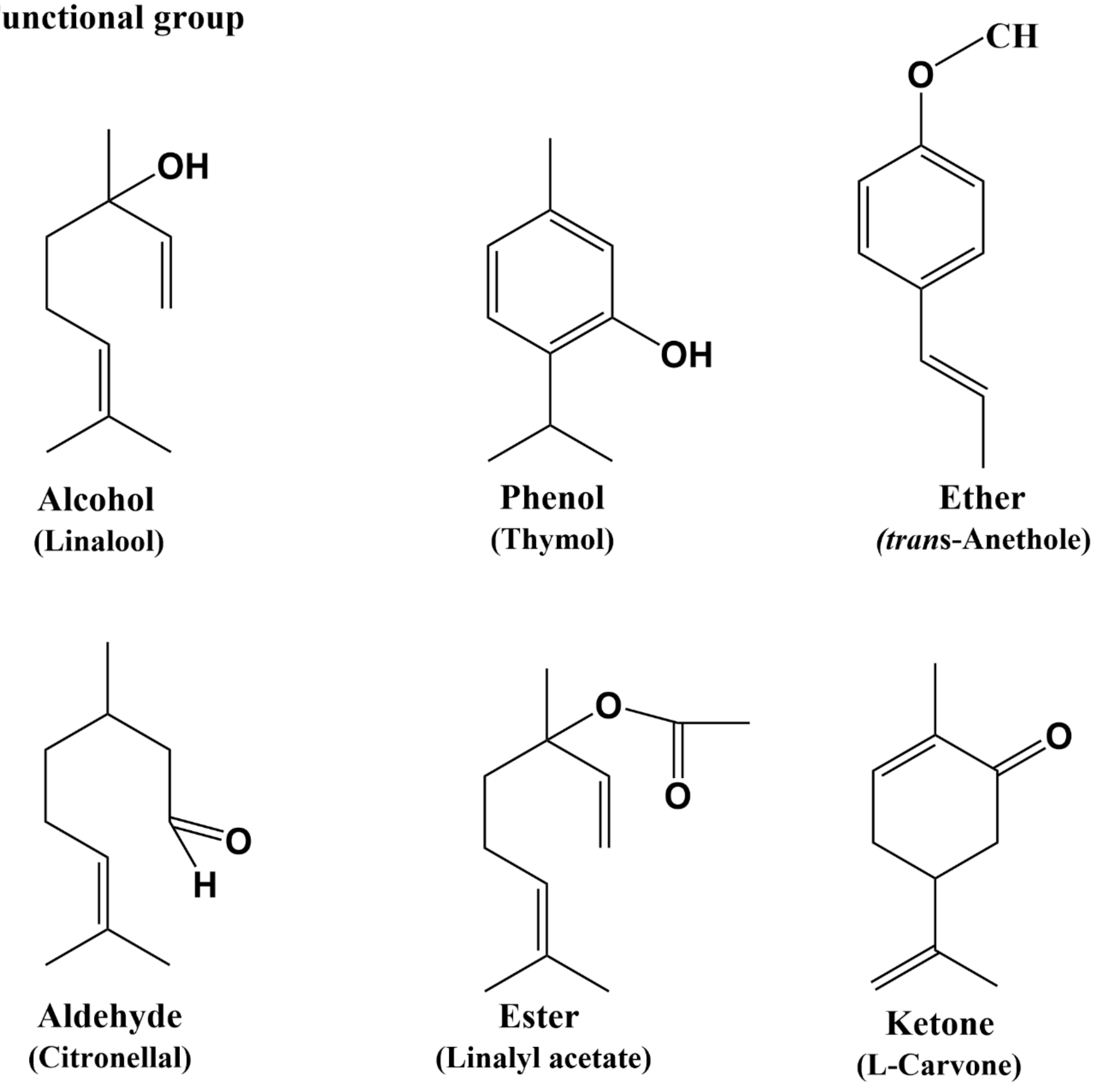


(a) Isoprene unit



(b) Functional group

Fig. 1. Classification of terpenes based on (a) isoprene unit and (b) functional group

Table 1. Insecticidal effects of plant essential oils and their components against ants

Family	Plant species	Plant part	Major component (%)	Species	Bioassay type		Insecticidal effect			
						Caste	Repellency (%)	Mortality (LC$_{50}$)^{a}	Knockdown (LT$_{50}$)*	References
Lauraceae	*Cinnamomum Camphora* (L.) J.Presl.	Leaf	Camphor (36.6)	*Solenopsis invicta* Buren	Fumigant (24 h exposure)	Minor worker		LC after 24 h; 1.67 µgmol^{-1}	10.82 h	Fu et al. (2015)
						Major worker		4.28 µgmol^{-1}	14.73 h	
	C. osmophloeum Kaneh	Leaf	*trans*-Cinnamaldehyde (79.9)	*S. invicta*	Exposure at 2%: Open	Worker			105.0 min	Cheng et al. (2008)
					Close	Worker			18.5 min	
Piperaceae	*Piper aduncum* L.	Aerial part	Dillapiole (64.4)	*S. saevissima* (Smith)	Contact; filter-paper technique (24 h)	Worker		LC; 114.4 mgL^{-1}		Souto et al. (2012)
	P. marginatum Jacq. A		(*E*)-β-Ocimene (9.8)			Worker		207.8 mgL^{-1}		
	P. marginatum B		(*E*)-Isoosmorhizole (32.2)			Worker		419. mgL^{-1}		
	P. divaricatum G. Mey		Methyleugenol (69.2)			Worker		552.2 mgL^{-1}		
	P. callosum Ruiz & Pav		Safrole (69.2)			Worker		571.1 mgL^{-1}		
Lamiaceae	Mint oil granules			*S. invicta*	Repellency	Worker	49–100 for 147.8 mgcm^{-2}		1.2–15.3 h with ~ 1.65 mgcm^{-2} of 2% mint oil granules	Appel et al. (2004)
	Pogostemon cablin Benth	Leaf	Patchoulol (36.6)	*Camponotus novogranadensis*	Topical application	Worker	24 h	LD; 2.31 µg/mg		Albuquerque et al. (2013)
						Worker	48 h	2.34 µg/mg		
				C. melanoticus	Topical application	Worker	4h	3.58 µg/mg		
						Worker	24 h	3.23 µg/mg		
						Worker	48 h	2.95 µg/mg		
				Dorymyrmex thoracicus	Topical application	Worker	4h	5.12 µg/mg		
						Worker	24 h	5.02 µg/mg		
						Worker	48 h	2.02 µg/mg		
Rutaceae	*Skimmia laureola* (DC.)	Leaf	D-Limonene (32.3)	*Lasius niger* L.	Contact; filter-paper technique	Worker		LC; 10.15 µL		Mehmood et al. (2016)
		Stem	β-Linalool (43.6)			Worker		10.15 µL		
		Root	1,3-Cycloheptadiene (36.9)			Worker		10.15 µL		
		Leaf	D-Limonene (32.3)		Repellency at 10%	Worker	3.5 h (100)			
		Stem	β-Linalool (43.6)			Worker	4 h (100)			
		Root	1,3-Cycloheptadiene (36.9)			Worker	4 h (100)			
Cuppressaceae	*Cuppressus nootkatensis* D.Don		Nootkatene (57)	*S. invicta* x *richteri*	Contact	Worker		LC; 0.26 %		Addesso et al. (2017)
					Fumigant	Worker		8.85 µl oil^{-}L		
					Digging	Worker				

							Digging suppressed by 50 %			
Cordiaceae	*Varronia curassavica* Jacq.	Leaf		*Dorymyrmex thoracius* Gallardo				LC;		de Oliveira et al. (2020)
	VAC-316		(E)-Caryophyllene (6.1)		Fumigant	Worker		$1.5\ \mu LL^{-1}$		
	VAC-324		(E)-Caryophyllene (22.3)		Fumigant	Worker		$2.5\ \mu L\ L^{-1}$		
	VAC-326		(E)-Caryophyllene (16.1)		Fumigant	Worker		$0.7\ \mu L\ L^{-1}$		
	VAC-503		(E)-Caryophyllene (10.8)		Fumigant	Worker		$1.5\ \mu L\ L^{-1}$		
	VAC-509`		(E)-Caryophyllene (20.8)		Fumigant	Worker		$2.5\ \mu L\ L^{-1}$		
	VAC-510		(E)-Caryophyllene (12.1)		Fumigant	Worker		$1.3\ \mu L\ L^{-1}$		

Commercial product/EOC										
(E)-Caryophyllene				*D. thoracius*	Fumigant	Worker		$1.5\ \mu LL^{-1}$		de Oliveira et al. (2020)
α-Humulene					Fumigant	Worker		$3.8\ \mu LL^{-1}$		
trans-Cinnamaldehyde				*S. invicta*	Exposure at 2%:					Cheng et al. (2008)
					Open	Worker		32.2 min		
					Close	Worker		21.2 min		
Camphor				*S. invicta*	Fumigant (24 h exposure)	Minor worker Major worker		$1.91\ \mu gmol^{-1}$ $5.59\ \mu gmol^{-}$		Fu et al. (2015)
Cineole				*S. invicta*	Fumigant (24 h exposure)	Minor worker Major worker		$2.34\ \mu gmol^{-1}$ $5.99\ \mu gmol^{-}$		

* Unless otherwise stated; knockdown time is expressed as LT_{50}; LT_{50} = lethal time required to kill 50% of the population.

[a] LC_{50} = lethal concentration required to kill 50% of the population.

Table 2. Insecticidal effects of plant essential oils and their components against bed bugs

Family	**Plant species**	**Plant part**	**Major component (%)**	**Species**	**Bioassay type**		**Insecticidal effect**		
						Life-stage	Repellency (%)	Mortality (% or LD$_{50,}$ or LC$_{50}$)[a]	References
Lamiaceae	*Oreganum vulgare* L.	Leaf	Terpineol (22.9)	*Cimex lectularius* L.	Treated surface method	Adult	At 10 %	*EC$_{50}$;	Sharififard et al. (2018)
						After 3 h	100	0.3 mgcm^{-2}	
						5 h	86	0.9 mgcm^{-2}	
						9 h	65	1.6 mgcm^{-2}	
						24 h	17	4.5 mgcm^{-2}	
Asteraceae	*Tagetes patula* L.	Aerial part	α-Terpinolene (15.5)	*C. lectularius*	Impregnated paper disk test	Adult		LC; 0.17 mgml^{-1}	Politi et al. (2017)
Schisandraceae	*Kadsuna coccinea Kaempf. ex Juss.*	NS**	β-Caryophyllene (24.7)	*C. lectularius*	Topical application	Post-treatment days		% Mortality at 100 µg	Rehman et al. (2019)
						1	Resistant	61.9	
						1	Susceptible	66.7	
						3	Resistant	61.9	
						3	Susceptible	66.7	
						5	Resistant	61.9	
						5	Susceptible	90.5	
						7	Resistant	61.9	
						7	Susceptible	90.5	
Commercial product/EOC									
EcoRaider				*C. lectularius*	Spray treatment	Adult	92% reduction after 12 wks		Wang et al. (2014)
								Mortality in Petri dishes	Feldlaufer and Ulrich (2015)
CirkilT RTU				*C. lectularius*	Fumigant	Adult		100	
Acetaphenone					Fumigant	Adult		100	
Cedarwood					Fumigant	Adult		0	
Cinnamon					Fumigant	Adult		100	
Citronella					Fumigant	Adult		86.9	
Clove					Fumigant	Adult		98.7	
Geranium					Fumigant	Adult		97.8	
Lemongrass					Fumigant	Adult		100	
Neem seed oil					Fumigant	Adult		2.4	
Peppermint					Fumigant	Adult		100	
Rosemary					Fumigant	Adult		100	
Thyme					Fumigant	Adult		100	
Methyl benzoate				*C. lectularius*	24 h exposure in flask (fumigant)	Susceptible adult		LC; 4.1 mgL^{-1}	Larson et al. (2020)
Acetaphenone								2.4 mgL^{-1}	
						Resistant adult		6.2 mgL^{-1}	
								4.1 mgL^{-1}	

Carvacrol					Topical toxicity				
Thymol					Topical toxicity	Adult male		LD; 27.5 μgmg^{-1}	Gaire et al. (2019)
Citronellic acid					Topical toxicity	Adult male		32.5 μgmg^{-1}	
Eugenol					Topical toxicity	Adult male		49 μgmg^{-1}	
Geraniol					Topical toxicity	Adult male		52 μgmg^{-1}	
α-Pinene					Topical toxicity	Adult male		64 μgmg^{-1}	
R(+)-Limonene					Topical toxicity	Adult male		70.5 μgmg^{-1}	
Linalool					Topical toxicity	Adult male		91.5 μgmg^{-1}	
Euclayptol					Topical toxicity	Adult male		112 μgmg^{-1}	
(-)-Terpinen-4-ol					Topical toxicity	Adult male		132 μgmg^{-1}	
trans-Cinnamaldehyde					Topical toxicity	Adult male		138.5 μgmg^{-1}	
Menthone					Topical toxicity	Adult male		138.5 μgmg^{-1}	
(±)-Citronellal					Topical toxicity	Adult male		165 μgmg^{-1}	
(±)-Camohor					Topical toxicity	Adult male		240 μgmg^{-1}	
Methyl eugenol					Topical toxicity	Adult male		515 μgmg^{-1}	
						Adult male		560 μgmg^{-1}	
Thymol					Fumigant (24 h exposure)				Gaire et al. (2019)
Carvacrol						Adult male		LC; 20.50 mgL^{-1}	
Linalool						Adult male		46.3 mgL^{-1}	
(±)-Camohor						Adult male		51.2 mgL^{-1}	
Menthone						Adult male		133.3 mgL^{-1}	
Euclayptol						Adult male		150.7 mgL^{-1}	
(-)-Terpinen-4-ol						Adult male		191.1 mgL^{-1}	
trans-Cinnamaldehyde						Adult male		388.3 mgL^{-1}	
R(+)-Limonene						Adult male		389.0 mgL^{-1}	
α-Pinene						Adult male		454.0 mgL^{-1}	
(±)-Citronellal						Adult male		488.8 mgL^{-1}	
						Adult male		1474.6 mgL^{-1}	
Carvacrol + Thyme + Eugenol								19 μgmg^{-1}	Gaire et al. (2020)

[a] LC$_{50}$ or LD$_{50}$ = lethal concentration or lethal dose required to kill 50% of the population.

[*] EC$_{50}$ = effective concentration required to cause 50% repellency against bed bug

[**] NS = not stated by the author.

Table 3. Insecticidal effects of plant essential oils and their components against cockroaches

Family	**Plant species**	**Plant part**	**Major component (%)**	**Species**	**Bioassay type**		**Insecticidal effect**			
						Life-stage (condition)	Repellency (%)	Mortality (LD$_{50}$ or LC$_{50}$)^{a}	Knockdown (LT$_{50}$)$^{\#}$	References
Piperaceae	*Piper nigrum* L.	Fruit	Piperine (34.8)	*Blattella germanica* (L.)	Repellency	Nymph Adult	49.1 at 12 h 55 at 12 h			Wagan et al. (2017)
Lamiaceae	*Nepeta cataria* L.	Aerial part	Z,E-nepetalactone (85)	*B. germanica*	Repellency	Adult male	55.6 at 800 µgcm^{-2}			Peterson et al. (2002)
	Pogostemon cablin (Blanco) Benth.	Leaf	Patchoulol (41.3)	*B. germanica*	Contact Repellency	Male Nymph	47.6 at 5 ppm after 4 h	LC; 23.5 µg/adult		Liu et al. (Liu et al. 2015)
	Thymus persicus (Ronniger ex Rech. f.)	Aerial part	ND*	*B. germanica*	Fumigant	Adult		LC after 24h; 28.8 µl/l		Rezaei et al. (2019)
Myrtaceae	*Eucalyptus camaldulensis* Dehn.	Aerial part	ND	*B. germanica*	Fumigant	Adult		LC after 24h; 21.8 µl/l		Rezaei et al. (2019)
Anacardiaceae	*Schinus molle* L.	Leaf	□-Cadinene (11.3)	*Blatta orientalis* L.	Repellency	Adult	100 at 176 ugcm^{-2}			Batista et al. (Batista et al. 2016)
Asteraceae	*Artemisia sieberi* Besser	Aerial part	ND	*B. germanica*	Fumigant	Adult		LC after 24h; 17.3 µl/l		Rezaei et al. (2019)
Chenopodiaceae	*Chenopodium ambrosioides* L.	Aerial part	*(Z)*-ascaridole (29.7)	*B. germanica*	Fumigant Topical	Male Male		After 24 h: LC; 4.1 mgL^{-1} LD; 64.5 µg/adult		Zhu et al. (Zhu et al. 2012)
Commercial products/EOC										
Oregano oil				*Supella longipalpa* Fabricius	Repellency	Nymph	96.5 at 30%			Sharififard et al. (2016)
Rosemary oil				*S. longipalpa*	Repellency	Nymph	94.5 at 2.5%			
Mint oil				*S. longipalpa*	Repellency	Nymph	63.3 at 30%			
Yarrow oil				*S. longipalpa*	Repellency	Nymph	86.7 at 30%			
Eucalyptus oil				*S. longipalpa*	Repellency	Nymph	27.7 at 30%			
Geranium oil				*B. germanica*	Contact	Adult		After 72 h exposure LC; 0.2 mgcm^{-2}		Werdin González et al. (Werdin Gonzalez et al. 2015)
Bergamot oil				*B. germanica*	Contact	Adult		After 72 h exposure LC; 0.4 mgcm^{-2}		
Red thyme oil				*Blatta lateralis (Walker)*	Topical Fumigant	Nymph Nymph		1.6 mg/nymph 160.5 mgL^{-1} air		Gaire et al. (2017)
Clove bud oil				*Blatta lateralis*	Topical Fumigant	Nymph Nymph		1.7 mg/nymph 319.0 mgL^{-1} air		
Java citronella oil				*Blatta lateralis*	Topical Fumigant	Nymph Nymph		7.9 mg/nymph 746.7 mgL^{-1} air		
Mint oil				*B. germanica* *Periplaneta Americana* (L.)	Topical Contact Topical Contact	Adult male Adult male Adult male Adult male		LD; 3.8 µl LT; 1 min at 100% LD; 2.6 µl LT; 11.1 min at 100%		Appel et al. (Appel et al. 2001)

Clove bud oil				*B. germanica*	Contact Repellency	Adult Adult	80% repellency at 2 mlcm^{-2} after 0.5 h	95% mortality at 4 mlcm^{-2}		Neupane et al. (Neupane et al. n.d.)
Z,*E*-nepetalactone				*B. germanica*	Repellency	Adult male	68.2 at 800 μgcm^{-2}			Peterson et al. (2002)
E,*Z*-nepetalactone				*B. germanica*	Repellency	Adult male	79.4 at 800 μgcm^{-2}			
(+)-α-Pinene				*B. germanica*	Repellency	First instar			11.8 min	Alzogaray et al. (Alzogaray et al. 2013)
				B. germanica	Fumigant	Male Female		LC; 11.8 mgL^{-1} 26.1 mgL^{-1}		Phillips and Appel (2010)
(-)-α-Pinene				*B. germanica*	Repellency	First instar			14.6 min	Alzogaray et al. (2013)
Limonene				*B. germanica*	Repellency	First instar			81.0 min	Alzogaray et al. (2013)
				B. germanica	Fumigant	Male Female		LC; 13 mgL^{-1} 15.3 mgL^{-1}		Phillips and Appel (2010)
Menthone				*B. germanica*	Repellency	First instar			141.0 min	Alzogaray et al. (2013)
				B. germanica	Fumigant	Male Female		LC; 7.4 mgL^{-1} 13.9 mgL^{-1}		Phillips and Appel (2010)
Linalool				*B. germanica*	Repellency	First instar			238.6 min	Alzogaray et al. (2013)
				B. germanica	Contact + fumigant Contact + fumigant	Susceptible Male Female Resistant Male Female		LD at 24 h exposure; 0.3 mgcm^{-2} 0.4 mgcm^{-2} 0.4 mgcm^{-2} 0.5 mgcm^{-2}		Chang et al. (Chang et al. 2012)
				B. germanica	Fumigant	Male Female		LC; 15.7 mgL^{-1} 142 mgL^{-1}		Phillips and Appel (2010)
Terpinolene				*B. germanica*	Vapor-phase	Female		87% at 0.06 mgcm^{-3}		Chang et al. (2012)
Nerol				*B. germanica*	Vapor-phase	Female		97% at 0.03 mgcm^{-3}		
1,8-cineole				*B. germanica*	Vapor-phase	Female		87% at 0.05 mgcm^{-3}		
				B. germanica	Fumigant	Male Female		LC; 6.8 mgL^{-1} 8.4 mgL^{-1}		Phillips and Appel (2010)
				B. germanica	Topical	Adult male Adult female		LD; 0.16 mg/insect 0.27 mg/insect		Philips et al. (2010)
p-cymene				*B. germanica*	Vapor-phase	Female		97% at 0.04 mgcm^{-3}		Chang et al. (2012
				B. germanica	Fumigant Topical	Male Male		After 24 h: LC; 9.92 mgL^{-1} LD; 119.9 μg/adult		Zhu et al. (2012)
				Blatta lateralis	Topical Fumigant	Nymph Nymph		9.9 mg/nymph 441.8 mgL^{-1} air		Gaire et al. (2017)

Pogostone				*B. germanica*	Contact Repellency	Male Nymph	62.4 at 5 ppm after 4 h	LC; 8.5 µg/adult		Liu et al. (Liu et al. 2015)
Caryophyllene				*B. germanica*	Contact Repellency	Male Nymph	55.2 at 5 ppm after 4 h	LC; 339.9 µg/adult		
Patchoulol				*B. germanica*	Contact Repellency	Male Nymph	40.5 at 5 ppm after 4 h	LC; 207.6 µg/adult		
Isoascaridole				*B. germanica*	Fumigant Topical	Male Male		After 24 h: LC; 2.1 mgL^{-1} LD; 96.3 µg/adult		Zhu et al. (2012)
Ascaridole				*B. germanica*	Fumigant Topical	Male Male		After 24 h: LC; 0.6 mgL^{-1} LD; 22 µg/adult		
Carvacrol				*B. germanica*	Fumigant	Male Female		LC; 80.7 mgL^{-1} > 1000 mgL^{-1}		Phillips and Appel (2010)
				B. germanica	Topical	Adult male Adult female		LD; 0.1 mg/insect 0.18 mg/insect		Philips et al. (2010)
trans-Cinnamaldehyde				*B. germanica*	Fumigant	Male Female		LC; 32 mgL^{-1} 34.4 mgL^{-1}		Phillips and Appel (2010)
				Blatta lateralis	Topical Fumigant	Nymph Nymph		1.0 mg/nymph 150.8 mgL^{-1} air		Gaire et al. (2017)
				B. germanica	Topical	Adult male Adult female		LD; 0.08 mg/insect 0.19 mg/insect		Philips et al. (2010)
Eugenol				*B. germanica*	Fumigant	Male Female		LC; 95.9 mgL^{-1} > 1000 mgL^{-1}		Phillips and Appel (2010)
				P. americana	Contact Repellency	Female Female	RP; 77.1 µgxm^{-2}	LC; 0.1 mgcm^{-2}		Ngoh et al. (1998)
				Blatta lateralis	Topical Fumigant	Nymph Nymph		1.6 mg/nymph 251.2 mgL^{-1} air		Gaire et al. (2017)
				B. germanica	Contact Repellency	Adult Adult	85% repellency at 1 mlcm^{-2} after 0.5 h	85% mortality at 4 mlcm^{-2}		Neupane et al. (2020)
				B. germanica	Topical	Adult male Adult female		LD; 0.11 mg/insect 0.29 mg/insect		Philips et al. (2010)
Eugenol acetate				*B. germanica*	Contact Repellency	Adult Adult	85% repellency at 2.5 mlcm^{-2} after 0.5 h	87% mortality at 4 mlcm^{-3}		Neupane et al. (2020)
Thymol				*B. germanica*	Fumigant	Male Female		LC;19.3 mgL^{-1} 142.9 mgL^{-1}		Phillips and Appel (2010)
				Blatta lateralis	Topical Fumigant	Nymph Nymph		0.3 mg/nymph 27.6 mgL^{-1} air		Gaire et al. (2017)

Safrole				*P. americana*	Fumigant Contact	Female Female		LC; 0.2 mgcm^{-2} 0.3 mgcm^{-2}		Ngoh et al. (1998)
Isosafrole				*P. americana*	Fumigant Contact	Female Female		LC; 0.3 mgcm^{-2} 0.2 mgcm^{-2}		
Citronellic acid				*B. germanica*	Topical	Adult male Adult female		LD; 0.25 mg/insect 0.49 mg/insect		Philips et al. (2010)
Geraniol				*B. germanica*	Topical	Adult male Adult female		LD; 0.26 mg/insect 0.83 mg/insect		Philips et al. (2010)
Thymol				*B. germanica*	Topical	Adult male Adult female		LD; 0.12 mg/insect 0.07 mg/insect		Phillips et al. (2010)

[a] LC_{50} or LD_{50} = lethal concentration or lethal dose required to kill 50% of the population.

[*] ND = not done by the authors.

[#] Knockdown time is expressed as LT_{50}; LT_{50} = lethal time required to kill 50% of the population.

Table 4. Insecticidal effects of plant essential oils and their components against flea

Family	**Plant species**	**Plant part**	**Major component (%)**	**Species**	**Bioassay type**		**Insecticidal effect**		
						Life-stage (condition)	Repellency (%)	Mortality (LD$_{50}$ or LC$_{50}$)[a]	References
Lauraceae	*Cinnamomum osmophloeum* Kaneh.	Leaf	*trans*-Cinnamaldehyde (87.1)	*Ctenocepalides felis felis* (Bouché)	Repellency	Adult	68.6–97.7		Su et al. (2014)
Lamiaceae	*Plectranthus amboinicus* (Lour)	Leaf	Thymol (58.1)	*C. felis felis*	Repellency	Adult	68.6–97.7		Su et al. (2014)
	Ocimum gratissimum L.	Leaf	Eugenol (74.5)	*C. felis felis*	Impregnated filter-paper test	Egg Larva Adult (24 h) Adult (48 h)		LC; 1.8 ugcm^{-2} 1.2 ugcm^{-2} 5.9 ugcm^{-2} 4.5 ugcm^{-2}	Barbosa dos Santos et al. (2020)
	Mentha spicata L.	Leaf	Carvone (83.3)	*C. felis felis*	Impregnated filter-paper test	Egg Larva Adult (24 h) Adult (48 h)		30.4 ugcm^{-2} 12.6 ugcm^{-2} 597.6 ugcm^{-2} 380.1 ugcm^{-2}	
	Laurus nobilis L.	Leaf	Eucalyptol (19.2)	*C. felis felis*	Impregnated filter-paper test	Egg Larva Adult (24 h) Adult (48 h)		2.4 ugcm^{-2} 0.5 ugcm^{-2} 412.1 ugcm^{-2} 454.9 ugcm^{-2}	
	Cinnamomum spp.	Leaf	(E)-Cinnamaldehyde (91.7)	*C. felis felis*	Impregnated filter-paper test	Egg Larva Adult (24 h) Adult (48 h)		1.8 ugcm^{-2} 0.4 ugcm^{-2} 67.9 ugcm^{-2} 41.9 ugcm^{-2}	
	Ziziphora tenuiore L.	Shoot	Thymol (36.3)	*Pulex irritans* L.	Repellency	Adult	At 1600 ugcm^{-2} = 98	[*]ED; 229 ugcm^{-2}	Ghavami et al. (2017)
	Mentha piperita L.	Shoot	Mentha (26.7)	*P. irritans*	Repellency	Adult	At 1600 ugcm^{-2} = 69.	776 ugcm^{-2}	
Cuppressaceae	*Calocedrus decurrens* (Torr.)	Heart wood	ND[**]	*Xenopsylla cheopis* (Rothchild)	Contact test	Adult (24 h)		LC; 0.24 mgml^{-1}	Dolan et al. (2007)
	Chamaecyparis lawsoniana (A. Murr.)	Heart wood	ND	*X. cheopis*	Contact test	Adult (24 h)		1.21 mgml^{-1}	
	Juniperus occidentalis (Hook)	Wood shavings	ND	*X. cheopis*	Contact test	Adult (24 h)		0.31 mgml^{-1}	
	Taiwania cryptomerioides Hayata	Heartwood	α-Cadinol (27.8)	*C. felis felis*	Repellency	Adult	68.6–97.7		Su et al. (2014)

Myrtaceae	*Syzigium aromaticum* Merr. & Perry	Stem	Eugenol (61.4)	*C. felis felis*	Filter-paper test	Egg Adult (24 h) Adult (48 h)		LC; 0.3 ugcm^{-2} 5.7 ugcm^{-2} 3.9 ugcm^{-2}	Lambert et al. (2020)
	Myrtus communis L.	Shoot	α-Pinene (32.5)	*P. irritans*	Repellency	Adult	At 1600 ugcm^{-2} = 96	ED; 229 ugcm^{-2}	Ghavami et al. (2017)
Poaceae	*Cymbopogon nardus* (L.) Rendl	Leaf	Citronellal (45.8)	*C. felis felis*	Impregnated filter-paper test	Egg Larva Adult (24 h) Adult (48 h)		LC; 12.0 ugcm^{-2} 7.3 ugcm^{-2} 597.1 ugcm^{-2} 486.1 ugcm^{-2}	Barbosa dos Santos et al. (2020)
Zingiberaceae	*Alpinia zerumbet* (Pers.)	Leaf	4-Terpineol (22.1)	*C. felis felis*	Impregnated filter-paper test	Egg Larva Adult (24 h) Adult (48 h)		LC; 13.1 ugcm^{-2} 7.3 ugcm^{-2} 553.3 ugcm^{-2} 456.3 ugcm^{-2}	Barbosa dos Santos et al. (2020)
Anacardiaceae	*Schinus molle* L.	Leaf	Cubenol (13.0)	*C. felis felis*	Impregnated filter-paper test	Adult (24 h) Adult (48 h)		LD; 12.0 ugcm^{-2} 9.1 ugcm^{-2}	Batista et al. (2016)
		Fruit	Myrtenal (20.9)	*C. felis felis*	Impregnated filter-paper test	Adult (24 h) Adult (48 h)		354.0 ugcm^{-2} 138.2 ugcm^{-2}	
Asteraceae	*Achillea wilhelmsii* L.	Shoot	Dimethylhepta (10.2)	*P. irritans*	Repellency	Adult	At 1600 ugcm^{-2} = 87	ED; 457 ugcm^{-2}	Ghavami et al. (2017)
Commercial product/EOC									
Eugenol				*C. felis felis*	Filter-paper test	Egg Adult (24 h) Adult (48 h)		0.1 ugcm^{-2} 1.4 ugcm^{-2} 2.4 ugcm^{-2}	Lambert et al. (2020)
trans-Cinnamaldehyde (2%)					Repellency	Adult	97.6		Su et al. (2014)
Thymol (0.5%)					Repellency	Adult	90.6		
Carvacrol				*X. cheopis*	Contact test	Adult (24 h)		LD; 0.01 (wt:vol)	Panella et al. (2005)
Valencene				*X. cheopis*	Contact test	Adult (24 h)		0.04(wt:vol)	
Nootkatene				*X. cheopis*	Contact test	Adult (24 h)		0.02 (wt:vol)	
Nootkatone (crystal)				*X. cheopis*	Contact test	Adult (24 h)		0.01 (wt:vol)	
Nootkatone				*X. cheopis*	Contact test	Adult (24 h)		0.003 (wt:vol)	
Valencene-13-ol				*X. cheopis*	Contact test	Adult (24 h)		0.01 (wt:vol)	
Valencene-13-aldehyde				*X. cheopis*	Contact test	Adult (24 h)		0.02 (wt:vol)	

[a] LC$_{50}$ or LD$_{50}$ = lethal concentration or lethal dose required to kill 50% of the population.

[*] ED$_{50}$ = effective dose required to kill 50% of the population.

[**] ND = not done by the authors.

Table 5. Insecticidal effects of plant essential oils and their components against head lice

Family	Plant species	Plant part	Major component (%)	Species	Bioassay type		Insecticidal effect			
						Life-stage (condition)	Repellency (%)	Mortality (LD$_{50}$ or LC$_{50}$)^{a}	Knockdown (KD$_{50}$)$^{\#}$	References
Lauraceae	*Cinnamomum porphyrium* Kosterm	Leaf	ND**	*P. humanus capitis*	Fumigant	Adult	49.5		> 1.1 min	Toloza et al. (2010b)
	C. aromaticum Nees	Bark	Cinnamaldehyde (70.1)	*P. humanus capitis*	Contact	Adult			11.4 min	Yones et al. (2016)
	C. zeylanicum J.Presl	Bark	Cinnamaldehyde (58.1)	*P. humanus capitis*	Contact	Adult			LT; 27.6 at 0.5 mgcm^{-2}	Yang et al. (2005)
	Laurus nobilis L.	Leaf	1,8-Cineole (50.8)	*P. humanus capitis*	Fumigant	Adult			24.4 min	Tolozo et al. (2006)
	Litsea cubeba (Lour.) Pers.	Leaf	Geraniol (36.7)	*P. humanus capitis*	Contact	Adult			LT; 30 min	Candy et al. (2018)
Lamiaceae	*Mentha spicata* L.	Aerial part	*l*-Carvone (32.8)	*P. humanus capitis*	Contact	Adult			8.8 min	Yones et al. (2016)
	Thymus vulgaris L.	Aerial part	Thymol (33.8)	*P. humanus capitis*	Contact	Adult			29.9 min	
	M. pulegum L.	Leaf	Pulegone (51.1)	*P. humanus capitis*	Fumigant	Adult	75.5		57.7 min	Tolozo et al. (2006)
	Origanum vulgare L.	Leaf	Carvacrol (80.5)	*P. humanus capitis*	Fumigant	Adult	34.5		> 60 min	
	Monarda fistulosa L.	Seed	Geraniol (91.7)	*P. humanus capitis*	Contact	Adult			LT; 180 min	Candy et al. (2018)
Myrtaceae	*Melaleuca* alternifolia (Maiden & Betche)	Leaf	ND	*P. humanus capitis*	Impregnated filter-paper test Ovicidal	Adult Eggs		100% mortality at 1% EO after 30 mins 50% abortive eggs at 25% EO after 4 d		Di Campli et al. (2012)
	Eucalyptus globulus L.	Leaf	1,8-Cineole (21.4)	*P. humanus capitis*	Contact	Adult			43.2 min	Yones et al. (2016)
	E. dunnii Maiden	Leaf	1,8-Cineole (49.6)	*P. humanus capitis*	Fumigant (closed container)	Adult			40 min	Toloza et al. (2010a)
	E. gunni Hook.f.	Leaf	1,8-Cineole (26.7)	*P. humanus capitis*	Fumigant	Adult			73. 4	
	E. cinerea F. Muell. Ex Benth.	Leaf	1,8-Cineole (62.1)	*P. humanus capitis*	Fumigant	Adult	50.2		12 min	Tolozo et al. (2006)
	E. viminalis Labill.	Leaf	1,8-Cineole (46.9)	*P. humanus capitis*	Fumigant	Adult	33.3		14.9 min	
	E. tereticornis Sm.	Leaf	1,8-Cineole (37.5)	*P. humanus capitis*	Fumigant	Adult	34.5		23.5 min	
	E. citriodora Hook	Leaf	Thymol (76)	*P. humanus capitis*	Fumigant	Adult	59.3		> 60 min	
	E. saligna Sm.	Leaf	1,8-Cineole (93.2)	*P. humanus capitis*	Fumigant	Adult	64.8		17.4 min	
	Eugenia aromaticum L.	Bud	Eugenol (72.9)	*P. humanus capitis*	Contact	Adult			19.7 min	Yones et al. (2016)
	E. aromaticum	Bud	Chavibetol (58.8)	*P. humanus capitis*	Fumigant (closed container)	Adult			LT; 5. 4 min	Bagavan et al. (2011)

				P. humanus capitis	Fumigant (open container)	Adult			LT; 47.9 min	
					Contact	Adult		6.54 min at 0.25 mgcm^{-2}		
		Bud	Eugenol (74.6)	*P. humanus capitis*	Contact	Adult			LT; 10 min	Candy et al. (2018)
	Myrcianthes cisplatensis (Cambess.) O.Berg	Leaf	1,8-Cineole (45.7)	*P. humanus capitis*	Fumigant	Adult			1.3 min	Tolozo et al. (2006)
Asteraceae	*Tagetes filifolia* Lag.	Leaf	ND	*P. humanus capitis*	Fumigant	Adult			> 60 min	Toloza et al. (2010b)
	T. mendocina Phil.	Leaf	ND	*P. humanus capitis*	Fumigant	Adult			> 60 min	
	Baccharis vernicosa Hook. & Arn.	Leaf	ND	*P. humanus capitis*	Fumigant	Adult			> 60 min	
	B. salicifolia Pers.	Leaf	ND	*P. humanus capitis*	Fumigant	Adult			> 60 min	
	Artemisia annua L.	Leaf	ND	*P. humanus capitis*	Fumigant	Adult			41.4 min	
	Argerantum conyzoides L.	Leaf	ND	*P. humanus capitis*	Fumigant	Adult		LD; 6.3 for 1 hr exposure		Shailajan et al. (2013)
Cucurbitaceae	*Momordica charantia* L.	Leaf	Nonacosone(NS)	*P. humanus capitis*	Contact	Adult		LC; 75.1 mgL^{-1}		Gandhi et al. (2017)
Verbenaceae	*Aloysia citrodora* Paláu	Leaf	ND	*P. humanus capitis*	Fumigant	Adult			3.02 min	Toloza et al. (2010b)
	A. polystachia (Griseb.)	Leaf	1,8-Cineole (84.6)	*P. humanus capitis*	Fumigant	Adult	30.7		23.4 min	Tolozo et al. (2006)
	Lippia multifora Moldenke	Leaf	Linalool (26.7)	*P. humanus capitis* *P. humanus capitis*	Contact Contact (close)	Adult Adult			At 10%; 22 min 16. 5 min	Oladimeji et al. (2000)
Apiaceae	*Pimpinella anisum* L.	Fruit	Anise camphor (85.2)	*P. humanus capitis*	Contact	Adult			45.4 min	Yones et al. (2016)
Pedaliaceae	*Sesamum indicum* L.	Seed	Undecane (8.2)	*P. humanus capitis*	Contact	Adult			> 180 min	Yones et al. (2016)
Commercial product/EOC										
Nerolidol				*P. humanus capitis*	Impregnated filter-paper test Ovicidal	Adult Eggs		<33% mortality at 2% after 30 mins 50% abortive eggs at 1% after 4 d		Di Campli et al. (2012)
Tea tree EO (10% v/v in EtOH)				*P. humanus capitis*	Impregnated filter-paper test	Adult		90% mortality after 210 mins		Williamson et al. (2007)
Lavender EO 10% v/v in EtOH)				*P. humanus capitis*	Impregnated filter-paper test	Adult		50% mortality after 210 mins		
Lemon EO 10% v/v in EtOH)				*P. humanus capitis*	Impregnated filter-paper test	Adult		10% mortality after 210 mins		
α-Pinene				*P. humanus capitis*	Fumigant (closed container)	Adult			42.7 min	Tolozo et al. (2010a)
p-Cymene				*P. humanus capitis*		Adult			> 80 min	
1,8-Cineole				*P. humanus capitis*		Adult			11.10 min	
Limonene				*P. humanus capitis*	Fumigant	Adult	5.1		27.2 min	Tolozo et al. (2006)
β-Myrcene				*P. humanus capitis*	Fumigant	Adult			48.9 min	
Menthone				*P. humanus capitis*	Fumigant	Adult	36.5		39.7 min	
Pulegone				*P. humanus capitis*	Fumigant	Adult	39.2		46.9 min	

Thymol				*P. humanus capitis*	Fumigant	Adult	52.7		60 min	

[a] LC_{50} or LD_{50} = lethal concentration or lethal dose required to kill 50% of the population.

[**] ND = not done by the authors.

[#] Unless otherwise stated; knockdown time is expressed as KT_{50}.; KT_{50} = Knockdown time required to kill 50% of the population. LT = Lethal time required to kill 50% of the population

Table 6. Insecticidal effects of plant essential oils and their components against silverfish and brown marmorated stink bug

Family	**Plant species**	**Plant part**	**Major component (%)**	**Species**	**Bioassay type**		**Insecticidal effect**		
						Life-stage (condition)	Repellency (%)	Mortality (LD$_{50}$ or LC$_{50}$)^{a}	References
Cuppressaceae	*Chamaecyparis formosensis* Matsum	Wood chip	Myrtenol (48.9)	*Lepisma saccharina* L.	Mortality test	Adult		0.16 mgcm^{-3} initiated 100% mortality after 2 h	Kuo et al. (2007)
Taxodioideae	*Cryptomeria japonica* (L.f.)	Leaf	Elemol (18.2)	*L. saccharina*.	Repellency Impregnated filter-paper test	Adult Adult	80% repellency at 0.01 mgcm^{-3}	LD; 0.087 mgcm^{-3}	Wang et al. (2006)
Lamiaceae	*Satureja spicigera* (C. Koch)	Aerial part	Carvacrol (32.1)	*Halyomorpha halys* Stål,	Contact	First instar Second instar Third instar Fourth instar Fifth instar Adult		LD; 0.63 µL/ml 0.78 µL/ml 1.02 µL/ml 1.47 µL/ml 2.87 µL/ml 4.66 µL/ml	Gokturk (2021)
Commercial Product/EOC									
Methyl benzoate				*H. halys*	Contact Ovicidal	Nymph (1st – 5th) Egg		LC; 1.01–2.39 µL/vial 0.020 mgcm^{-2}	Feng and Zhang (2017)
EcoSmart Neem oil				*H. halys*	Topical application	Nymph Adult		48 h post treatment; 100 % mortality 15 % mortality	Bergman and Raupp (2014)

^{a} LC$_{50}$ or LD$_{50}$ = lethal concentration or lethal dose required to kill 50% of the population.

Table 7. Insecticidal effects of plant essential oils and their components against stored product moths

Family	Plant species	Plant part	Major component (%)	Species	Bioassay type		Insecticidal effect			
						Life-stage (condition)	Repellency (%)	Mortality (LD$_{50}$ or LC$_{50}$)^{a}	Knockdown (LT$_{50}$)$^{\#}$	References
Lamiaceae	*Satureja hortensis* L.	Leaf	ND*	*Ephestia kuehniella* (Zell.)	Fumigant Contact	Adult Adult		After 9 h exposure; LC; 80.9 μlL^{-1} 0.27 μlcm^{-2}		Maedeh et al. (2011)
				Plodia interpunctella (Hubner)	Fumigant Contact	Adult Adult		139.8 μlL^{-1} 0.19 μlcm^{-2}		
	Ocimum basilicum L.	Leaf + fruit	Linalool (63.1)	*E. kuehniella*	Fumigant	Adult		100% mortality at 100 μL^{-1}		Pandır and Baş (2016)
	O. basilicum	NS**	Linalool (45.9)	*E. kuehniella*	Fumigant			After 24 h exposure,		Eliopoulos et al. (2015)
						Egg		LD 776 μlL^{-1}		
						Larva		2096 μlL^{-1}		
						Pupa		1567 μlL^{-1}		
						Adult		1.4 μlL^{-1}		
				P. interpunctella		Egg		779.2 μlL^{-1}		
						Larva		2036.2 μlL^{-1}		
						Pupa		1799.7 μlL^{-1}		
						Adult		1.2 μlL^{-1}		
	Mentha piperita L.	Leaf + fruit	Menthol (28.3)	*E. kuehniella*	Fumigant	Adult		100% mortality at 20 μL^{-1}		Pandır & Baş (2016)
	M. piperita	NS**	Isomenthone (48)	*P. interpunctella*	Contact	Adult		After 2 h, LD; 53.8 μgcm^{-2}		Jesser et al. (2017)
					Fumigant	Adult			KT; 27.1 min	
	M. spicata L.	NS	Carvone (67.1)	*E. kuehniella*	Fumigant			After 24 h exposure,		Eliopoulos et al. (2015)
						Egg		LD 896.5 μlL^{-1}		
						Larva		2277.6 μlL^{-1}		
						Pupa		1824.3 μlL^{-1}		
						Adult		0.5 μlL^{-1}		
				P. interpunctella		Egg		1231.4 μlL^{-1}		
						Larva		2437.5 μlL^{-1}		
						Pupa		1981.9 μlL^{-1}		
						Adult		0.4 μlL^{-1}		
	Lavandula angustifolia Mill.	NS	Linalool (40.5)	*P. interpunctella*	Contact	Adult		After 2 h, LD; 76.3 μgcm^{-2}		Jesser et al. (2017)
					Fumigant	Adult			KT; 35.2 min	
	Rosmarinus officinalis L.	Leaf + fruit	Cineole (25.7)	*E. kuehniella*	Fumigant	Adult		100% mortality at 10 μL^{-1}		Pandır & Baş (2016)
	R. officinalis	Aerial part	ND	*P. interpunctella*	Fumigant	Adult		After 24 h, LD = 0.93 μlL^{-1}		Mahmoudvand et al. (2011)
	Zataria multiflora Boiss	Aerial part	ND	*P. interpunctella*	Fumigant	Adult		After 24 h, LD = 1.75 μlL^{-1}		

	Origanum onites L.	Leaf	Carvacrol (70.3)	*E. kuehniella* *P. interpunctella*	Fumigant	Adult		LC after 24 h; 7.5 µlL^{-1} 4.1 µlL^{-1}		Ayvaz et al. (2009)
	Saturaja thymbra L.	Leaf	Carvacrol (53.7)	*E. kuehniella* *P. interpunctella*	Fumigant	Adult		LC after 24 h; 10.3 µlL^{-1} 3.4 µlL^{-1}		
	Vitex negundo L.	Leaf	1,8-Cineole (19.5)	*P. interpunctella*	Fumigant	Adult		LC after 24 h; 23.1 µlL^{-1}	3.1 h	Borzoui et al. (2016)
Myrtaceae	*Myrtus communis* L.	Leaf	Linalool (31.3)	*E. kuehniella* *P. interpunctella*	Fumigant	Adult		LC after 24 h; 12.7 µlL^{-1} 22.6 µlL^{-1}		Ayvaz et al. (2009)
Asteraceae	*Artemisia khorassanica* Podl.	Leaf	Camphor (23.4)	*P. interpunctella*	Fumigant	Adult		LC after 24 h; 9.6 µlL^{-1}	2.1 h	Borzoui et al. (2016)
Apiaceae	*Coriandrum sativum* L.	Seed	Linalool (66.8)	*P. interpunctella*	Fumigant	Adult		After 24 h; LD; 18.8 µgcm^{-3}		Lee et al. (2018)
	C. sativum	Seed	(+)-(s)-Carvone (40)	*E. kuehniella* *P. interpunctella*	Fumigant Fumigant	Adult Larva		After 24h; LD; 62.6 µlL^{-1} 55.2 µlL^{-1}		Maroufpoor et al. (2016)
	Petroselinum crispum L.	Aerial part	D-Limonene (18.8)	*E. kuehniella* *P. interpunctella*	Fumigant Fumigant	Adult Larva		After 24h; LD; 62.4 µlL^{-1} 55.1 µlL^{-1}		
	Prangos ferulacea (L.)	NS***	ND**	*E. kuehniella*	Fumigant	Egg Larva Adult		After 24h; LC; 320.4 µlL^{-1} 379.7 µlL^{-1} 0.6 µlL^{-1}		Ercan et al. (2013)
Zingiberaceae	*Zingiber officinale* (Roscoe)	Rhizome	ND	*E. kuehniella* *P. interpunctella*	Fumigant Contact Fumigant Contact	Larva Larva Larva Larva		After 9 h exposure; LC; 259 µlL^{-1} 0.61 µlcm^{-2} 69.1 µlL^{-1} 0.81 µlcm^{-2}		Maedeh et al. (2012)
Solanaceae	*Capsicum annuum* L.	Leaf + fruit	Capsaicin (35.4)	*E. kuehniella*	Fumigant	Adult		100% mortality at 5 µL^{-1}		Pandır & Baş (2016)
Geraniaceae	*Geranium maculatum* L	NS	Citronellol (26.1)	*P. interpunctella*	Contact Fumigant	Adult Adult		After 2 h, LD; 37.2 µgcm^{-2}	KT; 32.6 min	Jesser et al. (2017)
Poaceae	*Cymbopogon martini* (Roxb.) Wats.	NS	Geranyl acetate (59.4)	*P. interpunctella*	Contact Fumigant	Adult Adult		After 2 h, LD; 22.8 µgcm^{-2}	KT; 92.8 min	
Rutaceae	*Citrus bergamia* Risso	NS	Limonene (17.5)	*P. interpunctella*	Contact Fumigant	Adult Adult		After 2 h, LD; 116.2 µgcm^{-2}	KT; 68.7 min	
Brassicaceae	*Armoracia rusticana* (L.)	NS	Allyl isothiocyanate (97.8)	*P. interpunctella*	Fumigant	Egg Larva Pupa Adult		After 72 h exposure LD; 10 µlL^{-1} 17.2 µlL^{-1} 22.7 µlL^{-1} 4.5 µlL^{-1}		Chen et al. (2011)
Amaryllidaceae	*Allium sativum* L.	Bulb	ND	*P. interpunctella*	Fumigant	Egg		After 24 h exposure; LC; 17.7 µlL^{-1}		Isikber et al. (2009)

				E. kuehniella	Fumigant	Egg		6.6 µlL^{-1}		
Betulaceae	*Betula lenta L.*	Bark	ND	*P. interpunctella* *E. kuehniella*	Fumigant Fumigant	Egg Egg		After 24 h exposure; LC; 29.1 µlL^{-1} 20 µlL^{-1}		
Commercial product/EOC										
3-Carvomenthenone				*P. interpunctella*	Fumigant Fumigant	Larva Adult		After 24 h exposure; LC; 52.4 µgcm^{-3} 68.7 µgcm^{-3}		Park and Lee (2018)
Cyclohexenone				*P. interpunctella*	Fumigant Fumigant	Larva Adult		After 24 h exposure; LC; 2.5 µgcm^{-3} 3.6 µgcm^{-3}		
Methylcyclohexenone				*P. interpunctella*	Fumigant Fumigant	Larva Adult		After 24 h exposure; LC; 3.0 µgcm^{-3} 4.2 µgcm^{-3}		
Seudenone				*P. interpunctella*	Fumigant Fumigant	Larva Adult		After 24 h exposure; LC; 3.0 µgcm^{-3} 4.4 µgcm^{-3}		

[a] LC$_{50}$ or LD$_{50}$ = lethal concentration or lethal dose required to kill 50% of the population.

[**] ND = not done by the authors.

[***] NS = Not stated by authors.

[#] Unless otherwise stated; knockdown time is expressed as LT$_{50}$; LT$_{50}$ = lethal time required to kill 50% of the population. KT$_{50}$ = Knockdown time required to kill 50% of the population.

Table 8. Insecticidal effects of plant essential oils and their components against termite

Family	Plant species	Plant part	Major component (%)	Species	Bioassay type		Insecticidal effect			
						Life-stage (condition)	Repellency (%)	Mortality (LD$_{50}$ or LC$_{50}$)^{a}	Knockdown (LT$_{50}$)$^{\#}$	References
Lauraceae	*Cinnamomum parthenoxylon* Meisn.	Leaf	1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (31.2)	*Coptotermes curvignathus* (Holmgren)	No-choice test	Soldier & worker		10% ethanolic extract resulted in 53 and 72.7% mortality after 1 & 2 weeks, respectively		Adfa et al. (Adfa et al. 2017)
Lamiaceae	*Nepeta cataria* L.	NS**	Z,E-nepetalactone (64)	*Reticulitermes flavipes* (Kollar) *R. virginicus* (Banks)	Topical Contact Contact	Worker Worker Worker		After 7 d, LD; 8200 ugg^{-1} LC; 44.4 ugcm^{-2} LC; 21.1 ugcm^{-2}		Peterson and Ems-Wilson, (Peterson and Ems-Wilson 2003)
	Mentha arviensis L.	Leaf	Menthol (63.2)	*C. heimi* (Wasmann)	Fumigant	Soldier Worker		100% mortality after 3 h for both at 25 mg		Qureshi et al. (Qureshi et al. 2012)
Rutaceae	*Citrus latifolia* Tanaka	Wax	Limonene (59.6)	*Cryptotermes brevis* Walker	Antifeedant	Worker		Antiffedant index = 100 at 100 mgcm^{-3} EC*; 24.69 mgcm^{-3}		Sbeghen-Loss et al. (Sbeghen-Loss et al. 2011)
Cuppressaceae	*Cryptomeria fortunei* Hooibrenk	Leaf	α-Terpineol (NS**)	*R. chinensis* (Snyder)	Impregnated filter-paper	Worker		LC; 2.8 mgml^{-1}		Xie et al. (Xie et al. 2013)
	Cunninghamia konishii Hayata	Wood chip Leaf	α-Cedrol (53) α-Pinene (34.9)	*C. formosanus* Shiraki	No-choice	Worker Worker		100% mortality at 10 mgg^{-1} after 4 d for leaf and wood oil		Cheng et al. (Cheng et al. 2014)
Myrtaceae	*Eucalyptus camaldulensis* Dehnh.	Leaf	□-Terpinene (75.5)	*C. formosanus*	Contact Noncontact	Worker Worker		LC after 7 d, 15.4 mgg^{-1} of filter paper 17.5 mg/petri dish		Siramon et al. (Siramon et al. 2009)
	E. camaldulensis	Leaf	□D***	*C. gestroi* (Wasmann)	Impregnated filter-paper	Worker		LC; 3.2%	At 10%, 0.4 h	(Mikola et al., (Zampieri Mikola et al. 2017)
	E. citriodora.	Leaf	□D	*C. gestroi*	Impregnated filter-paper	Worker		0.6%	At 10%, <1 h	
	E. maidenii F. Muell.	Leaf	□D	*C. gestroi*	Impregnated filter-paper	Worker		3.1%	At 10%, 7 h	
	E. pseudoglobulus (Naudin)	Leaf	□D	*C. gestroi*	Impregnated filter-paper	Worker		3.7%	At 10%, 11.1 h	
	E. tereticornis Sm.	Leaf	□D	*C. gestroi*	Impregnated filter-paper	Worker		3.0%	At 10%, <1 h	
Zingiberaceae	*Alpinia galangal* (L.) Willd.	Rhizome	1,8-Cineole (61.9)	*C. gestroi*	Antifeedant Toxicity	Worker		At 2000 ppm, mean consumption = 3.3 mg LD; 5407 mgkg^{-1}		Abdullah et al. (Abdullah et al. 2015)

				C. curvignathus	Antifeedant Toxicity			At 2000 ppm = 3.3 mg LD; 3456 mgkg^{-1}		
Asteraceae	*Eclipta protasta* L.	Leaf	ND	*C, formosanus*	No-choice	Worker		LD 292 ppm at 24 h		Elango et al. (Elango et al. 2012)
Verbenaceae	*Lantana camara* L.	Growing plant	ND	*C. formosanus*	Barrier (plant + soil)	Soldier	No effect on termites foraging in 3 wks			Ding and Hu (2010)
				R. flavipes	Barrier (plant tissue + soil)	Soldier	Greater repellent effect on *C. formosanus* than *R. flavipes*			
	L. camara	Leaf	ND	*R. flavipes*	Antifeedant	78% feeding reduction at 0.21 mgcm^{-2}				Yuan and Hu (2012)
					Toxicity (no-choice paper test)		>90% mortality at 0.21 mgcm^{-2}			
Fabaceae	*Enterolobium cyclocarpum* (Jacq) Griseb	Heart wood	D-limonene (17.8)	*Incisitermes marginipennis* (Latreille)	Oral toxicity	Worker		After 5 wks., survival rate = 38% and feeding rate was 26% at 56.63 mg		Raya-Gonzalez et al. (Raya-Gonzalez et al. 2013)
Moraceae	*Morus alba* L.	Heart wood	Resorcinol (40.5)	*R. flavipes*	Filter-paper	Worker		LC; 1.71 mgml^{-1}		Hassan et al. (2018)
					Antifeedant	Worker		62% at 10 mgml^{-1}		
					Repellency	Worker	70% at 10 mgml^{-1}			
Acanthaceae	*Andrographis lineata* Wallich ex Nees.	Leaf	ND	*C. formosanus*	No-choice	Worker		At 24h; LD; 358 ppm		Elango et al. (2012)
	A. paniculata (Burm.f)	Leaf	ND	*C. formosanus*	No-choice	Worker		371 ppm		
Papaveraceae	*Argemone Mexicana*	Leaf	ND	*C. formosanus*	No-choice	Worker		253 ppm		
Aristolochiaceae	*Aristolochia bracteolate* Lam.	Leaf	ND	*C. formosanus*	No-choice	Worker		363 ppm		
Solanaceae	*Datura metel* L.	Leaf	ND	*C. formosanus*	No-choice	Worker		317 ppm		
Fibaceae	*Sesbania grandiflora* (L.)	Leaf	ND	*C. formosanus*	No-choice	Worker		289 ppm		
Compositae	*Tagetes erecta*	Leaf	ND	*C. formosanus*	No-choice	Worker		409 ppm		
Commercial products/EOC				*C. formosanus*						

Limonene				*C. brevis*	Antifeedant	Worker		Antiffedant index = 100 at 100 mgcm^{-3} EC; 44.43 mgcm^{-3}		Sbeghen-Loss et al. (2011)
α-Terpineol				*R. chinensis*	Impregnated filter-paper test	Worker		LC; 0.9 mgml^{-1}		Xie et al. (2013)
				C. formosanus	Contact Noncontact	Worker Worker		LC after 7 d, 1.5 mgg^{-1} of filter paper 0.8 mg/petri dish		Siramon et al. (2009)
p-Cymene				*C. formosanus*	Contact Noncontact	Worker Worker		3.8 mgg^{-1} of filter paper 92.2 mg/petri dish		Siramon et al. (2009)
□-Terpinene				*C. formosanus*	Contact Noncontact	Worker Worker		5.9 mgg^{-1} of filter paper 92542 mg/petri dish		Siramon et al. (2009)
1,8-Cineole				*C. formosanus*	Contact Noncontact	Worker Worker		6.7 mgg^{-1} of filter paper 52 mg/petri dish		Siramon et al. (2009)
				C. gestroi *C. curvignathus*	Repellent Toxicity Repellent Toxicity	Worker Worker Worker Worker	At 250 ppm, 50% At 750 ppm, 56%	LD; 1102 mgkg^{-1} LD; 945 mgkg^{-1}		Abdullah et al. (2015)
α-Pinene				*C. formosanus*	Contact Noncontact	Worker Worker		44.9 mgg^{-1} of filter paper 21.3 mg/petri dish		Siramon et al. (2009)
Terpinen-4-ol				*C. formosanus*	Contact Noncontact	Worker Worker		3.3 mgg^{-1} of filter paper 1.7 mg/petri dish		Siramon et al. (2009)
Cederene				*C. formosanus*	Consumption Survival	Worker Worker		72.1 mg 43.8%		Maistrello et al. (Maistrello et al. 2001)
Nootkatone				*C. formosanus*	Consumption Survival	Worker Worker		7.3 mg 15.3%		
Vetiver oil				*C. formosanus*	Consumption Survival	Worker Worker		15 mg 11.4%		
2-Phenyl-2-propanol				*C. formosanus*	Contact Vapor	Worker Worker		% mortality after 7 d, 93.7 98.4 after 3 d		Raina et al. (Raina et al. 2012)
Citral				*C. formosanus*	Contact	Worker		56.5		
l-Carvone				*C. formosanus*	Contact Vapor	Worker Worker		73.9 95.3 after 3 d		
l-Linalool				*C. formosanus*	Contact Vapor	Worker Worker		59.4 69.6 after 3 d		
Patchouli Oil				*C. formosanus*	Repellency Mortality Tunneling length	Worker Worker Worker	50 µg/g of sand after 24 h	11.6 µg/termite 10 cm at 50 µg/g of sand		Zhu et al. (Zhu et al. 2003)
Vetiver Oil				*C. formosanus*	Mortality	Worker		13% at 10 ugg^{-1}		Zhu et al. (2001)

					Tunneling length	Worker		19.87 at 10 ugg^{-1}		

[a] LC_{50} or LD_{50} = lethal concentration or lethal dose required to kill 50% of the population.

[*] EC_{50} = effective concentration required to cause 50% repellency against termite

[**] NS = Not stated by authors.

[***] ND = not done by the authors

[#] Knockdown time is expressed as LT_{50}; LT_{50} = lethal time required to kill 50% of the population.

Chapter 3

Topical Toxicity Profiles of Some Aliphatic and Aromatic Essential Oil Components against Insecticide-Susceptible and Resistant Strains of German Cockroach (Blattodea: Ectobiidae)

3.1 Abstract

Toxicity profiles of four aliphatic (α–pinene, cyclononanone, limonene, nerolidol), four aromatic (β–thujaplicin, carvacrol, eugenol, tropolone) essential oil components (EOCs), and permethrin were investigated against three strains of German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae). The strains include a susceptible strain (S), and two multi-resistant strains – strains D and E. Also, a synergism bioassay, using piperonyl butoxide (PBO) was conducted. The most toxic EOCs were aromatic EOCs carvacrol, eugenol, and tropolone, followed by aliphatic EOC limonene, all had LD_{50} values of < 0.7 mg/µl. Four of the EOCs were equally toxic against all the strains, with carvacrol being the most toxic, followed by eugenol, tropolone, and α–pinene. The other four EOCs were more toxic against strain S than against the two resistant strains. Permethrin was significantly more toxic to strain S (LD_{50} = 0.056 µg/µl) compared to the resistant strains (D = 2.138 µg/µl, E = 1.730 µg/µl). Toxicity of aliphatic EOCs correlated positively with their molecular weight against strain E only, while both molecular weight and vapor pressure of aromatic EOCs correlated significantly with toxicity in all strains. Strain D exhibited the greatest resistance (RR of 6.7) to EOCs, and synergism to the aliphatic EOC cyclononanone. Clear synergism with PBO was observed in permethrin against resistant strains, but not in all of the EOCs, suggesting multiple resistance mechanisms in the resistant cockroaches. These findings give insight on the potential of EOCs to be incorporated as parts of an IPM approach to managing insecticide resistant German cockroaches.

3.2 Introduction

The German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), inhabits voids between sinks and walls, bathrooms, utility cabinets, inside electrical appliances, and other places where food, water, and harborages are generally present (Schal et al. 1984, Bell et al. 2007). They can act as mechanical vectors of pathogenic microorganisms and as sources of allergens (Schal and Hamilton 1990, Fotedar et al. 1991) to humans and animals. In addition, their effects on human health, contamination of food and surfaces, and the revulsion they cause, have made the control of these pests critical. Some formulations of synthetic insecticides either as residual sprays or baits have been used extensively for German cockroach control, with good to moderate success (Wei, Appel Arthur G, et al. 2001), however, control failures due to development of resistance to contact insecticides, and environmental toxicity have made their further use undesirable (Wang et al. 2004, Wu and Appel 2017, DeVries et al. 2019). Therefore, there is a renewed interest in the development of control alternatives that are effective and sustainable.

As alternatives, natural insecticides, such as essential oils (EOs) derived from plants are good resources on which the development of these household and structural pest control agents can be based. There is an increased interest in essential oil and natural products research as evidenced by the many recent reports of efficacy, low mammalian toxicity, and environmental safety (Isman et al. 2008, Regnault-Roger et al. 2012). In the US, given a lower level of risk posed compared to conventional insecticides, natural insecticides require less or no toxicity data (EPA 24b list) and an average period of 12 months to get registered, saving both time and money. The identification of essential oil components (EOCs) from plants using gas chromatography-mass spectrometry (GC-MS), make it possible for them to be exploited for control purposes rapidly.

A number of essential oil components (EOCs) have been investigated against the German cockroach. Limonene, a monocyclic aliphatic compound, had a LD_{50} value of 0.285 mg/cockroach against adult males (Philips et al. 2010). Thymol, an aromatic monoterpernoid, was tested against the German cockroach for its potential application as a contact insecticide (Jang et al. 2005b). Thymol deposits exhibited higher contact toxicity (0.09 mg/cm^2) compared with propoxur (0.18 mg/cm^2), a carbamate insecticide. Other studies have demonstrated that α–pinene (bicyclic aliphatic compound), eugenol (aromatic compound), and carvacrol (aromatic compound) have insecticidal activity against cockroaches (Jang et al. 200ba, Phillips and Appel 2010b, Enan 2005). Collectively, these works have culminated into their commercialization as constituents of botanical insecticides (Table 1). For example, EcoPCO ACU (EcoSmart, Alpharetta, GA), an essential oil-based pesticide containing eugenol (EcoSmart, Alpharetta, GA), is registered for use as a contact insecticide against German cockroach.

Some studies with household and structural pests have reported that toxicity of some EOCs are comparable with conventional insecticides (Hori 2004, Saniewski et al. 2007). For example, toxicity of β–thujaplicin [obtained from leaf of *Chamaecyparis obtusa* (Siebold & Zucc.) Endl.: Cupressaceae] and pirimiphos- methyl were compared against three mosquito species. The LC_{50} value of β-thujaplicin was 2.91, 2.60, and 1.33 mg/L against *Aedes aegypti* (L.), *Ochlerotatus togoi* (T.), and *Culex pipiens*, respectively, while LC_{50} value of pirimiphos-methyl was 0.16, 0.22, 0.14 mg/L against *Ae. aegypti, Oc. togoi,* and *Cx.* (Jang et al 2005a). The toxicity of EOCs nerolidol, tropolone, and cyclononanone have also been reported (Lopes et al. 1999, Di Campli, Di Bartolomeo, Delli Pizzi, et al. 2012, Wang, Perumalsamy, et al. 2016).

Nerolidol, a natural aliphatic sequisterpene, is a hydrophobic compound that initiates a range of antimicrobial and antifungal activities (Ferreira et al. 2012, Tao et al. 2013, Mladineo et al. 2018, Souilem et al. 2018). The insecticidal properties of tropolone, an aromatic compound, and cyclononanone, an aliphatic monoterpene, have found promising application, as control agents, in a number of toxicity studies (INAMORI et al. 2000, Ema et al. 2004, Cho et al. 2011, Haney et al. 2019). These essential components have never been tested against the German cockroach.

An in depth analysis into the recent literature has shown that structural activity and physical properties of an EOC, rather than plants species from which they are obtained or extraction solvents used, may play a major role in explaining their bioactivity (Philips et al. 2010, Yeom et al. 2018). It has been argued that essential oil components containing a benzene ring are not easily metabolized and detoxified by insects, and by extension, are more toxic than aliphatic compounds (Rice and Coats 1994, Philips et al. 2010). Monocyclic aliphatic compounds are reported to be more toxic than bicyclic aliphatic compounds to all stages of German cockroach (Rice and Coats 1994, Philips et al. 2010). Rice and Coats (1994) and Tsao et al. (1995) demonstrated the role of plant compounds structural activity in toxicity towards structural and household pests. By derivatization of the hydroxyl group from selected monoterpenes and phenols, they showed enhanced toxicity from increased vapor pressure, leading to greater insecticidal action, and/or increased lipophilicity, leading to better penetration and bioavailability in the insect body. Jang et al. (2005b) reported that structural characteristics of EOCs such as degrees of saturation and types of functional groups rather than types of carbon skeleton appear to play a role in determining monoterpenoid toxicities to the German cockroach. It is clear that there are correlations between EOCs physical and structural properties and toxicity. This information would be useful in exploring essential oils that are as toxic or nearly as toxic as synthetic insecticides and that may help reduce the innate capacity of insects to develop resistance.

In view of these reports, this work was undertaken to investigate the toxicity of selected EOCs against susceptible and insecticide-resistant strains of the German cockroach. Additionally, a synergist, piperonyl butoxide (PBO), was used in combination with each EOC to possibly increase toxicity in all cockroach strains. Synergists are chemical substances that improve the toxicity (efficacy) of insecticides by reducing or eliminating resistance mechanisms such as detoxification (Bernard and Philogène 1993). PBO has been widely used to identify possible mechanism of resistance in German cockroaches, especially where the cytochrome P450 mono-oxygenase enzymes are a contributing factor to resistance ((Dong and Scott 1992, Scharf et al. 1997). These enzymes may act against a variety of xenobiotics including essential oils. Dehkordi et al. (Dehkordi et al. 2017) observed that PBO significantly reduced resistance to bendiocarb, a carbamate insecticide, in a population of German cockroaches collected around hospitals in Tehran. Chai and Lee (Chai and Lee 2010a) reported reduced resistance to deltamethrin and β–cyfluthrin, pyrethroid insecticides, in the field-collected strains exposed to PBO.

Insecticide resistance is associated with genetic changes that result in varied responses to insecticides between different populations. Finally, this study determines whether insecticide resistance, if present, can be extended to plant chemicals. Thus, we investigated the toxicity of EOCs against insecticide-susceptible and resistant strains of German cockroach to determine if there is cross resistance between permethrin and EOCs.

3.3 Materials and Methods

3.3.1 Chemicals and insecticide

Four aliphatic and four aromatic (Fig. 1) essential oil components, piperonyl butoxide (PBO), and permethrin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Four (α–pinene, limonene, carvacrol, and eugenol) of the EOCs were selected based on reported efficacies against German cockroach, and inclusion as constituents of commercially developed botanical formulations (Table 1). The other four EOCs (cyclononanone, nerolidol, β–thujaplicin, and tropolone) which have not been tested against the German cockroach, were selected based on reported effectiveness (Jang et al. 2005b, Phillips and Appel 2010b, Enan 2005) at controlling other household and structural pests (Table 2).

3.3.2 German cockroach strains

A susceptible strain (strain S) and two insecticide-resistant strains (strain D and strain E) of the German cockroach (Wu and Appel 2017b) were used in this study. The susceptible strain has been in continuous culture without exposure to insecticide at the Urban Entomology laboratory (Auburn University) for > 40 yr. The two resistant strains were originally collected from populations in residences from Franklin County, NC, USA (Wu and Appel 2017). These strains are multi-resistant to several classes of insecticides. Strain E displayed a 52-fold resistance to permethrin, 5-fold resistance to chlorpyrifos, 1-fold resistance to propoxur, and 4-fold resistance to fipronil. Strain D showed 37-fold resistance to permethrin, 7-fold resistance to chlorpyrifos, 1-fold resistance to propoxur, and 9-fold resistance to fipronil (Wu and Appel 2017).

The susceptible strain was kept in 32-gallon plastic bins while resistant strains were reared in 3.8-liter glass jars at 28 ± 2º C, with 40 – 55% RH, and a photoperiod of 12:12 (L: D) h. The cockroaches were provided harborage (corrugated cardboard), rodent food (Purina 500l lab diet from Purina LabDiet®, Inc. St. Louis, MO, USA) and clean water on a weekly basis. Adult males were used in this study because they have a more consistent body mass than other stages, and produce no eggs and associated hormone variations (Phillips et al. 2010b).

3.3.3 Bioassays

Topical application was used to determine the toxicity of the selected EOCs and permethrin against susceptible and insecticide-resistant strains. A preliminary bioassay was conducted on the susceptible strain to establish dose-mortality responses to each EOC and permethrin. Serial dilutions of essential oil components and permethrin were made in acetone (Fisher Certified ACS 99.7% purity; Fisher, Fair Lawn, NJ, USA) to obtain the various concentrations. Four to seven insecticide concentrations causing mortality in the range of > 0 and < 100 % mortality were used for toxicity study (S1). Cockroaches were lightly anaesthetized with CO_2 and arranged with ventral surface up on a disk of filter paper. A 25 µl Hamilton PB600-1 repeating dispenser (Hamilton Company, NV, USA) was used to topically apply a 1 µl dose of EOC solution, and permethrin between the metathoracic legs of each cockroach. A 25 µl syringe dispenses 0.5 µl, so 2 clicks were made to achieve 1 µl of each concentration. Control cockroaches were treated with 1 µl of acetone. Four replicate groups of six cockroaches each were used for each concentration and the control. After treatment, cockroaches were placed in 162.65 ml (5.5 oz) plastic cups (Georgia-Pacific, Atlanta, GA, USA) with a 1 cm length of wetted dental wick and covered with a perforated lid. Mortality was assessed 24 h after treatment.

The synergism bioassay began by delivering PBO at the maximum sublethal dose of 100 µg per cockroach (Valles et al. 1997) between the metathoracic legs 1 h before subsequently delivering previously tested concentrations for each EOC, and permethrin. Control cockroaches were given PBO before subsequently delivering 1 µl acetone an hour later.

3.3.4 Data analysis

There was no mortality in control. The toxicity (LD_{50}) of each EOC, and permethrin to each German cockroach strain was estimated by PoloPlus software (LeOra Software 2003, Robertson et al. 2003). The lethal dose ratio test was used to estimate significant differences among LD_{50} values (Robertson et al. 2003). The resistance ratio (RR) for each EOC, permethrin, and strains were calculated using:

$$\mathrm{RR} = \frac{\mathrm{LD_{50}\ of\ insecticide - resistant\ strain}}{\mathrm{LD_{50}\ of\ susceptible\ strain}}$$

Correlation analysis was used to relate essential oil toxicity (LD_{50}) with physical properties (molecular weight and vapor pressure) using R software (R Core Team 2018). The synergism ratio of each EOC, and permethrin was computed using the mathematical expression by (Wen and Scott 1997), with slight modification:

$$\mathrm{Synergism\ Ratio} = \frac{\mathrm{LD_{50}\ of\ essential\ oil\ component}}{\mathrm{LD_{50}\ of\ essential\ oil\ component + synergist}}$$

Where LD_{50} of essential oil component is the lethal dose for each EOC in the topical bioassay; and LD_{50} of essential oil component + synergist is the lethal dose for each EOC in the synergism bioassay. For permethrin, the synergism ratio was LD_{50} of permethrin divided by LD_{50} of permethrin + synergist. Synergism was considered significant based on the non-overlap of the confidence limits of the LD_{50} values.

3.4 Results

3.4.1 *Toxicity profiles of aliphatic and aromatic EOCs, and permethrin against German cockroach strains*

Toxicity (LD_{50}) of aliphatic essential oil components ranged from 0.025 mg/µl for cyclononanone against the susceptible strain S to > 12.5 mg/µl for nerolidol against strain E (Table 3). The order of toxicity of aliphatic EOC to strain S was cyclononanone > limonene > nerolidol > α–pinene. Limonene was the most toxic EOC to both the insecticide-resistant German cockroach strains investigated, followed by α–pinene, cyclononanone, and nerolidol. The three strains displayed similar level of susceptibility to limonene (strain S; LD_{50} = 0.063 mg/µl, strain D; LD_{50} = 0.062 mg/µl, and strain E; LD_{50} = 0.057 mg/µl, respectively). According to the lethal-dose ratio test, the resistant strains had significantly greater LD_{50} values for Nerolidol than the susceptible strain. Resistance ratios (RR) ranged from 0.9-fold for α–pinene against strain D to > 10-fold for nerolidol against strain E. The insecticide-resistant strains, D and E had 6.7-fold and 5.6-fold resistance, respectively, to cyclononanone. The slope value obtained from the probit line was shallowest (1.091) for strain E, indicating heterogeneous response to cyclononanone while steepest (19.902) for strain E, indicating homogenous response to limonene (Table 3).

For the aromatic EOCs, toxicity (LD_{50}) ranged from 0.0035 mg/µl for carvacrol against strain E to 0.096 mg/µl for β–thujaplicin against strain E (Table 4). In general, the aromatic EOCs were more toxic to strain S followed by strains E and D. Carvacrol was the most toxic essential oil component to all strains, followed by eugenol, tropolone, and β–thujaplicin. Carvacrol had similar toxicity between strain S (LD_{50} = 0.0075 mg/µl) and strain D (LD_{50} =

0.0065 mg/µl), but was about twice as toxic to strain E (LD_{50} = 0.0035 mg/µl). The resistant strains had significantly greater LD_{50} values to tropolone than strain S according to lethal-dose ratio test. RR ranged from 0.5-fold for carvacrol against strain E to 1.7- fold for β–thujaplicin against strain E. For the log-dosage probit line (slope), the response of strain D was heterogeneous to carvacrol (shallower slope; 1.387) while strain S had homogeneous response to eugenol (steeper slope; 10.223) (Table 4).

Permethrin was the most toxic treatment to all strains. Toxicity (LD_{50}) ranged from 0.056 µg/µl against strain S to 2.138 µg/µl against strain D (Table 4). According to the lethal-dose ratio test, the resistant strains had significantly greater LD_{50} values than the susceptible strain. Thus, the resistant strains showed as high as >30-fold resistance level, with strain D showing the highest, 38.2-fold resistance. The slope value obtained from the probit line was shallowest (0.462) for strain S and steepest for (0.632) for strain E indicating heterogeneity in response to permethrin in all populations (Table 5).

3.4.2 Correlation between EOCs Physical Properties and Toxicity.

There was a significant ($p < 0.05$) positive correlation between essential oil component molecular weight and toxicity for aliphatic EOCs against strain E only. For each increase in g/mol of the molecular weight of each essential oil component, there was a 0.14 mg/µl (±0.018; ±95% C.I) increase in toxicity ($p = 0.000788$; $r = 0.99$). However, both molecular weight ($r = 0.998$, $p = 0.0007$), and vapor pressure (S: $r = 0.850$, D: $r = 0.860$, E: $r = 0.773$) of the aromatic EOCs were significantly positively correlated with toxicity against all strains.

3.4.3 Synergistic effect of PBO on the toxicity of aliphatic and aromatic EOCs and permethrin

Application of the synergist PBO resulted in a significant reduction, based on the non-overlap of the confidence limits, in LD_{50} values for all aliphatic EOCs, and all strains with the exception of nerolidol against strain S (Table 2). The greatest reduction, as illustrated by the RR, was seen in strains D (LD_{50} of 0.168 mg/µl to 0.020 mg/µl i.e., 6.7-fold to 1.1-fold RR) and E (LD_{50} of 0.141 mg/µl to 0.021 mg/µl i.e, 5.6-fold to 1.1-fold RR) for cyclononanone. There was no significant reduction in RR values in the insecticide-resistant strains for limonene. Synergism ratio (SR) ranged from 1.0 for nerolidol against strain S to 8.4 for cyclononanone against strain D. Strain D had a shallower slope (1.645) for cyclononanone and a steeper slope (13.251) for α–pinene, indicating heterogeneity in response to cyclononanone homogeneity in response to α–pinene (Table 2).

The synergist PBO also reduced the LD_{50} values significantly (based on the non-overlap of the confidence limits) for only two aromatic EOCs, β–thujaplicin (in strains S, D and E) and carvacrol (in strain S) (Table 3). With PBO, the toxicity of tropolone decreased. In other words, the synergist-pretreated German cockroaches had an increase in LD_{50} values in all strains. Synergism ratios (SR) ranged from 0.7 for tropolone against strain S to 6.2 for β–thujaplicin against strain D. The slope of the log dose-probit line indicated that strain S had the shallowest slope (0.783) for carvacrol and the steepest slope (18.224) for eugenol indicating heterogeneous and homogeneous response to both EOCs respectively.

Upon pre-exposure to PBO, toxicity (LD_{50}) of permethrin against strain S (0.067 µg/µl) was within the 95% confidence limits of the non-PBO exposed cockroaches (Table 5). However, PBO pre-treated resistant strains had significant reduction in their LD_{50} values (strain D: 2.138 µg/µl to 1.195 µg/µl; strain E: 1.730 µg/µl to 1.135 µg/µl). Thus, PBO reduced RR's in strains D and E by approximately 50 % compared with permethrin alone. Nevertheless, strain D still showed the greatest level of tolerance with a resistance ratio of 17.8-fold while strain E had 16.9-fold resistance. The synergism ratio ranged from 0.8 in strain S to 1.8 in strain D. Strain S had the steepest slope (7.123), indicating homogeneous response to permethrin whereas strain E had the shallowest slope (1.107) – an indication of homogenous response to permethrin (Table 4).

3.5 Discussion

The need to provide effective, safe, and economically sound alternatives to conventional insecticides forms the basis of research to the toxicity of essential oils (Koul et al. 2008). Examples from plant-insect interactions, such as induced defenses used by plants (via phenolics and terpenoids) to ward off insect attack (Berenbaum et al. 1986, Li et al. 2002), show plant secondary metabolites can be exploited for insect control. In this study, we investigated the toxicity profiles of aliphatic and aromatic essential oil components (EOCs) against a susceptible strain (strain S) and two multi-resistant strains (strains D and E) of the German cockroach. Additionally, using PBO as a synergist, a potential resistance mechanism was investigated. To the best of our knowledge, this is the first description of contact or topical insecticidal activity of nerolidol and cyclonanone (aliphatic EOCs), and β–thujaplicin and tropolone (aromatic EOCs) against the German cockroach.

Between groups, aromatic EOCs were more toxic to the German cockroach strains than did aliphatic EOCs. Three of the four most toxic are aromatic EOCS, suggesting that the chemical structure of an EOC may determine toxicity. Additionally, the average of LD_{50}s for aromatic EOCs (strain S = 0.0331 mg/µl; D = 0.0461 mg/µl; E = 0.0461 mg/µl) were less than that obtained for aliphatic EOCs (strain S = 0.058 mg/µl; D = 0.099 mg/µl; E = 3.199 mg/µl) in all strains. Ngoh et al. (1998) investigated the toxic properties of nine EOCs based on functional groups against the American cockroach, *Periplaneta americana* (L.), and observed that benzene derivatives (aromatic EOCs) were more toxic than terpenoids (aliphatic EOCs). More so, it has been argued that essential oil components containing a benzene ring are not easily metabolized and detoxified in the insect (Rice and Coats 1994, Philips et al. 2010). This property may help explain why aromatic EOCs had superior toxicity to the cockroach strains.

Out of the eight EOCs evaluated in this study, four EOCs (aliphatic cyclononanone and nerolidol, and aromatic β–thujaplicin and tropolone), including permethrin, had lower toxicity against the two insecticide-resistant strains than against the susceptible strain, implying a likelihood of cross-resistance or multiple-resistance in these two insecticide-resistant strains with the four EOCs, and permethrin. Cross-resistance has been documented in some insects that demonstrate resistance to more than one class of insecticide (Silva et al. 2019, Reissert-Oppermann et al. 2019, DeVries et al. 2019). Across EOC groups (aliphatic/aromatic), strain D was resistant to 75% of the EOCs tested. Further, Wu and Appel (2017) reported that strain D showed 8.7 and 37.3-fold resistance to fipronil and permethrin, respectively. Therefore, strains D and E likely exhibit multiple resistance mechanisms.

Limonene, carvacrol, and eugenol were equally toxic to susceptible and insecticide-resistant strains. This is comparable with the findings of Intirach et al. (2016) who reported comparative toxicity of *Petroselinum crispum* (Mill.) essential oil to susceptible and insecticide resistant mosquitoes. However, the distinct superior topical toxicity of limonene to the German cockroach among other aliphatic EOCs is in disagreement with the findings of Yeom et al. (2012) who reported that limonene had greater toxicity only in fumigant tests. The observed difference might be due to strain and bioassay methods. For the aromatic EOCs, carvacrol had the greatest toxicity against all strains of the German cockroach, corroborating the results of

Philips and Appel (2010) who reported carvacrol had an LD_{50} value of 0.070 mg/µl compared to 0.075 mg/µl obtained in this study against strain S. Eugenol was also quite toxic to all strains. It has been reported that carvacrol and limonene inhibit AChE (Yeom et al. 2012) while eugenol binds octapomine receptors in German cockroaches (Enan 2005). Therefore, it is possible that even though strains D and E may have higher concentrations of cytochrome P450 and hydrolase enzymes that metabolize xenobiotics, EOCs such as limonene, carvacrol and eugenol are not significantly metabolized by these enzymes. These observations and our results indicate that these EOCs may affect many sites within German cockroach resulting in continued susceptibility to EOCs in the conventionally insecticide-resistant strains.

Structure activity relationship of EOCs against cockroaches is widespread in literature (Tsao et al 1995, Jang et al. 2005b, Philips et al. 2010, Yeom et al. 2018). Jang et al (2005b) noted that there was no relationship between monoterpenoids vapor pressure and eventual toxicity in German cockroaches. In this study, we found that the arrangement of carbon atoms (benzoid structure) and presence of pi-bonds in an EOC appear to be correlated with toxicity. This is expressed as significant positive correlations exist between aromatic EOCs vapor pressure and toxicity. Chang et al. (2012) found no significant correlation between molecular weight and toxicity of 38 constituents, including α–pinene and carvacrol, from *Cyperus rotundus* L. against the German cockroach in a filter paper test. Those results are in contrast to our findings – aliphatic and aromatic EOCs molecular weight significantly correlated with toxicity against the German cockroaches. The difference in findings may be a result of difference in constituents or method of chemical exposure to cockroaches. It is likely that EOCs with low molecular weight evaporate quickly. Thus, in their study, the EOCs might have evaporated before the cockroach obtained a lethal concentration, or the deposit might have been absorbed into the filter paper and was unavailable to the cockroach. A glass surface should have been used instead.

Based on the findings of this study and many others, synergists, such as PBO, clearly have a role in lowering tolerance to conventional insecticides and EOCs. In the topical application bioassay, strain D exhibited the greatest tolerance to the aliphatic EOC cyclononanone and to permethrin. Using the same strain, Wu and Appel (2017) reported a LD_{50} of 8.905 µg/µl and a RR of 37.3 for permethrin. Our result has a LD_{50} value in the range of Wu and Appel (2017) and a RR of 38.2. Pre-exposure of the same strain to PBO before cyclononanone and permethrin resulted in high synergism, i.e., SR of 8.4 and 1.8, respectively. Synergism ratio (SR) can be used to measure the success of a synergist used in predicting toxicity (Wang et al. 2018); with high synergism substantiating the contributory role of the mechanism blocked by the synergist thus increasing toxicity. Numerous studies have reported the usage of PBO to identify possible resistance mechanisms in German cockroaches (and other insects) particularly where the cytochrome P450 mono-oxygenase enzymes are a contributing factor to such resistance (Dong and Scott 1992, Scharf et al. 1997). Therefore, there is likely substantial P450 monooxygenase-mediated detoxification associated with the tolerance of strain D to cyclononanone.

While synergists can help identify resistance mechanisms going on in insect populations, the role of multiple insecticide resistance mechanisms complicates any straightforward conclusion. For example, we found that PBO suppressed tolerance, for nerolidol, from 10 to 1-fold in strain E, and reduced permethrin resistance from 37 to 17-fold in strain D. It is noteworthy that the resistant strains used in this study were derived from field populations and subjected to technical grade insecticides in a topical bioassay (Wu and Appel 2017). Compared with the findings of Wu and Appel (2017), strain D maintained its resistance level to permethrin (37.3 RR), but strain E had reduced resistance (from 52-fold to 30.9-fold), a reduction significant based on the non-overlap of confidence limits in both studies.

However, PBO did not increase toxicity of all EOCs in all cockroach strains. There was an increase in RR for the PBO-treated German cockroaches treated with β–thujaplicin and carvacrol, and decrease in LD_{50} of tropolone. This observation, however, is not without precedent. Sanchez-arroyo et al. (2001) provided evidence that PBO altered the pharmacokinetics of propoxur in German cockroach in a topical application bioassay thereby limiting the ability of the insecticide to penetrate through the cuticle. Thus, it may be that the pharmacokinetics of aromatic EOs were impaired by PBO. Nevertheless, further research needs to be conducted to substantiate this observation. It is also possible that cytochrome P450-dependent mixed-function oxidases (MFO) may slightly alter the structure of EOCs resulting in lesser toxicity (Tang et al. 2006).

It is commonly posited that resistance ratio values, RR, generated from studies with insecticide-susceptible populations maintained in the laboratory over multiple generations, are a worthy index of measuring performance of insecticides (Blanco et al. 2008, Klafke et al. 2010, Wu and Appel 2017). Consistent with this hypothesis, the resistance ratios of the EOCs and that of the synthetic insecticide, permethrin, showed that the German cockroaches strains investigated were more tolerant to permethrin than they were to EOCs. So, resistance to conventional insecticides, such as permethrin, may not be necessarily documented to EOCs.

Our current findings highlight that carvacrol, eugenol, and limonene, EOCs routinely included as constituents of commercialized botanical formulations, are most toxic to both susceptible and insecticide-resistant German cockroach. Also, the finding portends that aromatic

EOCs are more toxic than aliphatic EOs, and PBO can increase toxicity where P450 enzymes are present in a cockroach population. While there is a report on the synergistic effect EOCs and PBO against *Aedes aegypti* (Waliwitiya et al. 2012), this is the first work documenting moderate resistance to EOCs, and illustrating PBO synergism of EOCs against the German cockroach. Further studies, however, are required to investigate the biochemical mechanisms between the interaction(s) of PBO and EOCs to understand role played in moderating toxicity.

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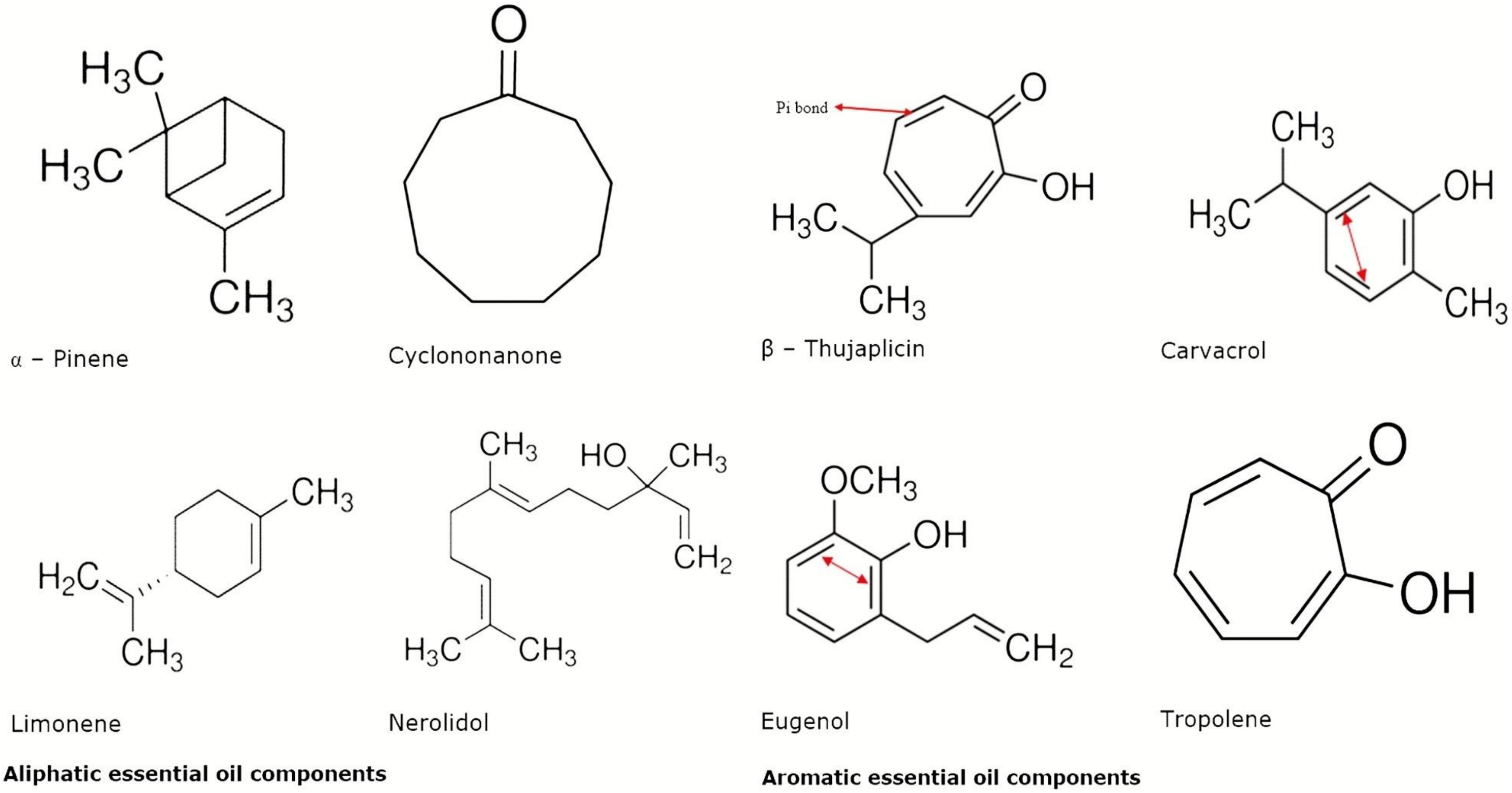

α – Pinene
Cyclononanone
β – Thujaplicin
Carvacrol
Limonene
Nerolidol
Eugenol
Tropolene

Aliphatic essential oil components

Aromatic essential oil components

Fig. 1. Structures of the essential oil components (EOCs)

Table 1. Essential oil components commercially used against German cockroach

Essential oil component	Brand name of commercial product	Plant family	Plant(s) obtainable[a]	Density (g/ml)	Purity (%)	Boiling point (˚C)	Vapor pressure (mmHg)	Solubility (g/L)	Molecular weight (g/mol)	Log P
α–pinene	(Ed Rosenthal's Zero Tolerance)	Pinaceae	*Pinus spp* L.	0.860	98	155	3.489	0.0059	136.230	4.320
Limonene	(D-Liminite orange oil)	Rutaceae	*Citrus limonum* Osbeck	0.840	≥95	175	1.541	0.0034	136.240	4.550
Carvacrol	(EcoSmart)	Lamiaceae	*Thymus vulgaris* L.	0.980	98	236	0.030	0.960	150.217	3.160
Eugenol	(EcoPCO ACU, essentria G)	Myrtaceae	*Syzigium aromaticum* Merrill & Perry	1.070	99	254	0.010	1.790	164.200	2.400

[a] Palmer 1942, Wang et al. 2015, Dudai et al. 2001, Mockute and Bernotiene 1999, respectively

Table 2. Essential oil components not previously tested against the German cockroach

Essential oil component	**Plant family**	**Plant (s) obtainable**[a]	**Density (g/ml)**	**Purity (%)**	**Boiling point (˚C)**	**Vapor pressure (mm Hg)**	**Solubility (g/L)**	**Molecular Weight (g/mol)**	**Log P**
Cyclononanone	Lamiaceae	*Lavandula pubescens* Decne.	0.963	95	95 – 97	0.099	503.800	140.225	2.321
Nerolidol	Piperaceae Lamiaceae	*Piper gaudichaudianum* Kunth *Thymus serpyllum* L.	0.875	98	114	0.001	0.014^{-1}	222.198	4.600
β-thujaplicin	Cupressaceae	*Thuja plicata* Donn.	1.004	99	140	0.089^{-3}	1.200	164.200	1.137
Tropolone	Cupressaceae	*Cupressus lusitanica* Mill.; *Thuja plicata*; *Thuja occidentalis* L.	1.148	98	80 – 84	6.790^{-4}	40.900	122.123	0.500

(NB: Physical properties of oil were adapted from PubChem and Sigma-Aldrich web page)

[a] Plants were described by Al-Badani et al. 2017, Chaaban et al. 2018 and Paaver et al. 2008, Daniels and Russel 2007, Hori 2004 and Saniewski et al. 2007, respectively

Table 3. Toxicity of aliphatic EOCs, and aliphatic EOCs + PBO topically applied to German cockroaches

Essential oil component	Bioassay	Strain[a]	LD_{50} (95% CI; mg/µl)	Slope (SE)	χ^2	df	RR[b]	SR[c]
α–pinene	EOC	S	0.081 (0.073–0.093)	6.228 (1.902)	5.076	4	–	–
		D	0.075 (0.069–0.081)	9.721 (2.209)	6.668	3	0.9	–
		E	0.098 (0.094–0.130)*	14.962 (5.293)	6.398	3	1.2	–
	EOC + PBO	S	0.056 (0.035–0.063)	10.007 (2.984)	15.589	4	–	1.4
		D	0.056 (0.040–0.061)	13.251 (4.440)	3.882	4	1.0	1.3
		E	0.057 (0.043–0.063)	10.128 (2.944)	5.794	4	1.0	1.7
Cyclononanone	EOC	S	0.025 (0.015–0.047)	1.648 (0. 400)	27.338	5	–	–
		D	0.168 (0.075–3.776)	1.180 (0.379)	9.284	5	6.7	–
		E	0.141 (0.064–4.408)	1.091 (0.375)	8.633	5	5.6	–
	EOC + PBO	S	0.019 (0.017–0.020)	2.421 (0.764)	4.777	4	–	1.3
		D	0.020 (0.017–0.020)	1.645 (0.587)	1.273	4	1.1	8.4
		E	0.021 (0.020–0.022)*	2.270 (0.682)	2.255	4	1.1	6.7
Limonene	EOC	S	0.063 (0.060–0.067)	16.178(2.701)	14.750	4	–	–
		D	0.062 (0.053–0.069)	8.144 (2.207)	18.337	4	1.0	–
		E	0.057 (0.053–0.060)	19.902 (3.716)	16.491	4	0.9	–
	EOC + PBO	S	0.049 (0.034–0.054)	11.233 (3.671)	4.088	4	–	1.3
		D	0.047 (0.029–0.053)	10.174 (3.490)	4.185	4	1.0	1.3
		E	0.049 (0.034–0.053)	10.159 (3.291)	4.797	4	1.0	1.2
Nerolidol	EOC	S	0.064 (0.059–0.068)	9.039 (2.108)	14.149	5	–	–

	D	0.091 (0.081–0.148)*	7.316 (2.400)	9.468	5	1.4	–
	E	12.5 (**)*	2.245 (2.922)	11.817	4	>10	–
EOC + PBO	S	0.064 (0.052–0.069)	8.486 (2.285)	10.943	4	–	1.0
	D	0.079 (0.071–0.099)*	6.138 (2.172)	2.145	4	1.2	1.2
	E	0.067 (0.054–0.073)	7.401 (2.224)	3.690	4	1.0	>10

LD$_{50}$ values followed by * are significantly different than the LD$_{50}$ value of Strain S (susceptible strain) for each essential oil component according to the lethal-dose ratio test (Robertson et al. 2003)

** Values were not computed by PoloPlus software

[a] Strain S is the susceptible strain; strains D and E are the resistant strains

[b] RR – Resistance ratio

[c] SR = synergist ratio

Table 4. Toxicity of aromatic EOCs, and aromatic EOCs + PBO topically applied to German cockroaches

Essential oil component	**Bioassay**	**Strain**[a]	**LD$_{50}$ (95% CI; mg/µl)**	**Slope (SE)**	**χ^2**	**df**	**RR**[b]	**SR**[c]
β– thujaplicin	EOC	S	0.056 (0.034–0.302)	1.673 (0.566)	5.943	3	–	–
		D	0.080 (0.050–0.503)	2.035 (0.682)	4.615	4	1.4	–
		E	0.096 (0.059–1.062)	2.352 (0.840)	6.206	4	1.7	–
	EOC + PBO	S	0.010 (0.001–0.015)	1.938 (0.678)	5.434	4	–	5.6
		D	0.013 (0.003–0.021)	1.676 (0.558)	4.721	4	1.3	6.2
		E	0.043 (0.028–0.154)*	1.715 (0.614)	1.778	4	4.3	2.2
Carvacrol	EOC	S	0.0075 (0.0047–0.0131)	1.999 (0.334)	5.483	3	–	–
		D	0.0065 (0.0041–0.0119)	1.387 (0.279)	4.406	4	0.9	–
		E	0.0035 (0.0024–0.0102)	2.816 (0.814)	4.044	3	0.5	–
	EOC + PBO	S	0.0022 (0.003–0.0050)	0.783 (0.272)	2.448	4	–	3.4
		D	0.0045 (0.0015–0.0166)	0.812 (0.290)	2.208	4	2.0	1.4
		E	0.0035 (0.0013–0.0105)*	0.882 (0.297)	4.764	4	1.6	1.0
Eugenol	EOC	S	0.023 (0.018–0.025)	10.223 (2.778)	4.834	4	–	–
		D	0.031 (0.026–0.035)*	5.161 (1.592)	4.407	4	1.3	–
		E	0.025 (0.021–0.028)	7.800 (2.235)	4.222	4	1.1	–
	EOC + PBO	S	0.023 (0.013–0.026)	5.815 (1.647)	18.224	4	–	1.0
		D	0.030 (0.035–0.034)*	4.946 (1.469)	11.025	4	1.3	1.0
		E	0.027 (0.018–0.031)*	4.869 (1.746)	1.032	4	1.2	0.9

Tropolone	EOC	S	0.046 (0.039–0.050)	5.165 (1.112)	13.705	6	–	–
		D	0.067 (0.060–0.076)*	5.516 (1.174)	7.321	5	1.5	–
		E	0.060 (0.055–0.067)*	5.666 (1.046)	10.980	6	1.3	–
	EOC + PBO	S	0.067 (0.057–0.073)	7.123 (1.888)	9.612	4	–	0.7
		D	0.071 (0.063–0.078)	8.759 (2.071)	16.725	4	1.1	0.9
		E	0.065 (0.055–0.071)	7.555 (2.089)	4.851	4	1.0	0.9

LD_{50} values followed by * are significantly different than the LD_{50} value of Strain S (susceptible strain) for each essential oil component according to the lethal-dose ratio test (Robertson et al. 2003)

[a] Strain S is the susceptible strain; strains D and E are the resistant strains

[b] RR – Resistance ratio

[c] SR = synergist ratio

Table 5. Toxicity of permethrin, and permethrin + PBO topically applied to German cockroaches

Bioassay	**Strain**[a]	**LD_{50} (95% CI; µg/µl)**	**Slope (SE)**	**χ^2**	**df**	**RR**[b]	**SR**[c]
Permethrin (P)	S	0.056 (0.004–0.179)	0.632 (0.167)	9.987	4	–	–
	D	2.138 (1.142–11.378)*	0.478 (0.202)	5.601	4	38.2	–
	E	1.730 (2.782–4.430)*	0.462 (0.169)	2.784	4	30.9	–
P + PBO	S	0.067 (0.057–0.073)	7.123 (1.888)	9.612	4	–	0.8
	D	1.195 (0.825–1.742)*	1.863 (0.350)	9.857	4	17.8	1.8
	E	1.135 (0.583–2.026)*	1.107 (0.301)	6.180	4	16.9	1.5

LD_{50} values followed by * are significantly different than the LD_{50} value of Strain S (susceptible strain) for each essential oil component according to the lethal-dose ratio test (Robertson et al. 2003)

[a] Strain S is the susceptible strain; strains D and E are the resistant strains.

[b] RR – Resistance ratio

[c] SR = synergist ratio

Chapter 4

Essential Oil Components in Superabsorbent Polymer Gel Modify Reproduction of *Blattella germanica* (L.) (Blattodea: Ectobiidae)

4.1 Abstract

The use of essential oil components (EOCs) against the German cockroach, *Blattella germanica* (L.), has increasingly received attention from researchers. However, the determination of lethal doses/concentrations alone does not provide enough information on the range of biological effects of these EOCs. To improve our understanding of the potential effects of EOCs, we examined biological parameters of *B. germanica* exposed to sublethal EOCs formulated in gels. This study employed superabsorbent polymer (SAP) gel to prolong bioavailability of limonene, carvacrol, and β–thujaplicin, and evaluated how these EOCsshape biological parameters of *B. germanica*. Overall, median survival days ranged from 57 – 69.5 d for males and 73 – 99 d for females. The survival day ranking for the EOCs was limonene > β–thujaplicin > carvacrol. Carvacrol and β–thujaplicin gels reduced male longevity by at least 34% and 39%, respectively, while limonene had no effect. The longevity of females was reduced by limonene, but not carvacrol and β–thujaplicin gels. EOCs significantly suppressed overall adult females' reproductive period, oothecal hatchability, reduced fecundity, and interoothecal period, but not number of oothecae formed and egg incubation period. The preoviposition period (mean: 2 – 25 d) ranking was limonene < carvacrol < β–thujaplicin. Based on these results, limonene, carvacrol, and β–thujaplicin in superabsorbent polymer gels show promising potential to reduce adult male survival/longevity, suppress egg hatchability, and female fecundity, and to delay the

interoothecal period. These findings may represent the basis for the practical use of EOCs as a tactic in integrated pest management systems for *B. germanica*.

4.2 Introduction

In homes, the German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), spreads filth, ruins foodstuffs, damages fabrics, and destroys book-bindings (Schal et al. 1984, Bell et al. 2007). Often, *B. germanica* disgorges portions of partially digested food, or discharge nauseous secretions from their bodies. Because *B. germanica* moves freely between filth and food, within human habitation, it can spread disease-causing organisms (Jalil et al. 2012, Menasria et al. 2014). Moreover, this indoor pest has been implicated in increasing childhood asthma morbidity (Cohn et al. 2006, Togias et al. 2010). The United States Office of Housing and Urban Development (HUD) reported the proportion of homes in low-cost housing with cockroach allergens at 63.7 % (Ii et al. 2009). Consequently, the burden imposed by *B. germanica* on public health has made its control critically important. Typically, control strategies rely on conventional insecticides due to cost and efficiency. This involves the use of bait formulations, insecticides, insect growth regulators, and potentially essential oils (Ko et al. 2016, Rabito et al. 2017, DeVries et al. 2019, Oladipupo et al. 2019a). These control agents are often designed as contact insecticides (either as foggers or aerosols) or as a bait which incorporates an insecticide in a food base that insects must generally consume to be affected. Presently, baits are the most popular tactic for *B. germanica* control (Nalyanya et al. 2001)

However, not all *B. germanica* that are exposed to a toxicant are killed. It may be that the threshold required to induce mortality was never reached. Beyond that, some slow-acting agents are designed to facilitate secondary kill (Ko et al. 2016). That is, the active ingredient (AI) is expected to be translocated in the cockroach feces or other residues and cause further mortality within the population – even if the dose/concentration acquired by the secondary individuals is much lower than the initially exposed ones. In the advent of survival, *B. germanica* may experience aftereffects that may impair various physiological, biological, and behavioral traits of the insect (Abd-Elghafar and Appel 1992, Biondi et al. 2013, González et al. 2016).

Substantial information exists on the sublethal effects of conventional insecticides on *B. germanica*. Stürmer et al. (2014) observed that sublethal doses of trichlorfon altered cockroach physiology and behavior by increasing both spontaneous locomotory activities and grooming time. Moreover, sublethal exposure of conventional insecticides in *B. germanica* disrupts fecundity, stimulates premature oothecal drop, and reduces egg hatchability (Abd-Elghafar and Appel 1992, Lee et al. 1998, Ko et al. 2016, Tengfei et al. 2019). Populations of *B. germanica* exposed to sublethal doses of spinosad and indoxacarb showed a reduction in the number of oocytes, basal oocyte volume, and the number of laid and hatched eggs per ootheca (Maiza et al. 2013).

These observations outline the magnitude and potential sublethal effects of conventional insecticides used for *B. germanica* management. However, the development of insecticide resistance and environmental concerns have motivated the consideration of plant-based products (such as essential oils) as alternatives (Wang et al. 2004, Wu and Appel 2017, DeVries et al. 2019, Fardisi et al. 2019). Indeed, many studies have evaluated the bioactivity of essential oils against numerous insects including *B. germanica* (see review by Regnault-Roger et al. 2012, Isman and Grieneisen 2014, Oladipupo et al. 2019a, Oladipupo et al. 2019b), yet only a few consider their sublethal effects (González et al. 2016). For example, sublethal effects of essential oils include retardation of development and fecundity in *Spodoptera littoralis* (Boisduval) and

Acanthoscelides obtectus (Say) (Papachristos and Stamopoulos 2009, Pavela 2012). The evaluation of the toxicological profiles, especially sublethal effects, is a key point to further understanding, development, and commercialization of essential oil-based products.

Such studies would give scientific and practical insights that can be leveraged for *B. germanica* control. González et al. (2016) reported that polymer-based nanoformulations of essential oils could improve stability and increase potency. Numerous studies have reported using encapsulated nanoparticles to improve the dispersibility and insecticidal activity of essential oils in water (Asbahani et al. 2015, Barradas et al. 2016, Ferreira et al. 2019). Superabsorbent polymer (SAP) gel, also known as hydrogel, can retain and store water for a considerable period. SAP gel is easy to apply and cost-effective (Buczkowski et al. 2014). If employed as a carrier, SAP gel requires a relatively small amount of active ingredient. SAP gels have been evaluated as a bait delivery option for urban pests such as the Argentine ant, *Linepithema humile* (Mayr) (Rust et al. 2015, Boser et al. 2017, Merrill et al. 2018).

In this study, we investigated the sublethal effects of essential oil components incorporated in SAP gels against *B. germanica*. The objectives of this study were to test the ability of SAP gels to deliver three essential oil components (EOCs) and determine how the sublethal concentrations of these EOCs shape the biological parameters of *B. germanica*. This is to assist in further developing of EOCs for *B. germanica* control. There have been a few essential oil-based products in the market targeting German cockroaches (Appel et al. 2001). Favorable results would provide an available reference for understanding the influence of EOCs on aspects of the developmental biology of *B. germanica,* optimizing commercialization of EOCs, and rationalizing inclusion of EOCs in IPM control interventions for managing *B. germanica*.

4.3 Materials and Methods

4.3.1 Chemicals

Three essential oil components (EOCs), limonene, carvacrol, and β–thujaplicin, were purchased from Sigma-Aldrich (St. Louis, MO). Four sublethal concentrations of each EOCs (Table 1) were used based on data from a previous study (Oladipupo et al. 2019a). Serial dilutions of each EOC were made in an emulsifier, Tween-80 (Control 603550; DIFCO laboratories, Detroit, MI), to ensure the EOCs (Table 1) become miscible (dispersible) in water (Tengfei et al. 2019).

4.3.2 Superabsorbent gel preparation

Water storing crystals (CAS No.: 25608-12-2, purity of 99%), potassium polyacrylate $(C_3H_3KO_2)_n$, were purchased from Wormy worms LLC (Humble, TX). A suspension was prepared by adding 50 mg of water storing crystals to 50 ml of distilled water. One ml of an EOC sublethal concentration was added to the suspension and allowed 30 min for the suspension to form a Superabsorbent polymer (SAP) gel.

4.3.3 Insects

An insecticide-susceptible *B. germanica* strain was used in this study. This strain has been in continuous laboratory culture without exposure to insecticides for > 50 yr in the Urban Entomology laboratory at Auburn University, AL. *B. germanica* were collected from this stock culture and reared in 3.8-liter glass jars at 28 ± 2º C, with 40 – 55% RH, and a photoperiod of 12:12 (L: D) h. The cockroaches were provided harborage (corrugated cardboard), rodent food (Purina 500l lab diet from Purina LabDiet®, Inc. St. Louis, MO), and clean water weekly.

Nymphs were selected from the colony and sexes separated into different jars (3.8-liter glass jars) to prevent mating. From these, 3 – 7 d old adult males and females were selected and used for the experiment. This was done to increase the likelihood of mating while reducing the number of infertile oothecae (Abd-Elghafar and Appel 1992).

4.3.4 Biological parameters bioassay

Four sublethal concentrations (Table 1) of each EOC were used. Each sublethal concentration was delivered into a water-storing crystal (as described above) to form SAP gel. A pair of virgin male and female adult *B. germanica* were placed in a plastic Petri dish (10 cm x 1.5 cm) provided 3 pieces of dog chow, 4 g of SAP gel, and a piece of cardboard harborage (5 cm x 3 cm). The Petri dishes were covered and greased on the inside vertical surfaces. The SAP gel was replaced with a piece of water-soaked cotton wick (3.5 cm) on day 7. The wick was replaced every 4 d throughout the experiment. There were 10 replicates for each treatment. A blank control with only Tween-80 in SAP gel was run simultaneously with the EOC treatments. The experiment was conducted at 28 ± 2° C, with 40 – 55% RH, and a photoperiod of 12:12 (L: D) h. Male *B. germanica* were observed daily for longevity. Female *B. germanica* were observed daily to determine the preoviposition and incubation period of each ootheca, number of oothecae formed, number of oothecae hatched, number of oothecae dropped, fecundity, and longevity. Longevity was defined as the number of days from the start of the experiment to *B. germanica* mortality. The number of oothecae formed is the count of oothecae produced per female *B. germanica*, while oothecae hatched and oothecae dropped is the number of oothecae that produced nymphs and inviable oothecae, respectively. Pre-oviposition periods were grouped into two phases: the period between mating to the formation of the first ootheca, and the subsequent period between the hatching of one ootheca and formation of the next. Incubation and pre-oviposition periods were recorded for all oothecae produced by female *B. germanica*

4.3.5 Statistical analyses

All data were processed in Microsoft Excel 2016. One-way ANOVA was used to determine the relationship between sublethal concentrations of EOCs in superabsorbent polymer gel, and all the variables collected (male and female longevity, number of oothecae produced, oothecae hatched, oothecae dropped, fecundity, preoviposition period, and incubation period). Where significant, means were separated using Tukey's HSD test at $P < 0.05$. All figures were drawn using SigmaPlot 14.0 (Systat Software, Inc. 2017). Survival analysis was used to estimate the sublethal effect of EOCs against adult male and female *B. germanica* survival (days). Survival analysis focuses on the expected duration of time until the occurrence of an event of interest. This approach is well suited to analyze the time a toxicant can persist in a population (In and Lee 2018). This method is advantageous over LT_{50} (of probit analysis) because all observations do not need to have an exact starting and ending points. Moreover, the censorship feature (of survival analysis) allows for measurement of lifetimes for a population that either has not experienced the event of interest or its lifetime is greater than the duration of the study, a feature absent in probit analysis. Kaplan-Meier estimators were used to fit models [EOC treatment concentrations, longevity (in days), and event (occurrence of mortality or otherwise)] in R (v. 3.5.3) using the *surviminer* package (0.3.0) to calculate the median survival days. The median survival days is the time at which the survivorship function equals 0.5. The log-rank test was used to test the null hypothesis that there were no differences in survival between adult *B.*

germanica exposed to EOCs in SAP gel and the control. One-way ANOVA and survival analysis were conducted in R studio version 3.6.1. (R Core Team, 2018).

4.4 Results

4.4.1 Sublethal effect of EOCs in SAP gel on survival days and longevity of adult male and female B. germanica

The survival probability of adult males and females over time is presented in Fig. 1. Median survival days of adult males [69.5 d ± 12.5 at 95% confidence limits (95% CL)] and females [99.0 d ± 20 (95% CL)] exposed to all concentrations of limonene SAP gel was significantly lower (log-rank statistic: male; χ^2 (df = 4, N = 50) = 11.1, P = 0.025, female; χ^2 (df = 4, N = 48) = 15, P = 0.005) than those of the control males [120 d ± 34 (95% CL)] and females [130 d ± 9 (95% CL)], respectively (Fig. 1A). There was no difference between the median survival days of adult males and females exposed to all concentrations of carvacrol SAP gel compared with control males and females (log-rank statistic: male; χ^2 (df = 4, N = 50) = 8.7, P = 0.068, female; χ^2 (df = 4, N = 48) = 3.6, P = 0.47) (Fig. 1B). However, adult males exposed to all concentrations of β–thujaplicin SAP gel had a significantly lower (log-rank statistic: χ^2 (df = 4, N = 48) = 15.8, P = 0.003) median survival [61 d ± 9 (95% CL)] compared with control males [120 d ± 34 (95% CL)]. There was also no difference between adult females exposed to β–thujaplicin SAP gel compared with control females (log-rank statistic: χ^2 (df = 4, N = 40) = 7.4, P = 0.12) (Fig. 1C).

The sublethal effects of limonene, carvacrol, and β–thujaplicin SAP gels at four sublethal concentrations on the longevity of adult male and female *B. germanica* are presented in Fig. 2. The longevity of male *B. germanica* was control > limonene > β–thujaplicin > carvacrol. The mean longevity of control males was 100.7 ± 11.13 d. The mean longevities of male *B. germanica* exposed to limonene ranged between 64.7 ± 5.65 d for 10.18 mg/g and 77.8 ± 9.13 d for 9.36 mg/g, which was significantly different from the control group ($F_{1,48}$ = 7.887, P = 0.007). Carvacrol reduced the longevity of male *B. germanica* by at least 34% at 5.38 mg/g ($F_{1,48}$ = 4.090, P = 0.006). β–thujaplicin also significantly reduced the longevity of male *B. germanica* by at least 39% at 5.33 mg/g ($F_{1,48}$ = 3.752, P = 0.010).

The longevity of female *B. germanica* was control > β–thujaplicin > carvacrol > limonene (Fig. 2). Mean longevity of control females of *B. germanica* was 105.5 ± 10.12 d and was not significantly different ($F_{1,48}$ = 0.2, P = 0.66) from limonene at 9.36 mg/g (120 ± 9.72 d). However, longevity of females decreased at higher limonene concentrations ($F_{1,48}$ = 3.359, P = 0.017; range: 69.8 ± 13.33 – 81.9 ± 13.34 d). Longevity of female was unaffected by increasing carvacrol concentration ($F_{1,48}$ = 2.013, P = 0.109; range: 59.7 ± 12.86 – 90.4 ± 14.75 d). After exposure to β–thujaplicin SAP gels, the mean longevity of female *B. germanica* was not significantly different from the control ($F_{1,48}$ = 1.067, P = 0.384; range: 73.4 ± 14.46 – 103.1 ± 14.80 d).

4.4.2 Sublethal effect of EOCs in SAP gel on number of oothecae formed, dropped, and hatched

Overall, the mean number of oothecae produced by adult *B. germanica* females varied among the treatments (Fig. 3). Control *B. germanica* females produced a mean of 4.2 ± 0.59 (n = 10) oothecae. The mean number of oothecae produced by adult females exposed to limonene SAP gel decreased significantly with increasing concentration ($F_{1,48}$ = 4.686, P = 0.035; range: 1.80 ± 0.63 – 4.30 ± 0.63). The number of oothecae from females exposed to carvacrol SAP gel increased with increasing concentration ($F_{1,48}$ = 1.819, P = 0.184; range: 1.8 ± 0.63 – 2.7 ± 0.79). The mean number of oothecae in adult females exposed to β–thujaplicin SAP gel ranged from 2.1 ± 0.48 to 3.2 ± 0.59 and was significantly lower than the control ($F_{1,48}$ = 5.879, P = 0.019).

The mean number of dropped oothecae in the control treatment was 0.90 ± 0.10 (n = 10) (Fig. 4). The number of oothecal dropped was significantly greater in *B. germanica* exposed to carvacrol SAP gel at 5.38 and 7.90 mg/g ($F_{1,48}$ = 3.195, P = 0.0216; range: 0.80 ± 0.29 – 2.30 ± 0.56), and less in *B. germanica* exposed to carvacrol SAP gel at 10.82 and 14.55 mg/g, respectively. There was no significant difference in the mean number of oothecae dropped by females exposed to limonene ($F_{1,48}$ = 0.224, P = 0.923; range: 0.80 ± 0.36 – 1.30 ± 0.47) and β–thujaplicin SAP gels ($F_{1,48}$ = 0.411, P = 0.800; range: 1.10 ± 0.18 – 1.70 ± 0.68).

The mean number of hatched control oothecae was 3.67 ± 0.62 (n = 10) (Fig. 5). Hatchability of oothecae was significantly affected by limonene and carvacrol SAP gels. The mean number of hatches declined in later-produced oothecae and there was no egg-hatch from any of the 6th oothecae. In limonene SAP gel treatments, oothecal hatch decreased with increasing concentration (F = 2.479, P = 0.007; range: 0.61 ± 0.34 – 1.9 ± 0.48). The numbers of oothecae hatched (range: 0.5 ± 0.22 – 1.6 ± 0.62) in carvacrol treatments were significantly less than in the control treatment (F = 2.471, P = 0.014). The mean number of ootheca hatched in β–thujaplicin SAP gel treatments was also significantly less than that of the control (F = 2.181, P = 0.010; range: 0.7 ± 0.39 – 2.1 ± 0.63).

4.4.3 Sublethal effect of EOCs in SAP gel on fecundity of female B. germanica

Fecundity (total number of offspring per adult female) of control females was 102.1 ± 18.75 nymphs (Fig. 6). The EOC SAP gels significantly ($P < 0.05$) reduced fecundity. Fecundity of females exposed to limonene SAP gel at 10.18 mg/g (30.6 ± 12.78) and 10.54 mg/g (24.8 ± 13.34) was significantly lower than females in other treatment concentrations ($F_{1,48} = 11.340$, $P < 0.0001$). Fecundity of females exposed to carvacrol SAP gel was significantly lower than the control ($F_{1,48} = 10.740$, $P = 0.0003$; range: 11.2 ± 6.61 – 47.9 ± 18.76). Fecundity of females exposed to β–thujaplicin SAP gel was significantly lower than the control ($F_{1,48} = 10.410$, $P = 0.0001$; range: 21.2 ± 12.07 – 61.7 ± 17.53).

4.4.4 Sublethal effect of EOCs in SAP gel on preoviposition and egg incubation period of B. germanica

Pre-oviposition periods were grouped into two phases: the time between mating to the formation of the first ootheca, and subsequent periods between the hatching of one ootheca and formation of the next. The shortest mean initial preoviposition period was 6.10 ± 0.72 d (n = 10) for the control females (Table 2). The mean initial preoviposition period was control < limonene < carvacrol < β–thujaplicin. The interoothecal period for the control increased with successive oothecae but dropped sharply for the 4th and 5th interoothecal periods. In most cases, the longest interoothecal periods were between the first and second oothecae in females exposed to EOC SAP gels.

Females exposed to limonene SAP gel had longer initial preoviposition periods as the concentration of increased ($F_{1,48} = 1.675$, $P = 0.177$). The period between the first and second oothecae, at 0.063 mg/µl was significantly longer than in the control ($F_{1,18} = 2.803$, $P = 0.045$).

As with the control, there was a reduction in the 4th and 5th interoothecal periods at 14.55 mg/g. Carvacrol SAP gel increased the interoothecal period between the first and second ($F_{1,18}$ = 4.542, P = 0.007), and fourth and fifth ($F_{1,18}$ = 13.050, P = 0.001) ootheca. Exposure to β–thujaplicin SAP gel resulted in the longest mean initial oviposition period ($F_{1,18}$ = 2.738, P = 0.041). Additionally, the interoothecal period between first and second ($F_{1,18}$ = 3.560, P = 0.003), fourth and fifth ($F_{1,18}$ = 22.838, $P < 0.0001$), and fifth and sixth ($F_{1,48}$ = 19.58, P = 0.017) oothecae were the longest.

The incubation period is marked by the time between the formation of an ootheca and hatch (Table 3). The incubation period from the control groups ranged from 18 – 22 d. The periods between oothecae decreased from the first to fifth oothecae in the control groups. The incubation periods for limonene, carvacrol, and β–thujaplicin ranged from 15.75 to 22.50 d, 15.00 to 38.00 d, and 16.33 to 25.00 d, respectively. In general, these interval periods were not significantly different ($P > 0.05$) from the control.

4.5 Discussion

The estimation of median lethal doses/concentrations of a toxicant in toxicity studies does not provide a comprehensive assessment of the range of bioactivity of a toxicant. There may be undescribed costs of sublethal exposure unaccounted for. Several sublethal reproductive costs for three essential oil components (EOCs) against *B. germanica* were investigated in this study. The results presented here suggest that such sublethal or biological costs might give insights on strategies for *B. germanica* population suppression, which may inspire further development of EOCs as *B. germanica* control agents.

The mean control longevity of *B. germanica* males and females reported in this study was similar to that reported by Abd-Elghafar and Appel (1992), Lee et al. (1998), and Ang and Lee (2011). The data on the sublethal effects of limonene, carvacrol, and β–thujaplicin in superabsorbent polymer (SAP) gel on adult male and female *B. germanica* show that the longevity of males was reduced while females were only affected by limonene at higher concentrations (i.e. > than 9.36 mg/g). Our result agrees with Karr and Coats (1992) who reported that EOCs such as *d*–limonene, linalool, β–myrcene, and □–terpineol failed to affect the female lifespan. Ang and Lee (2011) reported no significant difference between the longevity of male and female susceptible populations of *B. germanica* in an unexposed fitness test. Thus, the difference reported in our study between sexes suggests significant sublethal effects of the EOCs in SAP gel. Females exposed to EOCs had longevity 30% shorter than those reported by Hamilton and Schal (1990) after treatment with sublethal concentrations of chlorpyrifos-methyl. The likely explanation for females not being significantly affected by all EOCs may be due to their physiology. Cha et al. (1970) reported a distinct difference in the magnitude of resistance between female and male *B. germanica* exposed to conventional insecticides. Besides, females are much larger than males and receive a proportionately lower dose (mg EOC/g body weight) than smaller males. Moreover, the fat body and the presence of female-specific proteins (yolk proteins) in the hemolymph could have a role in the storage and metabolism of the EOCs (Roma et al. 2010). Reproductive females have greater general metabolic activity and may store EOC or move EOC into the 1st or 2nd ootheca. We speculate such transovarial EOC movement may be responsible for the significantly longer interoothecal period between the first and second oothecae observed in this study.

The mean number of control oothecae formed in this study (4.2 ± 0.59) was similar to that reported by Abd-Elghafar and Appel (1992) but less (6.6 ± 0.5) than those reported by Willis et al. (1958) for other insecticide susceptible *B. germanica* strains. The number of oothecae formed by *B. germanica* females exposed to limonene and β–thujaplicin SAP gels decreased with increasing EOC concentration. This may be due to the poor physiological conditions of the female *B. germanica* after exposure to higher dosages of limonene and β–thujaplicin SAP gels. *B. germanica* were generally lethargic with wings displayed awkwardly (personal observation, Oladipupo). Similarly, exposure to carvacrol reduced the mean number of oothecae. Similar observations between toxicant exposure and oothecae formation have been reported previously (Hamilton and Schal 1990, Abd-elghafar et al. 1992). Additionally, plant metabolites such as essential oil components can inhibit the juvenile hormone (JH) in cockroaches (Karr and Coats 1992). William (1969) described the role of JH in activating ovaries to incorporate vitellogenins necessary for yolk formation. A clear correlation between JH inhibition and plant metabolites such as limonene and other monoterpenes has been reported (Module and Blackwell 1993, Yu 2000). Thus, it possible that higher concentrations of monoterpenes such as limonene and β–thujaplicin might reduce yolk formation or absorption in *B. germanica*.

Control oothecal hatch in this study (3.67 ± 0.62) was smaller than that reported by Abd-Elghafar and Appel (1992). We found that limonene, carvacrol, and β–thujaplicin significantly suppressed hatchability. Reduced hatch may be due to the intoxication of the eggs by the EOCs. Females were killed with their ootheca attached when the concentration was high, and the oothecae rapidly desiccated suggesting insufficient water-exchange between oothecae and dead female. On the one hand, Zhou and Patourel (1989) reported oothecal hatch in dead females exposed to hydramethylnon and boric acid. On the other hand, Karr and Coats (1992) reported no significance between reproductive parameters of *B. germanica exposed to* EOCs linalool, *d*-limonene, α–terpineol. The *B. germanica* used in Karr and Coats (1992) were topically exposed to the sublethal EOCs, while in this study, we focused on exposure of EOC in SAP gels to *B. germanica* to which probably include consumption (oral), topical and possibly vapor routes of entry. Besides, *B. germanica* were observed to feed on the EOC SAP gels (personal observation, Oladipupo). These additional rates of entry may have been responsible for the difference in results. Frequency and rapidity of premature oothecal drop (Fig. 5) may be an important component in the evaluation of EOCs for *B. germanica* control. Mean control fecundity in this study (102 ± 18.75) was similar to that reported by Abd-Elghafar and Appel (1992). The impact of limonene, carvacrol, and β–thujaplicin in reducing the total number of offspring produced (fecundity) should be considered as parts of their total efficacy.

We found that the longest interoothecal period (range: 6.80 – 15.00 days) was between first and second oothecae for most of the EOC SAP gels. Abd-Elghafar and Appel (1992) reported this period to range from 7.50 – 17.78 days for *B. germanica* exposed to chlorpyrifos, cyfluthrin, and hydramethylnon while Willis et al. (1958) reported 17 days. It is a common occurrence for sublethal exposure to delay post-oviposition periods in insects (Ang and Lee 2011, Crawley et al. 2017). In fact, on average, fewer females produced oothecae in the EOC SAP gels than were produced in the control, though this effect was not statistically significant (Table 2). Surprisingly, the interoothecal period between the 4th and 5th oothecal became significantly longer for females exposed to carvacrol and β–thujaplicin (aromatic EOCs), and shorter for limonene (aliphatic EOC) SAP gels. In *B. germanica*, the feeding cycle intensifies before oothecae production, and food deprivation limits oothecal production (Cochran 1983). This study found that a greater number of oothecae were produced in female *B. germanica* exposed to carvacrol and β–thujaplicin than limonene. So, it could be that aliphatic EOCs such as limonene exhibit moderate antifeeding activity. Overall, the mean egg incubation periods between the treatments and the control were not significantly different and could result from the short exposure period of this study. EOC SAP gels were removed after 7-d exposure. Females might have not acquired sufficient EOC doses to affect oothecae. Cockroach females are known to share nutrients and water with the developing oothecae (Cochran 1983).

The outstanding reproductive suppression of these EOCs suggests use against adult insect populations that insect growth regulators (IGRs) do not affect. Physiologically, IGRs exploit hormonal titers in embryonic and immature stages to interfere with insect growth and development (Graf 1993). Moreover, treatments with IGRs neither act immediately nor kill the pest at the most nuisance stage. As such, for *B. germanica* populations where generations overlap and different life stages are present, IGRs alone would be ineffective. Conversely, EOCs such as limonene, carvacrol, and β–thujaplicin are toxic to adult *B. germanica* in both contact and fumigant tests (Philips and Appel 2010, Philips et al. 2010, Oladipupo et al. 2019a). The reproductive suppression observations made in this study provide additional insight into the total efficacy of these EOCs.

One important limitation of our study is the large number of different endpoints that the determination of the sublethal effects of a toxicant against an insect may follow. Here we focused on reproductive effects induced by exposure of *B. germanica* to EOC in SAP gels to which probably include possible oral (consumption), topical (walk-on), and vapor route (fumigant). So, it might be difficult to attribute precisely which route was responsible for the observations made in this study. The sublethal concentrations used in this study are nowhere near enough to elicit death via fumigation (Philips and Appel 2010) or contact (Philips et al. 2010, Oladipupo et al. 2019a). Future work could use this as a study as a reference point, and design assays that test for each of these possible pathways independently.

Nevertheless, because there seems to be little or no residual activity at currently formulated and used concentrations associated with essential oils, despite the obvious level of toxicity (see review by Isman 2019), new strategies must be employed to deliver EOCs for a prolonged period. Onesuch strategy would include the incorporation of EOCs in materials, such as superabsorbent polymers, that can prolong bioavailability. Then, the subsequent goal would be to evaluate such an approach with further emphasis on how these EOCs shape biological parameters of insect pests. Our findings suggest that sublethal concentrations of limonene, carvacrol, and β–thujaplicin in SAP gels can affect *B. germanica* in three ways: (1) reduce adult male survival/longevity, (2) suppress egg hatchability and female fecundity, and (3) delay interoothecal period. In addition, the EOCs could affect behaviors: feeding, foraging, activity, mate location, courtship, etc. that might result in even more net activity. These findings may represent the basis for the practical use of EOCs and the inclusion of new EOC formulations into integrated pest management systems for *B. germanica*.

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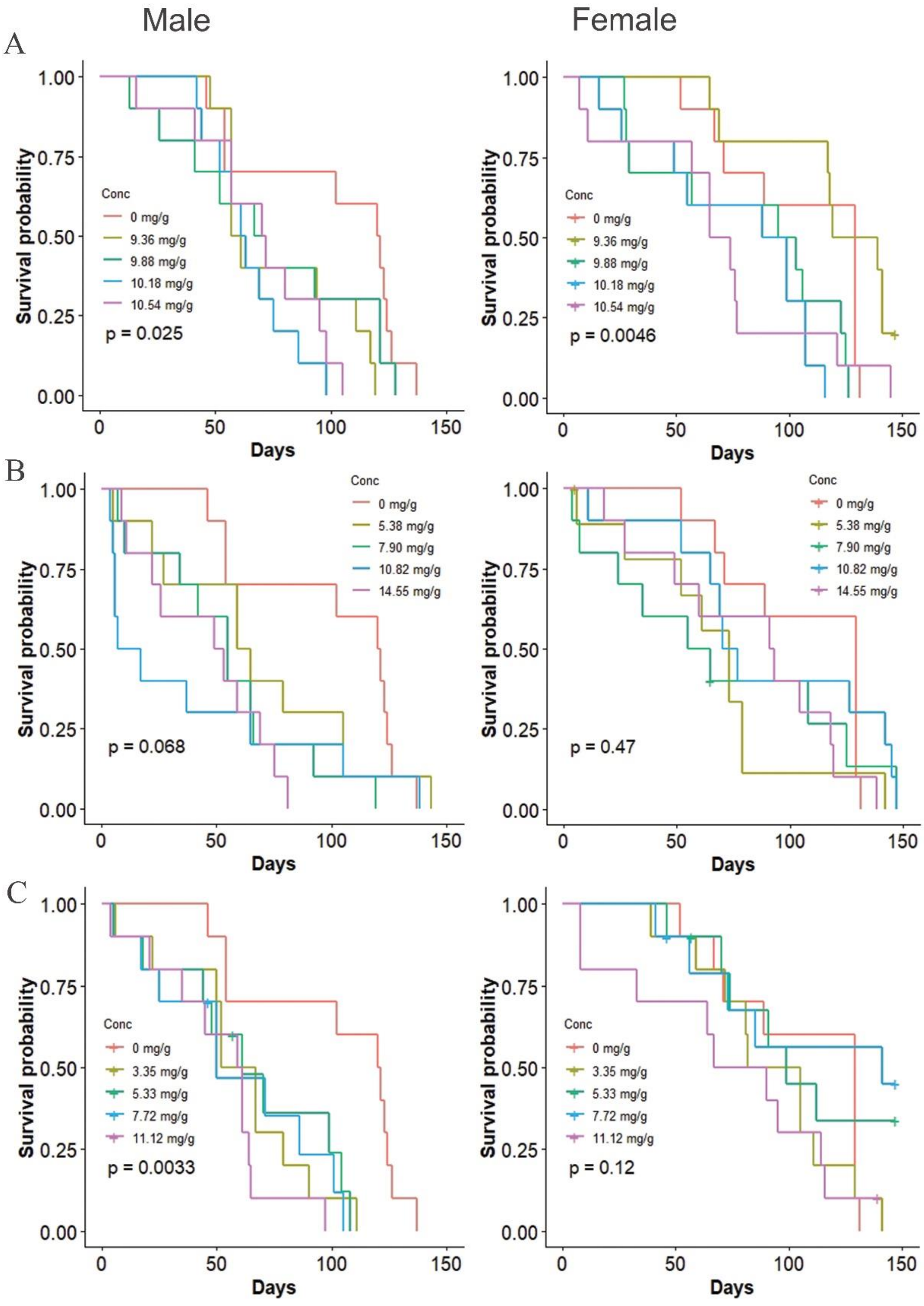

Fig. 1. Sublethal effect of superabsorbent polymer gel of (A) limonene, (B) carvacrol, and (C) β–thujaplicin on median survival days of adult male and female *B. germanica*. Ten replicates were used per concentration

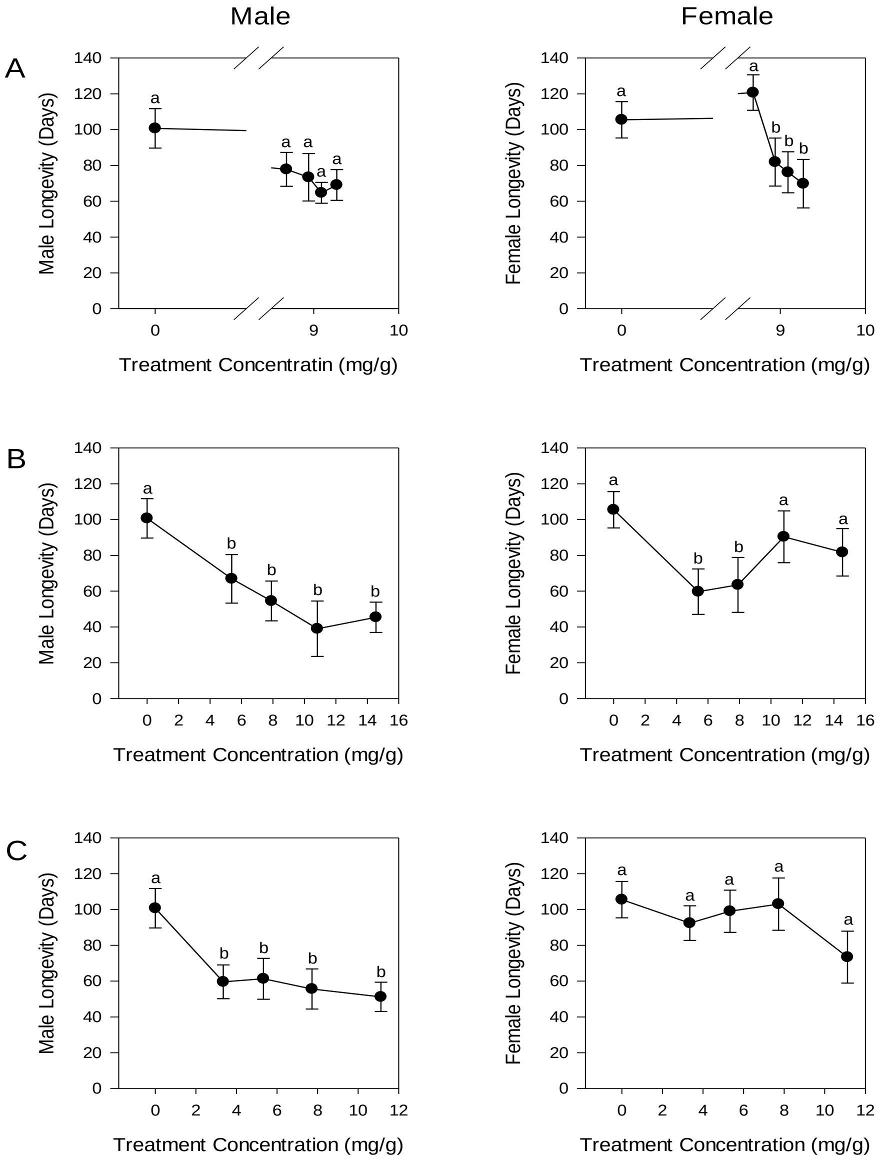

Fig. 2. Sublethal effect of superabsorbent polymer gel of (A) limonene, (B) carvacrol, and (C) β–thujaplicin on mean longevity of adult male and female *B. germanica*. SEM showed for each mean. Error bars followed by the same letters are not significantly ($P > 0.05$) different from one another. Ten replicates were used per concentration

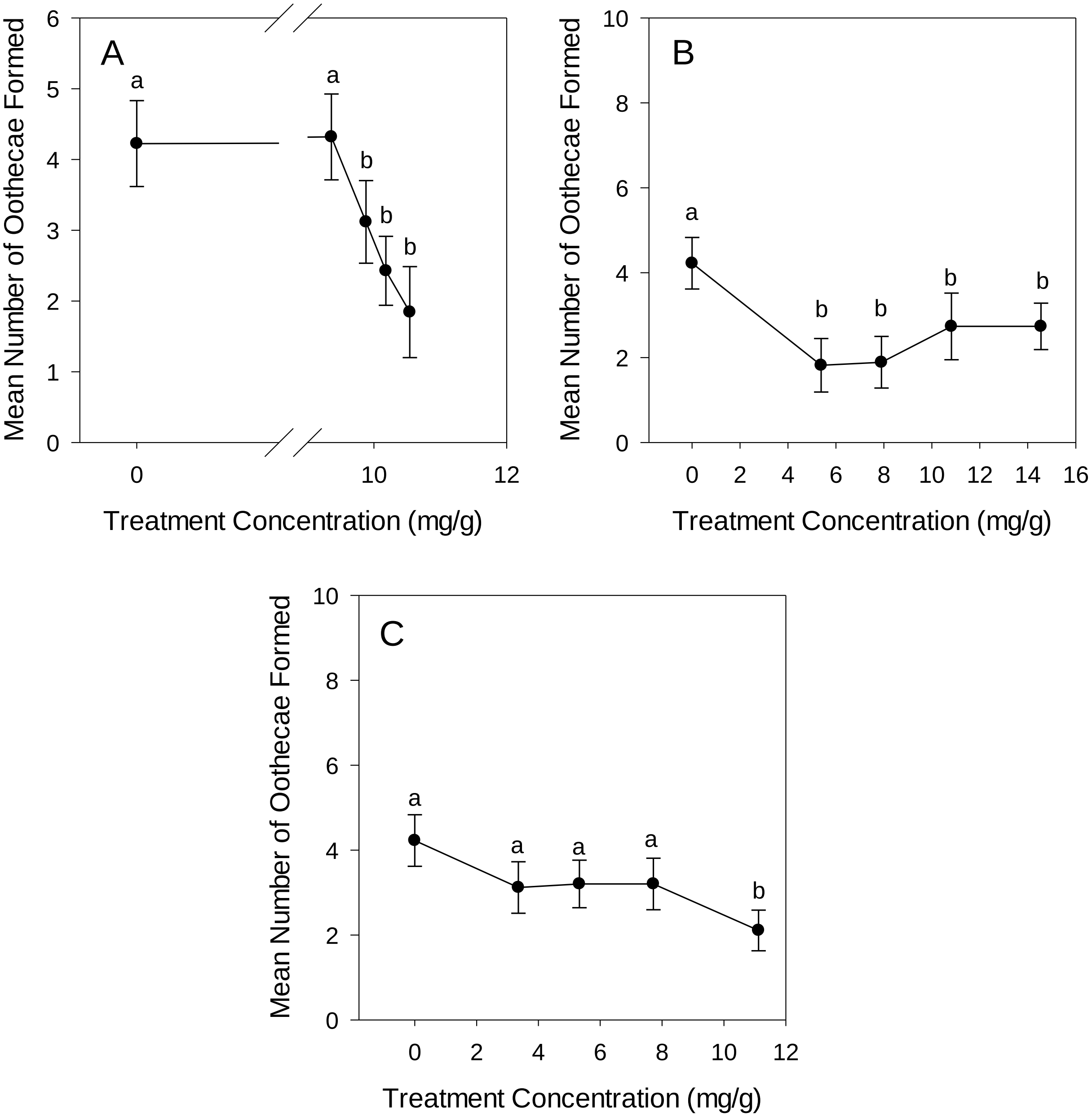

Fig. 3. Sublethal effect of superabsorbent polymer gel of (A) limonene, (B) carvacrol, and (C) β–thujaplicin on the mean number of oothecae formed. SEM showed for each mean. Ten replicates were used per concentration

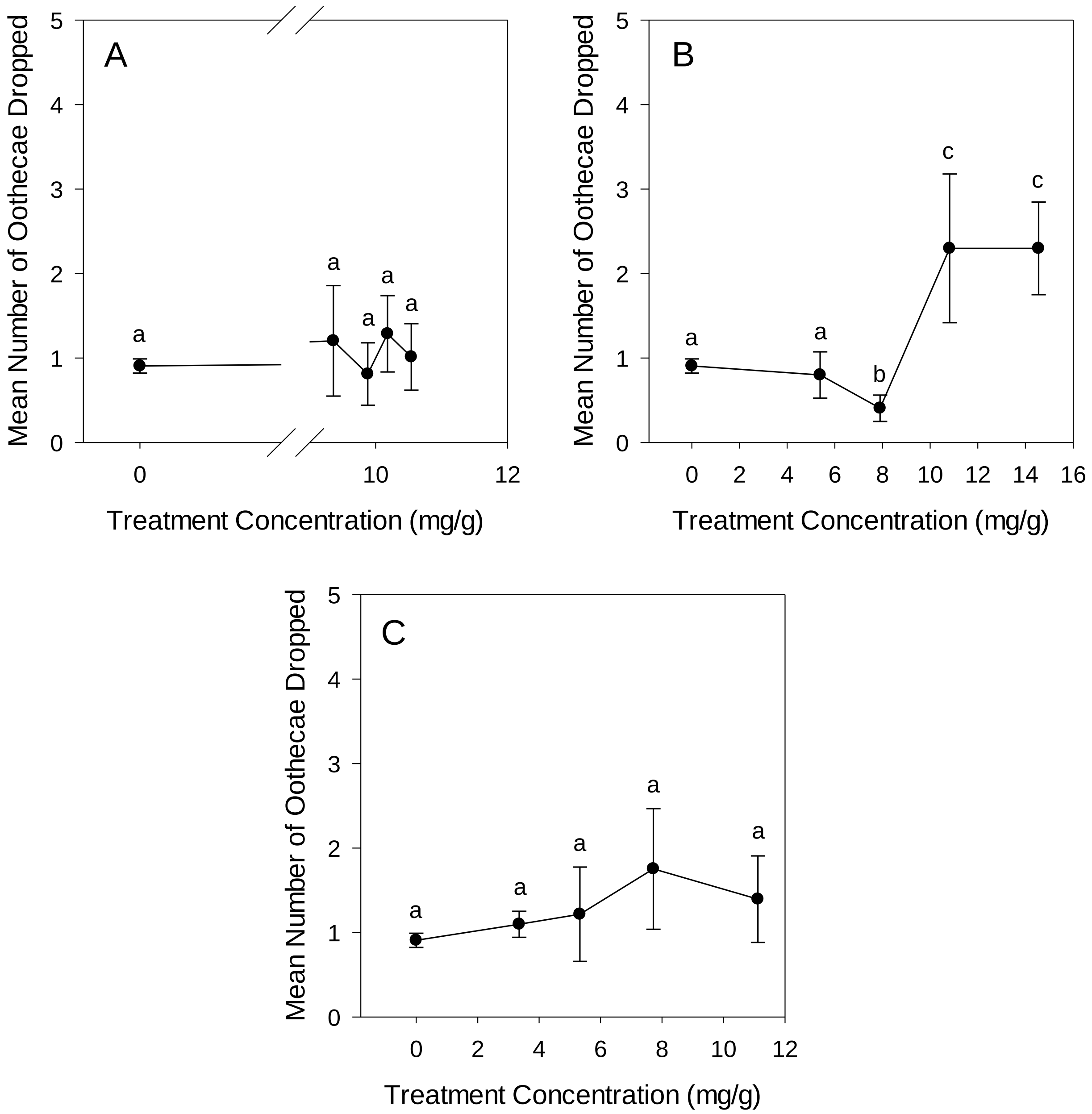

Fig. 4. Sublethal effect of superabsorbent polymer gel of (A) limonene, (B) carvacrol, and (C) β–thujaplicin on the mean number of oothecae dropped. SEM showed for each mean. Ten replicates were used per concentration

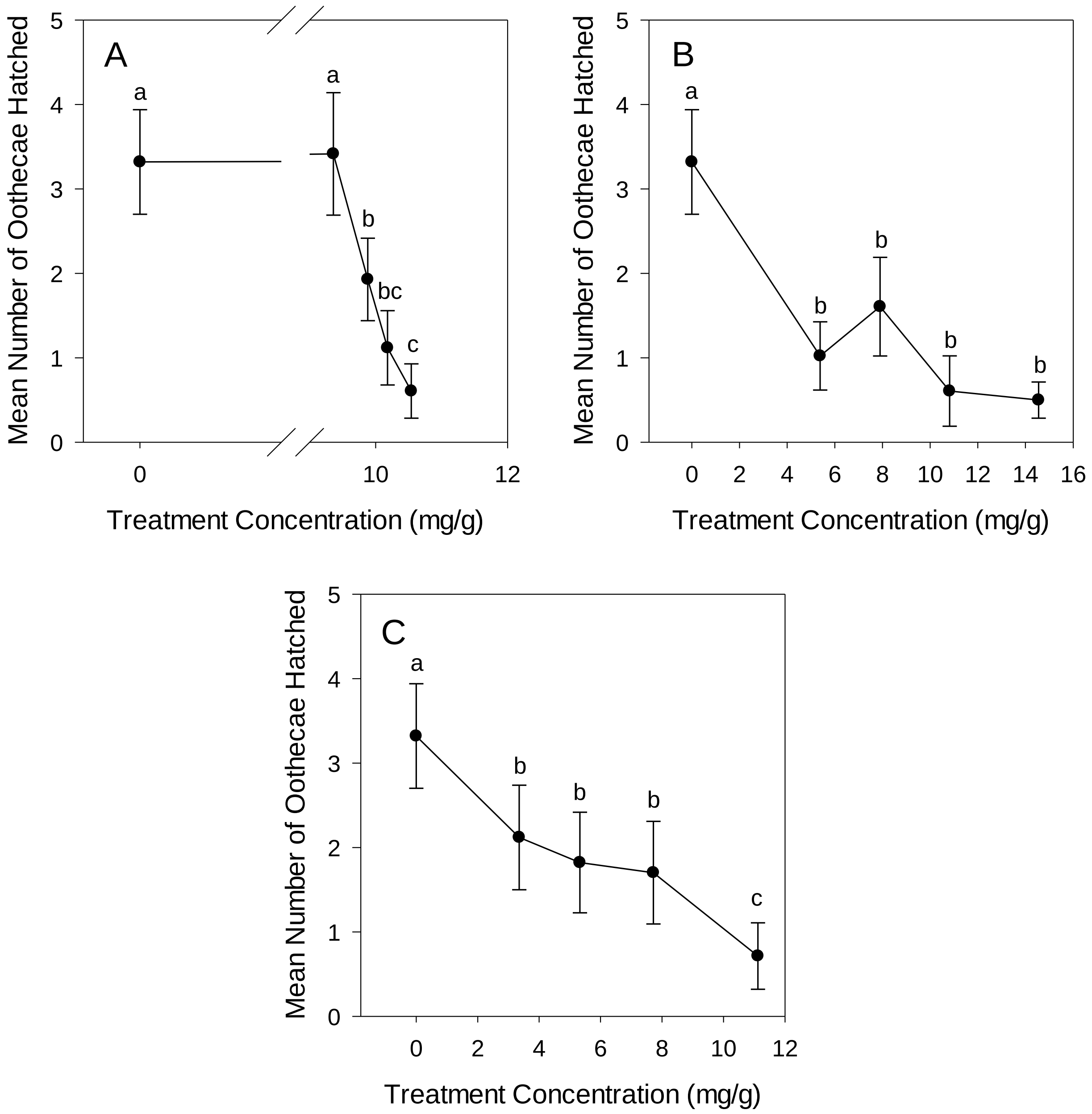

Fig. 5. Sublethal effect of superabsorbent polymer gel of (A) limonene, (B) carvacrol, and (C) β–thujaplicin on the mean number of oothecae hatched. SEM showed for each mean. Ten replicates were used per concentration

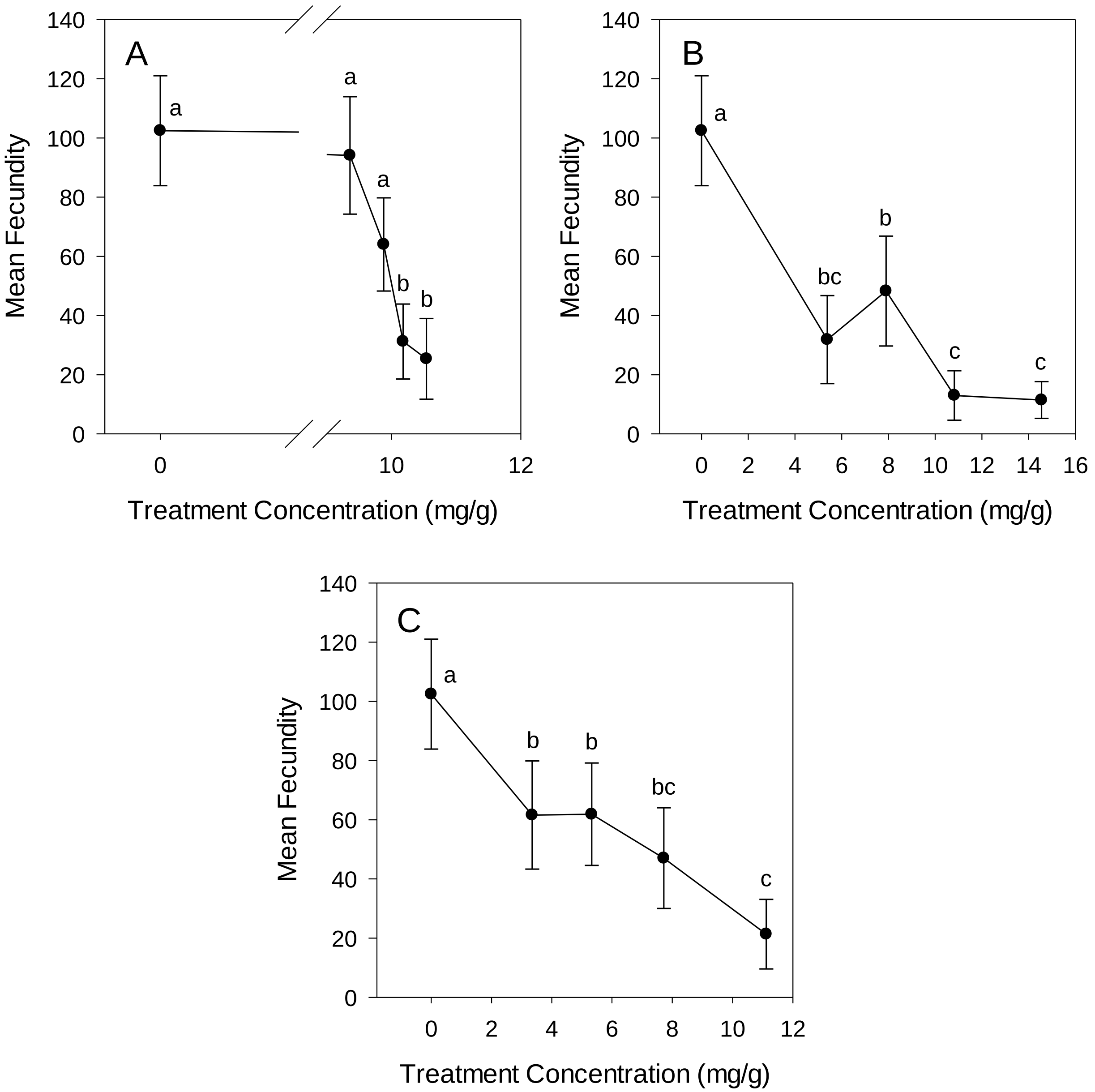

Fig. 6. Sublethal effect of superabsorbent polymer gel of (A) limonene, (B) carvacrol, and (C) β–thujaplicin on *B. germanica* fecundity. SEM showed for each mean. Ten replicates were used per concentration

Table 1. Sublethal concentrations (mg/g) of essential oil components in superabsorbent polymer gel applied against adult *B. germanica*

Concentration	Limonene (≥95)*	Carvacrol(98)*	β–thujaplicin(99%)*
1	9.36	5.38	3.35
2	9.88	7.90	5.33
3	10.18	10.82	7.72
4	10.54	14.55	11.12

[a] Values are recalculated from lethal dose values presented in Oladipupo et al. 2019a

* = values in parentheses are percentage purity values for each essential oil components.

Table 2. Sublethal effect of essential oil components in superabsorbent polymer gel on the pre-oviposition periods (day) in *B. germanica*

Essential oil Component	Treatment Conc. (mg/g)	St – F$_1$^{a}	n^b	H$_1$ – F$_2$^{c}	n	H$_2$ – F$_3$	n	H$_3$ – F$_4$	n	H$_4$ – F$_5$	n	H$_5$ – F$_6$	n
Control	0	6.10 ± 0.72^{aA}	10	6.00 ± 0.55^{aA}	9	7.38 ± 0.84^{aA}	8	8.00 ± 1.00^{aA}	6	2.00 ± 0.00^{aB}	5	3.25 ± 0.48^{aB}	4
Limonene	9.36	6.8 ± 0.61^{aA}	10	6.63 ± 1.22^{aA}	8	7.75 ± 1.54^{aA}	8	6.14 ± 1.01^{aA}	7	6.00 ± 0.44^{bA}	7	8.60 ± 2.09^{bA}	6
	9.88	7.3 ± 1.54^{aA}	10	7.60 ± 1.31^{aA}	10	5.80 ± 0.58^{aA}	5	8.20 ± 1.32^{aA}	5	6.00 ± 1.00^{bA}	2	–	
	10.18	6.43 ± 1.54^{aA}	7	6.43 ± 0.48^{aA}	7	6.33 ± 0.21^{aA}	6	6.75 ± 0.75^{aA}	4	–	–	–	–
	10.54	8.00 ± 0.82^{aA}	7	10.50 ± 1.66^{bA}	4	5.33 ± 0.67^{aA}	3	6.50 ± 0.50^{aA}	2	2.00 ± 0.00^{aB}	1	4.00 ± 0.00^{aB}	1
Carvacrol	5.38	9.00 ± 2.94^{aA}	9	8.00 ± 1.15^{bA}	4	5.00 ± 0.58^{aA}	3	4.00 ± 2.00^{aA}	2	4.00 ± 0.00^{bA}	1	6.00 ± 0.00^{bA}	1
	7.90	8.57 ± 1.56^{aA}	7	9.50 ± 1.94^{bA}	4	6.33 ± 0.33^{aA}	3	6.00 ± 0.58^{aA}	3	8.50 ± 2.5^{cA}	2	–	
	10.82	11.90 ± 2.51^{bA}	8	7.40 ± 0.75^{bA}	5	5.50 ± 1.50^{aA}	4	4.00 ± 0.82^{aA}	4	4.75 ± 0.95^{bA}	4	10.67 ± 3.28^{bA}	3
	14.55	8.70 ± 2.59^{aA}	9	12.57 ± 1.88^{bA}	7	6.17 ± 2.45^{aA}	6	7.75 ± 1.03^{aA}	4	14.00 ± 0.00^{dA}	1	–	–
β–thujaplicin	3.35	9.00 ± 2.02^{aA}	10	10.00 ± 0.58^{bA}	7	5.60 ± 0.93^{aA}	5	5.00 ± 1.48^{aA}	5	6.67 ± 1.73^{bA}	3	5.00 ± 1.00^{bA}	2
	5.33	12.10 ± 1.42^{bB}	10	22.00 ± 0.34^{cC}	8	7.00 ± 1.18^{aA}	5	5.75 ± 0.25^{aA}	4	9.67 ± 2.33^{cB}	3	8.00 ± 0.00^{bAB}	1
	7.72	15.00 ± 3.25^{bA}	10	9.14 ± 2.03^{bA}	7	6.17 ± 0.54^{aA}	6	7.00 ± 2.05^{aA}	6	5.67 ± 0.33^{bA}	3	10.33 ± 0.88^{bA}	3
	11.12	10.12 ± 2.15^{bA}	8	9.00 ± 1.46^{bA}	6	7.80 ± 0.49^{aA}	5	7.00 ± 5.00^{aA}	2	25.00 ± 0.00^{dB}	1	–	–

Values presented are mean (± SEM). SEM followed by the same letter (lower case) in the same column are not significantly different ($P > 0.05$) from one another. SEM followed by the same letter (upper case) across the row are not significantly different ($P > 0.05$) from one another. Ten replicates were used per concentration.

[a] Initial preoviposition period i.e. mating to the formation of the first ootheca (start [St] to first [F$_1$] ootheca).

[b] Number of females that produced oothecae.

[c] Inter-oothecae period i.e. period from the hatch of one ootheca to the formation of another (hatch [H$_x$] of x ootheca to the formation [F$_x$] of the next ootheca, with x indicating the number of oothecae).

Table 3. Sublethal effect of essential oil components in superabsorbent polymer gel on the egg incubation periods (day) of oothecae produced by female *B. germanica* during a lifetime

Essential oil Component	Treatment Conc. (mg/g)	First ootheca	n^a	Second ootheca	n	Third ootheca	n	Fourth ootheca	n	Fifth ootheca	n
Control	0	22.00 ± 3.12^{aA}	6	20.63 ± 0.84^{aA}	6	19.17 ± 0.17^{bA}	5	19.00 ± 0.00^{aA}	3	18.00 ± 0.00^{aA}	1
Limonene	9.36	19.75 ± 0.31^{aAB}	8	19.14 ± 1.08^{aAB}	7	18.00 ± 0.52^{aAB}	6	17.17 ± 1.14^{aA}	6	20.80 ± 1.46^{aB}	5
	9.88	20.00 ± 0.82^{aA}	8	19.00 ± 1.18^{aA}	5	19.00 ± 0.58^{aA}	4	20.00 ± 1.00^{aA}	2	–	–
	10.18	18.00 ± 0.91^{aA}	4	19.75 ± 0.95^{aA}	4	17.67 ± 0.88^{aA}	3	–	–	–	–
	10.54	15.75 ± 1.25^{aA}	4	22.50 ± 1.50^{aA}	2	18.00 ± 0.00^{aA}	1	17.00 ± 0.00^{aA}	1	–	–
Carvacrol	5.38	19.60 ± 1.43^{Aa}	5	21.00 ± 4.00^{aA}	3	38.00 ± 0.00^{cA}	1	19.00 ± 0.00^{aA}	1	–	–
	7.90	21.50 ± 4.86^{aA}	6	17.67 ± 0.88^{aA}	3	17.00 ± 0.58^{aA}	3	17.00 ± 0.58^{bA}	3	–	–
	10.82	16.67 ± 2.60^{aA}	3	21.33 ± 0.88^{aA}	3	15.00 ± 0.00^{aA}	1	19.00 ± 0.00^{aA}	1	–	–
	14.55	23.33 ± 1.33^{aA}	3	16.00 ± 0.00^{aA}	1	–	–	–	–	–	–
β–thujaplicin	3.35	19.50 ± 1.96^{aA}	6	18.17 ± 0.60^{aA}	6	17.80 ± 1.36^{aA}	5	20.00 ± 2.00^{aAA}	3	19.00 ± 0.00^{aA}	1
	5.33	18.12 ± 0.79^{aA}	8	18.20 ± 0.66^{aA}	5	18.33 ± 0.33^{aA}	3	16.33 ± 0.88^{aA}	3	25.00 ± 0.00^{bB}	1
	7.72	19.50 ± 0.34^{aA}	6	18.50 ± 0.50^{aA}	4	18.67 ± 0.33^{aA}	3	18.00 ± 0.002^{aA}	2	21.00 ± 2.00^{aA}	2
	11.12	18.33 ± 1.45^{aA}	3	23.00 ± 2.00^{bA}	2	19.50 ± 0.50^{aA}	2	–	–	–	–

Values presented are mean (± SEM). SEM followed by the same letters (lower case) down the column are not significantly different ($P > 0.05$) from one another. SEM followed by the same letters (upper case) across the row are not significantly different ($P > 0.05$) from one another. Ten replicates were used per concentration.

[a] Number of females that produced oothecae.

Chapter 5

Essential Oil Components Disrupt the Gas Exchange Patterns of Insecticide-Susceptible and Multi-Resistant Strains of *Blattella Germanica* (L.) (Blattodea: Ectobiidae)

5.1 Abstract

The discontinuous gas exchange cycle (DGC) is hypothesized to conserve water or facilitate gaseous exchange in hypoxic/hypercapnic environments. Most of the research on essential oil components (EOCs) focuses on demonstrating their toxicity against insect pests such as *Blattella germanica* (L.), none has explored the physiological effects of EOCs on DGC of *B. germanica*. Consequently, we investigated the effects of limonene, carvacrol, and β-thujaplicin on the DGC of insecticide-susceptible (strain S) and multi-resistant (strains D and E) *B. germanica*. Before EOC treatment, there were no significant differences ($F_{2,77} = 0.735$, $P = 0.483$) among strains in the rate of CO_2 emission ($\dot{V}_{CO_2}$; ml h^{-1}), the mean rate of mass loss ($\dot{V}_{H_20}$; mg/h), and CO_2 burst duration (sec). Following EOC treatment, there were significant differences ($P < 0.05$) in these characteristics. The DGC recovery percentage among strains was inversely proportional to EOC dose with the least recovery (20–42.86%) observed in strains treated with carvacrol. A significantly lower proportion ($P < 0.05$) of strain S recovered than the multi-resistant strains. Respiratory (RWL) and cuticular (CWL) water loss increased significantly following treatment with EOCs for all strains. This water loss cost was notable in strains treated with limonene and least observed when treated with β-thujaplicin. This study highlights the physiological effects of these EOCs on the respiratory patterns and water loss of *B. germanica*. Such information improves our understanding and gives cues on the potential application of these EOCs in urban insect pest management.

5.2 Introduction

Coordinated spiracular opening and closing results in several patterns of gas exchange (Marias et al. 2005). Based on carbondioxide (CO_2) emission, these patterns can be classified as cyclic, continuous, and discontinuous gas exchange (Marias et al. 2005, Contreras et al. 2014). The discontinuous gas exchange (DGC) differs from cyclic and continuous gas exchange patterns in that the release of CO_2 and uptake of oxygen (O_2) is at distinct intervals (Shelton and Appel 2000, Vtar et al. 2018). In addition, DGC has periods of CO_2 release that approach zero while CO_2 release for continuous and cyclic are variable and never approaches zero (Lighton 1994). DGC can be broadly categorized into burst and interburst periods along 3 phases. The "C phase" is when spiracles are shut firmly and gaseous (O_2, CO_2, and respiratory water i.e., H_2O) exchange with the environment is negligible. The "F-phase" is when CO_2 and H_2O accumulate within the insect and leak out during the rapid opening and closing of the spiracles. O_2 can diffuse during the "F-phase". The "O-phase" is when spiracles are thrust open and there is a flow of CO_2 and H_2O out of the tracheal system and O_2 into the insect (Kestler 1984, Chown and Nicolson 2004, Quinlan and Gibbs 2006). In locusts, and many other insects, the control of the spiracles is regulated by central interneurons as wells as local effects such as O_2, CO_2, and a toxicant (Harrison et al. 2013).

The effects of a toxicant on the periods and phases of the DGC can be estimated via flow-through respirometry (Schilman 2017). Jõgar et al. (2008) demonstrated that neem extracts induced flaccid paralysis and lethal desiccation leading to water loss at a daily rate of 5.26% in *Pieris brassicae* L., during DGC. Neem caused the abolishment of DGC and increased metabolic rate in the diapausing adult of *Leptinotarsa decemlineata* (Say) (Kuusik et al. 2001). Gibbs (2003) reported a strong correlation between water loss and metabolic rate in *Drosophila spp.*

suggesting that the type of gas pattern exhibited helps to regulate respiratory water loss rate. Of the gas patterns (i.e., cyclic, continuous, and DGC), DGC has received the most attention. To date, eight adaptive hypotheses including reduction in respiratory water loss (RWL) and two nonadaptive hypotheses have been posited to explain the occurrence of DGC in insects, even so, no single hypothesis has received unequivocal support (Mattews 2018, Contreras et al. 2014, Terblanche and Woods 2018)

For relatively small insects like *B. germanica*, maintaining a water balance is critical (Mullins 2015). Mullins (2015) reported that water balance in *B. germanica* is not just maintained by the spiracles but the cuticle, hypopharynx, salivary glands, tergal glands, and hindgut. Bioactive compounds can be employed as stressors to determine how *B. germanica* responds to disturbances in their gas exchange patterns. Stressful conditions, such as insecticide exposure, offer an opportunity to understand how the insect mitigates the effect of DGC disruption (Bustami et al. 2002, Sláma et al. 2007, Contreras and Bradley 2010). The ability of an insect to maintain DGC will likely be based on three factors: the temperature of the external environment, the dose of the stressor – the stressor, in this case, an essential oil component (EOC), and the size of the insect. Of these factors, we manipulated EOC dose to estimate the physiological state of *B. germanica* post stressor exposure.

Toxicity of EOC is usually evaluated in topical application, continuous exposure, or fumigation assays (Philips et al. 2010, Philips and Appel 2010, Oladipupo et al. 2020a, Gaire et at. 2020). This information only provides the gram per bodyweight of an EOC required to initiate kill at a given probability. However, the poisoning process of EOCs is poorly understood (Enan 2001). Several reports indicate that EOCs such as limonene, carvacrol, and β-thujaplicin show inhibitory action on acetylcholinesterases, octopamine receptors, and block synaptic transmissions, respectively (Kostyukovsky et al. 2002, Tong et al. 2013, Abd El-Latif 2015, Politi et al. 2017, Jankowska et al. 2017, da Silva et al. 2018). Additionally, EOCs are possible neuromodulators or neurohormones by modulating levels of dopamine, tyramine, and serotonin in eusocial insects (Enan 2001, Mannino et al. 2018). Little to no information exists on the identification of the target site(s) responsible for EOCs' insecticidal/kill effects. Together with observations on the speed of kill and dying behavior, it is reasonable to assume the EOCs have neurotoxic effects. For example, eugenol can decrease spontaneous spike activity in the dorsal unpaired median neurons of the ventral nerve cord of the American cockroach (Price and Berry 2006) and bind to octopamine receptors in several insects including the German cockroach (Enan 2001, Kostyukovsky et al. 2002). If EOCs trigger loss of spiracular coordination through action on nerves, it may increase the frequency or prolong the opening and closing of spiracles, thereby enhancing further penetration into the insect body (Harak et al. 1999, Sibul et al. 2004). Reid et al. (2017) noted that the toxicity of EOCs against *Dendroctonus ponderosae* (Hopkins) is associated with water loss. However, comparisons of the effects of EOCs on RWL and cuticular water loss (CWL) are lacking. Such information represents a way of understanding the direct detrimental and physiological effects of EOCs.

This study investigated the influence of limonene, carvacrol, and β-thujaplicin on the DGC characteristics and water conservation in *B. germanica* strains. Since the content and composition of cuticles in insects is likely to vary with populations (Blomquist and Bagneres 2010), we compared the effects of these EOCs on the DGC characteristics (i.e., mean $\dot{V}_{CO_2}$ and water loss rates) among an insecticide-susceptible and two resistant *B. germanica* strains.

5.3 Materials and methods

5.3.1 Chemicals

Limonene, carvacrol, and β-thujaplicin (> 95% purity) were purchased from Sigma-Aldrich (St. Louis, MO). Four doses (mg/µl) of each EOC were used based on data from a previous study (Oladipupo et al. 2020b). Serial dilutions of each EOC were made in acetone (Fisher Certified ACS 99.7% purity; Fisher, Fair Lawn, NJ) to obtain the final desired concentrations.

5.3.2 Experimental animals

Adult males of three *B. germanica* strains were used in this study. Males were selected to avoid complications arising from metabolic demands due to oogenesis in females (Dingha et al. 2005) and the molting process of immature. Strain S was an insecticide-susceptible strain collected from a colony maintained in culture at UC Riverside, CA and Auburn University, AL for > 50 yr. Strains D and E were insecticide-resistant strains initially sourced from field populations in Franklin County, NC, and observed to be resistant to different insecticides including permethrin and fipronil (Wu and Appel 2017). Strains D and E were 37- and 52-fold resistant to permethrin, respectively (Wu and Appel 2017). All strains were reared at 28 ± 2 ºC, with 40–55% RH and a photoperiod of 12:12 (L: D) h. They were provided harborage (corrugated cardboard), rodent food (Purina 500l lab diet from Purina LabDiet®, Inc. St. Louis, MO), and clean water weekly. Cockroaches were starved for 2–3 days before the experiments to avoid digestion effects on their metabolic rate during experimental trials (Leis et al. 2016).

5.3.3 Treatments and respiratory measurements

Individuals of each strain were subjected to one of three different treatment groups before respiratory measurements were conducted. Group 1 was the untreated control, group 2 was treated with acetone only, and group 3 was subjected to a dose of EOC. Group 1 measurements were used to determine the optimal period for a given strain to establish DGC for groups 2 and 3. For each strain, individual *B. germanica* were weighed in a pre-weighed and perforated 1.5 ml Eppendorf tube, and placed in an incubator with 100 ml/min airflow to acclimate for two days before an experimental trial commenced.

Flow-through respirometry was used to record the pattern of CO_2 and water vapor emission. For each EOC and strain, nine individuals were used for each dose and the controls (acetone and untreated controls). The respirometer chamber consisted of an 8 cm length of Ynomid Tygon tubing (2.3 mm diameter) housed in a Sable Systems (Sable Systems; Henderson, NV) PT-1 Peltier-effect temperature-controlled cabinet at 25 °C. Dry CO_2–free air was pulled through 1-meter length of copper tubing and across a pre-weighed *B. germanica* enclosed in the tubing. The air was pulled through a Li-Cor CO_2 and H_2O analyzer (LI-6262 or LI-7000; LiCor Inc., Lincoln, NE) at 100 ml/min with a Sable Systems mass flow system (MFS 2).

A baseline measurement of CO_2 and water vapor was recorded for 3 minutes for the empty tubing. The recording was paused and a pre-weighed *B. germanica* was allowed to crawl from an Eppendorf tube into the Tygon tubing and remained motionless for five minutes before resuming the recording. After 40–60 minutes, depending on CO_2 emission pattern, a 1 µl dose of treatment (i.e., acetone for group 2 and EOC for group 3) was topically applied using a 25-µl Hamilton PB600-1 repeating dispenser (Hamilton Company, Reno, NV). Specifically, the Tygon tubing was carefully pierced with the syringe needle and a 1 µl dose (i.e., 2 clicks of the repeating dispenser) of treatment was delivered on the segment between the metathoracic legs of *B. germanica*. The internal diameter and length of the Tygon tubing were such that the cockroaches could not turn to avoid the treatment. The tubing self-sealed upon removal of the needle and remained an airtight barrier to the outside air. The recording lasted at least 180 minutes before the experiment was terminated.

Data from the CO_2 and H_2O analyzer were recorded using Sable Systems data acquisition and analysis software, ExpeData (ExpeData Release 1.9.22; Henderson, NV). After readings were discontinued, *B. germanica* was transferred back to the Eppendorf tube and the final weight was recorded. Data were baseline corrected and converted into either ml (for $\dot{V}_{CO_2}$) or µg (for $\dot{V}_{H_20}$) using a macro (Lighton 2018). Data from those *B. germanica* that failed to exhibit DGC, or exhibited DGC then transitioned to continuous or died after topical treatments were not included. Data were only reported for those that exhibited DGC (i.e., burst and interburst phases) and for those that resumed DGC following DGC loss after topical application. Six cycles of the three phases of DGC were analyzed from each *B.germanica* replicate. DGC characteristics including mean $\dot{V}_{CO_2}$ (ml/min), volume (ml), duration (min), standard deviation, coefficient of variation, and recovery time were recorded for the burst and interburst phases before and after topical treatment. The recovery time represents the period required for *B. germanica* to revert to DGC from other patterns following EOC application. For uniformity, the percent number of *B. germanica* per strain that reverted to DGC following topical application (i.e., recovery percent) was calculated. Mean $\dot{V}_{H_2O}$ (µg/min), its corresponding volume (ml), duration (min), standard deviation, coefficient of variation of $\dot{V}_{CO_2}$, and recovery time were also recorded. We calculated CWL and RWL according to Gibbs and Johnson (2004) and Ciancio et al. (2020). Briefly, we regressed $\dot{V}_{H_2O}$ against $\dot{V}_{CO_2}$ and estimated CWL from the intercept of the plot. Consequenly, RWL was estimated by deducting CWL from the averaged $\dot{V}_{H_2O}$.

5.3.4 Data analysis

Data from ExpeData were processed in Microsoft Excel 2016. The summary of the gas pattern (i.e., discontinuous or otherwise) exhibited by each *B. germanica* strain was calculated in SigmaPlot 14.0 (Systat Software, Inc. 2017). Mean $\dot{V}_{CO_2}$ (ml/min), volume (ml), and duration (min), for burst and interburst phases before and after exposure, were summarized using repeated analysis of variance. For each of these parameters, a paired t-test was conducted to compare before and after each EOC application at each EOC treatment dose. A paired t-test was also used to compare the coefficient of variation before and after the EOC application. CWL and RWL before and after EOC application were similarly compared using a t-test. The effect of strain on these water loss parameters was analyzed using One-way ANOVA. For each strain, mean $\dot{V}_{CO_2}$ recorded before EOC applications were subjected to a one-way analysis of variance (ANOVA). One-way ANOVA was conducted on the mass-loss rate for each dose and strain. Cuticular permeability (CP) was calculated using:

$$\text{CP}=\frac{\text{Total Water Loss }(\mu\text{g})}{\text{Surface Area }(\text{cm}^2)\text{ X Run time (h) X Saturation deficit (mmHg)}}\text{ (Edney 1977).}$$

Surface area was estimated for each cockroach strain using the constant SA = 12.17 $W^{0.63}$ (Simanton, 1933). Where SA = surface area and W= weight of *B. germanica* in grams. T-tests and one-way ANOVA were conducted in R studio version 3.6.1. (RStudio, 2020) The recovery time for each *B. germanica* was plotted against the dose for each strain using SigmaPlot 14.0.

5.4 Results

5.4.1 *Effects of three EOCs on DGC recovery rate and time of insecticide-susceptible and resistant* B. germanica *strains*

Individuals of each strain exhibited all of the three general gas exchange patterns (i.e., DGC, cyclic, and continuous) at different times. For DGC, 53.4% of the susceptible strain exhibited DGC while 59.6% and 57.8% exhibited DGC in multi-resistant strains D and E, respectively. There was no significant difference between the mean body mass of *B. germanica* among strains (43.97–49.16 mg). For each EOC, DGC percent recovery (%) after EOC application was inversely proportional to the EOC dose (Fig. 1). Unsurprisingly, acetone produced the greatest recovery (94–100%) for all strains. The least DGC recovery (20–42.86%) was observed in carvacrol treatments. DGC recovery was comparable in β–thujaplicin (33.3–83.3%) and limonene (25–80%) treatments. Among strains, recovery was strain D > S > E.

For each EOC, the recovery time varied among the three strains (Fig. 2). Acetone treatment had a mean recovery time of 2.36 ± 0.21 min (Fig. 2A). For limonene, the recovery time significantly differed among strains at 9.88 mg μl^{-1} i.e., LD_{30} ($F_{2,10} = 9.386$, $P = 0.005$; range: 1.16–5.05 min), 10.18 mg μl^{-1} i.e., LD_{40} ($F_{2,7} = 19.72$, $P = 0.001$; range: 4.93–11.89 min), and 10.54 mg μl^{-1} i.e., LD_{50} ($F_{2,7} = 47.95$, $P < 0.0001$; range: 9.52–61.48 min) (Fig. 2A). The insecticide susceptible strain took significantly longer ($P < 0.05$), from 2.36 to 50 min, to recover than the multi-resistant strains. For carvacrol, the results were mixed (Figure 2B). Recovery time was significantly different among strains at 5.38 mg μl^{-1} i.e., LD_{20} ($F_{2,10} = 8.469$, $P = 0.007$; range: 2.19–3.18 min) and 10.82 mg μl^{-1} i.e., LD_{40} ($F_{2,8} = 5.089$, $P = 0.04$; range: 5.44–8.95 min). For β-thujaplicin (Fig. 2C), strain S recovered more slowly than strains D and E at 5.33 mg μl^{-1} i.e., LD_{30} ($F_{2,5} = 63.39$, $P = 0.0002$; range: 2.83–6.75 min), 7.72 mg μl^{-1} i.e., LD_{40} ($F_{2,9} =$

70.9, $P < 0.0001$; range: 3.90–11.23 min), and 11.12 mg µl^{-1} i.e., LD$_{50}$ ($F_{2,10} = 57.8$, $P = 0.0002$; range: 2.82–13.83 min). Additionally, differences were observed between the recovery time of the multi-resistant strains at LD$_{40}$ (E > D; $P < 0.0001$) and LD$_{50}$ (D > E; $P = 0.009$).

5.4.2 *DGC characteristics of insecticide-susceptible and resistant* B. germanica *strains*

DGC recordings of susceptible and multi-resistant *B. germanica* strains measured at 25ºC before and after exposure to each EOC are presented in Figures 3–6. Overall, the frequency of DGC increased following topical application of an EOC. In individuals where DGC was restored, a consistent trend was observed with the interruption period (i.e., interruption period ranking was S > D > E) was observed (Figs. 3–6). Characteristics of DGC shown in Table 1 were calculated from 6 DGCs from 3–6 replicates of each strain. Rate of CO$_2$ emission ($\dot{V}_{CO_2}$; ml h^{-1}) between the insecticide susceptible and resistant strains was not significantly different ($F_{2,77} = 0.735$, $P = 0.483$) (Table 1). The CO$_2$ emission rate corresponds to the summation of the burst and interburst phases for each DGC. However, strain E had a significantly longer ($P < 0.05$) interburst duration than the susceptible strain (Table 1). Also, the burst duration generally accounted for a greater percentage (range: 53–61%) of the cycle duration for all strains. Mean $\dot{V}_{CO_2}$ (ml h^{-1}) and mean $\dot{V}_{H_2O}$ (µg/min) together with their corresponding duration (min) and volume (ml) values for the interburst and burst phases were not significantly different ($P > 0.05$) among strains (S1). There was also no significant difference ($P < 0.05$) in the rate of mass loss over time for the susceptible and multi-resistant strains following topical treatment (S2).

5.4.3 *Effects of three EOCs on DGC characteristics of insecticide-susceptible and resistant* B. germanica *strains*

DGC characteristics (i.e., mean $\dot{V}_{CO_2}$, volume, duration, and coefficient of variation) were compared before and after the topical application of an EOC (Table 2). Since the statistical conclusions between before and after topical applications per dose were similar, DGC characteristics compared at LD_{50} were reported. Distinctions were made between the interburst and burst phases. After the topical application of limonene, strain S had significantly increased ($P < 0.05$) CO_2 emission rate, volume, and duration, whereas the resistant strain D showed significantly higher duration, volume, and mean, while strain E had a significantly higher mean $\dot{V}_{CO_2}$ (interburst) and volume and duration (burst) (Table 2). For carvacrol, burst volume and interburst mean CO_2 emission rates and duration significantly increased between the before and after across strains. Interburst and burst volume and duration, significantly increased following β–thujaplicin application for the susceptible and multi-resistant strains (Table 2).

5.4.4 *Effects of three EOCs on respiratory and cuticular water loss in insecticide-susceptible and resistant* B. germanica *strains*

Respiratory water loss (µg/min) following EOC treatment is presented in Figure 7. There was no significant difference (t = -1.602; $P = 0.250$) between respiratory water loss before and after treatment with acetone control for all strains (Fig. 7). Following treatment with limonene, respiratory water loss significantly increased from 0.78 ug min^{-1} in acetone control to 15–31.26 µg/min for strain S and 6.52–28.65 ug min^{-1} for strain E. Significantly higher respiratory water was lost by strain D when treated with 9.88 mg μl^{-1} (i.e., LD_{30}; t = -6.789; $P = 0.021$) and above.

All treatment with carvacrol beyond 5.38 mg µl^{-1} resulted in a significant ($P < 0.05$) increase in respiratory water loss for both susceptible and resistant strains. β-thujaplicin treatment resulted in a significantly higher ($P < 0.05$) water loss in strain S at LD_{40} i.e.,7.72 mg µl^{-1}. β-thujaplicin resulted in a significantly higher respiratory water loss across concentrations against strain E. At LD_{30} i.e., 5.33 mg µl^{-1} (t = -4.211; $P = 0.05$) and 11.12 mg µl^{-1} (t = -10.369; $P = 0.009$), β-thujaplicin resulted in a significantly higher respiratory water loss for strain D.

Cuticular water loss values following treatment with EOCs are presented in Figure 8. There was no significant difference between cuticular water loss before and after topical application of acetone (t = -0.302; $P = 0.791$). For all strains, treatment with limonene at 10.18 mg µl^{-1} and 10.54 mg µl^{-1} signficantly ($P < 0.05$) increased cuticular water loss from 0.81 µg min^{-1} to 5.45–21.23 µg/min. Only the highest dose (LD_{50} i.e., 14.55 mg µl^{-1}) of carvacrol resulted in significantly higher water loss for strain S (t = -10.879; $P = 0.008$) and strain E (t = -7.675; $P = 0.02$). There was no statistically significant difference ($P > 0.05$) between cuticular water loss before and after treatment with carcvacrol for strain D. Significantly higher cuticular water loss were observed at 11. 12 mg µl^{-1} for strain S (t = -5.6878; $P = 0.03$) and at 7.72 mg µl^{-1} for strain D (t = -10.369; $P = 0.009$) following treatment with β-thujaplicin. CP values were 16.79 µg cm^{-2} h^{-1} mmHg^{-1} for strain S while strains E and D had CP values of 18.06 and 16.57 µg cm^{-2} h^{-1} mmHg^{-1}, respectively.

5.5 Discussion

One goal of this study was to describe the physiological effects of three essential oil components (EOCs) on the discontinuous gas exchange cycle (DGC) characteristics of insecticide-susceptible and multi-resistant strains of *B. germanica*. Our results establish that before exposure to an EOC, there was no difference in the rate of CO_2 emission among insecticide-susceptible and multi-resistant strains. This is consistent with findings from other studies on *B. germanica* (Hostetler and Brenner 1994, Dingha et al. 2004, Dingha et al. 2005). Neither was there a difference in RWL and CWL nor the rate of mass loss over duration of each *B. germanica* among strains. Thus, the differences observed on *B. germanica*'s DGC after EOC treatments are caused by the EOCs

The exhibition of the DGC by an insect species can be influenced by several factors including temperature, life-stage, activity, and localized chemical stimuli (Schneiderman 1960, Dingha et al. 2005, Contreras and Bradley 2010). At 25 °C, more than 50% of *B. germanica* exhibited DGCs, regardless of strain. Similar observations were documented in the DGCs of susceptible and resistant populations of *B. germanica* at 10 °C, (Dingha et al. 2004) and 26 °C (Hostetler and Brenner 1994). The rest of the individuals showed either continuous, cyclic, or died after topical application. These data implicate that DGC is an innate process, not just one exhibited during calm behavior (i.e., irregular movement within the Tygon tubing) to influence the ability of *B. germanica* to DGC. The range of metabolic rate ($\dot{V}_{CO_2}$) recorded in this study (0.021 – 0.025 ml h^{-1}) is comparable with other studies on *B. germanica* (Dingha et al. 2004; Dingha et al. 2005). One common explanation for the similarity in metabolic rate in strains regardless of their resistance profile is that insecticide-resistant insects may just have higher levels of detoxifying enzymes without any induction to affect $\dot{V}_{CO_2}$ (Terriere 1983).

Not all *B. germanica* restored DGC following topical application of an EOC. Change in the gas pattern from DGC to either continuous or cyclic in response to exposure to a toxicant is not new (Kestler 1991, Kuusik et al. 2001). We hypothesize that one way via which these EOCs exert their effect against *B. germanica* is through their effects on nerves which in term result in a lack of coordination of spiracular opening and closing thereby disrupting the DGC. Disruption/change in a gas pattern, particularly DGC, is usually accompanied by a surge in the frequency of spiracular opening. Typically, *B. germanica* has 16 spiracles on its abdomen (Dingha et al. 2005). Thus, we hypothesize that the neurotoxic mode of action of these EOCs resulted in the loss of coordination among segments thereby triggering gas pattern change (i.e., DGC to continuous).

But the focus of this study was on the individuals that restored DGC following an EOC treatment. In *B. germanica* that restored DGC, there was an inverse relationship between EOC dose and percent recovery DGC (Fig. 1). In general, fewer *B. germanica* recovered DGC upon exposure to carvacrol. Carvacrol can increase the membrane permeability of epidermal cells leading to more insecticide in the target insect pest (Lambert et al. 2001) supporting the potential of it being employed as a synergist. Second, carvacrol interferes with *B. germanica* and *Drosophila* fecundity (Nesterkina et al. 2020, Oladipupo et al. 2020a). Moreover, carvacrol has been demonstrated to bind to a novel binding site in housefly nicotinic acetylcholine receptors (Tong et al. 2013). This finding provides promising evidence about the possible inclusion of carvacrol in pest management strategies.

As highlighted earlier, our results show similarities in the DGC characteristics between the insecticide-susceptible and multi-resistant *B. germanica* strains before treatment with EOC. Consequently, we evaluated these characteristics after treatment with an EOC. One general trend observed was the increase in metabolic rate of all strains, as measured by $\dot{V}_{CO_2}$, after treatment with EOCs (Table 2). Such a trend is consistent with findings from earlier studies on insects (Kestler 1991, Jõgar et al. 2006) and non-insect arthropods (Gromysz-Kalkowska and Szubartowska 1994). For example, increased metabolic rate in *L. decemlineata* ensued following treatment with a bioinsecticide, Neem EC (Jõgar et al. 2008). In another insect model, only pyrethroid-resistant populations of *Sitophilus zeamais* Motschulsky exhibited reduced $\dot{V}_{CO_2}$ following exposure to clove and cinnamon EOs (Gonzales Correa et al. 2015). We suggest that the difference in $\dot{V}_{CO_2}$ effects between our study and the aforementioned studies following treatment with an EO may be a reflection of the resistance mechanisms of insects used. The insects used in this study are multi-resistant to several classes of insecticides (Wu and Appel 2017, Oladipupo et al. 2020b). Further, our results demonstrate that, from a control standpoint, an increase in metabolic rate vis-à-vis oxygen consumption is likely to drive substantial respiratory water loss (Schilman et al. 2008) and possibly improve insecticide/EO uptake assuming they act as a "semi-fumigant". Thus, it seems reasonable to add desiccation to the effects of these EOCs against *B. germanica*.

There were notable differences in the DGC cycle patterns of the *B. germanica* strains following EOC treatment (see Figures 3-6). First, was an immediate cessation of DGC following EOC treatment. This represents the first visible pathophysiological effects of these EOCs (Sanchez-Arroyo et al. 2001; Jõgar et al. 2008). Such cessation is most likely triggered by neurological/neurotoxic effects of these EOCs against *B. germanica*. Woodman et al. (2008) demonstrated that hyperactivity in cockroaches following a toxicant treatment is due to an increase in the spike frequency of the major central nervous system. This appears to be consistent with the excitatory motion observed during topical application of the EOCs to all the *B. germanica* strains (Oladipupo, personal observation). Besides, certain EOCs act as an excitatory neurotransmitter in insect nervous system (Jankowska et al. 2017). Such an observation makes the gamma-aminobutyric acid (GABA) receptor a realistic candidate/target for limonene, carvacrol, and β-thujaplicin mode of action investigations since in individuals that recovered

DGC, the CO_2 baseline did not return to normal following EOC treatment. Thus, it seems reasonable to conclude that these alterations in DGC rhythms are reflective of the neurotoxic effects of these EOCs.

While there are conflicting opinions about the adaptive significance of DGC in insects (Kestler 1984, Kestler 1991, Jõgar et al. 2006), one unequivocal truth remains: compared to cyclic and continuous gas exchange cycles, the DGC serves as a water-saving mechanism (Kestler 1984, Quinlan and Gibbs 2006, Huang et al. 2015). For example, substantial water loss was recorded in *L. decemlineata*, *Pieris brassicae* (L.), and *Bombus terrestris* L., following the cessation/abolishment of DGC (Jõgar et al. 2008, Schilman et al. 2008, Muljar et al. 2012). In all these examples, the change in the gas exchange pattern was a result of exposure to an insecticide. Thus, the comparison of the respiratory (RWL) and cuticular (CWL) water loss between periods of a DGC-to-DGC abolishment, in our study, offers the chance to evaluate this hypothesis relative to the insecticide resistance profile of *B. germanica*. Dingha et al. (2005) compared water loss rates between insecticide-susceptible and insecticide-resistant *B. germanica* strains during DGC. To the best of our knowledge, our study is the first to compare the period during DGC to the period after DGC abolishment with *B. germanica* of different insecticide resistance profiles.

Our results substantiate previous findings that the cost of DGC loss includes increased respiratory water loss (Kestler 1984, Quinlan and Gibbs 2006, Jõgar et al. 2008, Schilman et al. 2008, Nuljar et al. 2012, Abbas et al. 2020). For example, in the grasshopper *Tmethis pulchripennis* (Serville 1838), lower RWL was recorded when the grasshopper was expressing DGC (Huang et al. 2015). Our data show that following treatment with an EOC, RWL increases. CWL reflects the amount of water loss due to evaporation and is thus unavoidable. The EOCs also influenced the CWL water lost during DGC for the *B. germanica* strains. This suggests that the EOCs can influence the physical parameters of the insect's respiratory system such as spiracular conductance, tracheal volume, and CO_2 buffering capacity (Matthews 2018).

Additionally, in an earlier study using the same *B. germanica* strains, the CP values of strain S and the resistant strains were 3.42 and 2.26 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$, respectively, at 10 ºC. Here, we report a higher value of 16.79 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ for strain S and 16.75 – 18.05 in the resistant strains at 25 ºC after treatment with the EOCs. Taken together, we conclude that the cost of limonene, carvacrol, and β-thujaplicin on *B. germanica* strains includes water loss. This water loss cost was most notable in strains treated with limonene and least observed when treated with β-thujaplicin.

In summary, our data demonstrate that the DGC can be used as a metric to infer the physiological effects of EOCs. These effects are likely to be influenced by the insecticide resistance profile of an insect. In the case of *B. germanica*, one general trend observed was the increase in $\dot{V}_{CO_2}$ of all strains after treatment with EOCs. In individuals that reinstated DGC following EOC exposure, an inverse relationship was observed between dose and DGC recovery rate. The EOCs resulted in significantly higher respiratory and cuticular water loss. Additionally, there was an unexplainable marked increase in CWL and CP after treatment with these EOCs. This water loss cost was most notable in strains treated with limonene and least observed when treated with β-thujaplicin. Among strains, strain E is likely to exhibit the least water loss in comparison to strains S and D. Resultantly, we argue that the physiological cost of these EOCs against *B. germanica* is embedded in water titer imbalance. This study offers insights into the possible trade-off costs of these EOCs on the respiratory physiology of *B. germanica*.

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Table 1. Characteristics (mean ± SD) of the discontinuous gas exchange cycle (DGC) in male susceptible (Strain S) and multi-resistant *B. germanica* strains (Strains D and E) measured at 25 °C before treatment

	Strains		
	S	D	E
Mass (mg)	43.97±3.82a	49.16±3.74a	49.01±3.95a
n*	55	64	59
Rate of burst CO_2 emission $\dot{V}_{CO_2}$ (ml h^{-1})	0.021±0.003a	0.025±0.001a	0.023±0.004a
Burst duration (min)	1.773±0.147a	1.758±0.124a	1.716±0.036a
Burst duration (% of DGC duration)	60.82%	60.85%	53.24%
Rate of interburst CO_2 emission $\dot{V}_{CO_2}$ (ml h^{-1})	0.011±.002a	0.012±.003a	0.008±.001a
Interburst duration (min)	1.143±0.264a	1.131±0.104a	1.507±0.173b
Interbursturst duration (% of DGC duration)	39.20%	39.15%	46.76%
Coefficient of variation	2.253±1.079a	2.114±0.639a	2.468±1.100a

* = the number of *B. germanica* individuals measured exhibiting DGC in strains before treatment. Values were calculated from 6 cycles per individual for each n. Means across rows followed by the same letter are not significantly different at $P < 0.05$.

Table 2. Paired *t*-test comparing the mean (ml h^{-1}), volume (ml), duration (min), and coefficient of variation parameters of discontinuous gas exchange of an LD_{50} dose of essential oil components between susceptible and multi-resistant strains of *B. germanica*

Essential oil component$^{\#}$		**DGC parameter**	**Strain S**			**Strain D**			**Strain E**		
			Before	**After**	**t-value**	**Before**	**After**	**t-value**	**Before**	**After**	**t-value**
Limonene	Burst	Mean	0.023	0.029	-3.488*	0.0240	0.025	-0.587	0.030	0.032	-0.641
		Volume	0.001	0.028	3.997*	0.001	0.045	0.668	0.001	0.054	2.141*
		Duration	2.164	0.811	5.759*	1.952	1.880	0.102	1.885	1.763	2.456*
	Inter-burst	Mean	0.011	0.034	-15.232*	0.013	0.047	-2.856*	0.011	0.015	-2.352*
		Volume	0.001	0.019	4.611*	0.001	0.001	2.713*	0.001	0.027	-0.896
		Duration	1.842	0.600	9.722*	1.076	1.146	2.349*	1.898	1.639	1.885
	Coefficient of variation		2.069	10.540	-2.163	1.563	4.369	-2.970*	3.217	7.933	4.935*
Carvacrol	Burst	Mean	0.0204	0.028	-3.488	0.020	0.027	-3.734*	0.026	0.028	0.391
		Volume	0.001	0.107	3.836*	0.001	0.019	5.548*	0.001	0.037	8.018*
		Duration	1.792	5.223	-2.127	1.823	0.762	6.353*	1.842	1.267	2.106
	Inter-burst	Mean	0.012	0.045	-66.901*	0.010	0.019	-4.363*	0.008	0.017	-14.510*
		Volume	0.001	0.009	-3.965*	0.001	0.006	1.561	0.001	0.019	-4.515*
		Duration	1.858	0.217	2.856*	1.172	0.345	7.369*	1.734	1.211	5.273*
	Coefficient of variation		1.930	6.043	-2.115	2.915	4.943	-3.142	1.921	6.043	-1.489
β–thujaplicin	Burst	Mean	0.028	0.027	1.076	0.047	0.035	-2.039	0.022	0.0264	-5.102*
		Volume	0.001	0.034	4.562*	0.001	0.051	-2.474*	0.001	0.056	-7.326*
		Duration	1.654	1.364	3.404*	1.146	1.319	-2.440*	1.737	2.130	-2.625*
	Inter-burst	Mean	0.019	0.019	0.049	0.018	0.016	1.203	0.011	0.016	-7.211*
		Volume	0.001	0.012	3.336*	0.001	0.015	2.340*	0.001	0.020	-0.316

		Duration	1.172	0.773	6.431*	1.186	0.939	1.964*	1.684	1.237	4.267*
	Coefficient of variation		2.657	4.938	-0.689	1.646	2.560	0.394	2.460	4.880	-2.023

= comparison was made after topical application of 1 µl of LD_{50} of an essential oil component. The t-value (i.e., t) gives an index of the magnitude of the difference between the before and after topical compared values. The *P*-value annotates the statistical significance of such a difference. Consequently, * = *P*-value < 0.05.

Table 3. Comparison of the average water loss (mg min^{-1}) for male susceptible (Strain S) and multi-resistant *B. germanica* strains during DGC and immediately after topical application of an essential oil component

Essential oil component#	**Dose (mg/µl)**	**Strain S**			**Strain D**			**Strain E**		
		During DGC	**After Topical application**	**t-value***	**During DGC**	**After Topical application**	**t-value***	**During DGC**	**After Topical application**	**t-value***
Limonene	9.36	0.002	0.012	-1.384	0.004	0.032	-1.109	0.004	0.018	-6.605*
	9.88	0.005	0.024	-2.227	0.003	0.040	-1.080	0.005	0.010	-0.606
	10.18	0.003	0.036	-1.934	0.003	0.023	-2.117	0.005	0.012	-2.344
	10.54	0.003	0.011	-1.406	0.004	0.008	-0.979	0.004	0.008	-2.344
Carvacrol	3.35	0.013	0.015	-1.529	0.007	0.008	-2.455	0.003	0.011	-4.044
	5.33	0.004	0.008	-2.412	0.007	0.014	-4.004*	0.008	0.020	-1.435
	7.72	0.005	0.010	-3.714	0.007	0.016	-2.042*	0.005	0.011	-2.087
	11.12	0.003	0.010	-37.673*	0.002	0.003	-2.901*	0.003	0.011	-11.242*
β–thujaplicin	5.38	0.004	0.005	-3.373*	0.002	0.007	-3.370	0.004	0.006	-0.807
	7.90	0.003	0.008	-0.875	0.021	0.017	-2.562	0.004	0.005	-1.326
	10.82	0.005	0.007	-1.751	0.002	0.004	-0.476	0.002	0.006	-2.839
	14.55	0.002	0.017	-1.243	0.002	0.017	-11.136*	0.003	0.008	-1.820

Average means were computed 20 minutes during DGC and 20 minutes immediately after topical applications. Means are averages of three-five independent replicates. A paired t.test was used to compare during and after topical application water loss values. The T-value (i.e., t) gives an index of the magnitude of the difference between the during DGC and after topical application values. T-value followed by * is significant (i.e., *P*-value < 0.05).

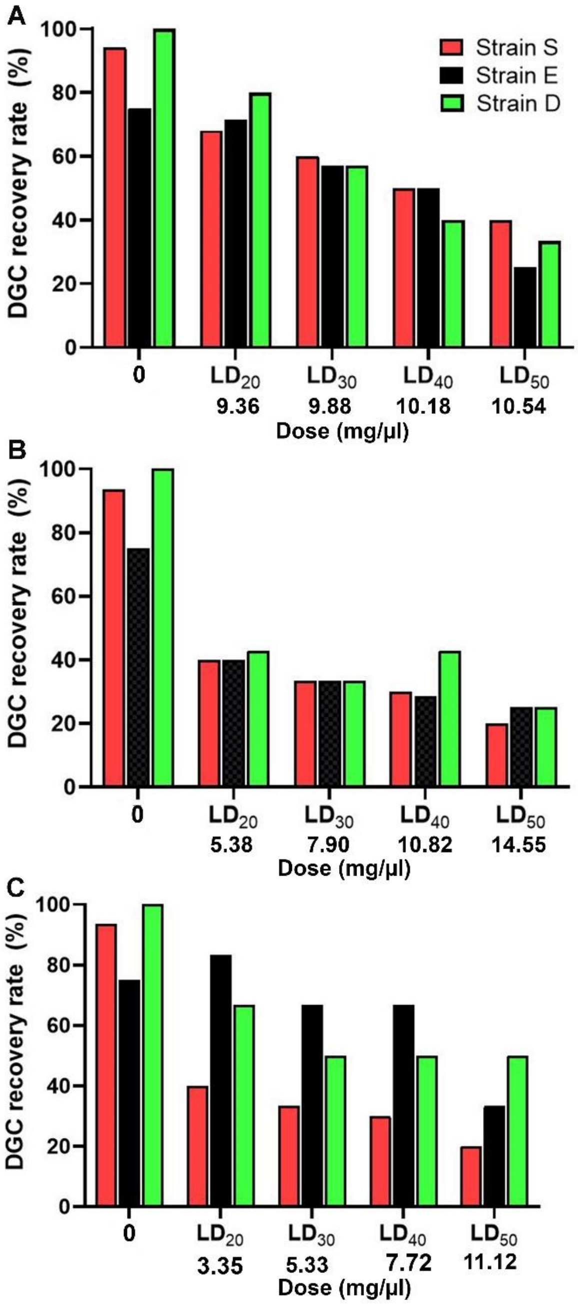

Fig. 1. DGC recovery rate in susceptible (Strain S) and multi-resistant (Strains D and E) strains topically treated with (A) limonene, (B) carvacrol, and (C) β-thujaplicin. N = 135 individuals for each EOC

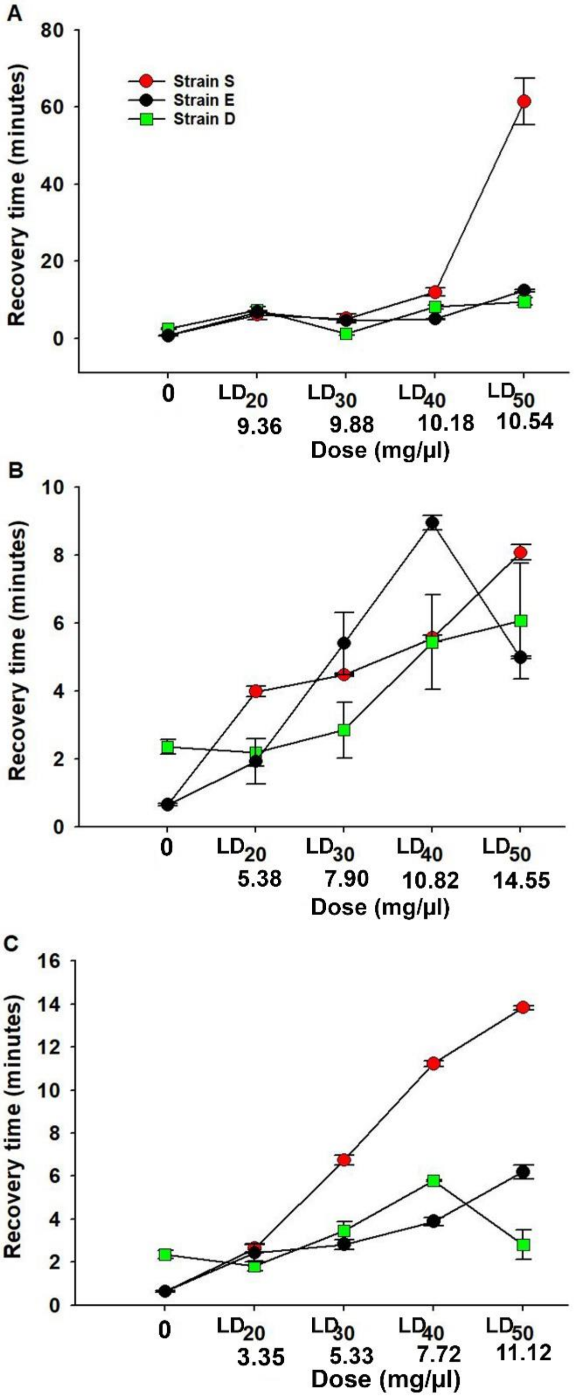

Fig. 2. DGC recovery time in susceptible (Strain S) and multi-resistant (Strains D and E) *B. germanica* strains topically treated with (A) limonene, (B) carvacrol, and (C) β-thujaplicin. N = 135 individuals for each EOC

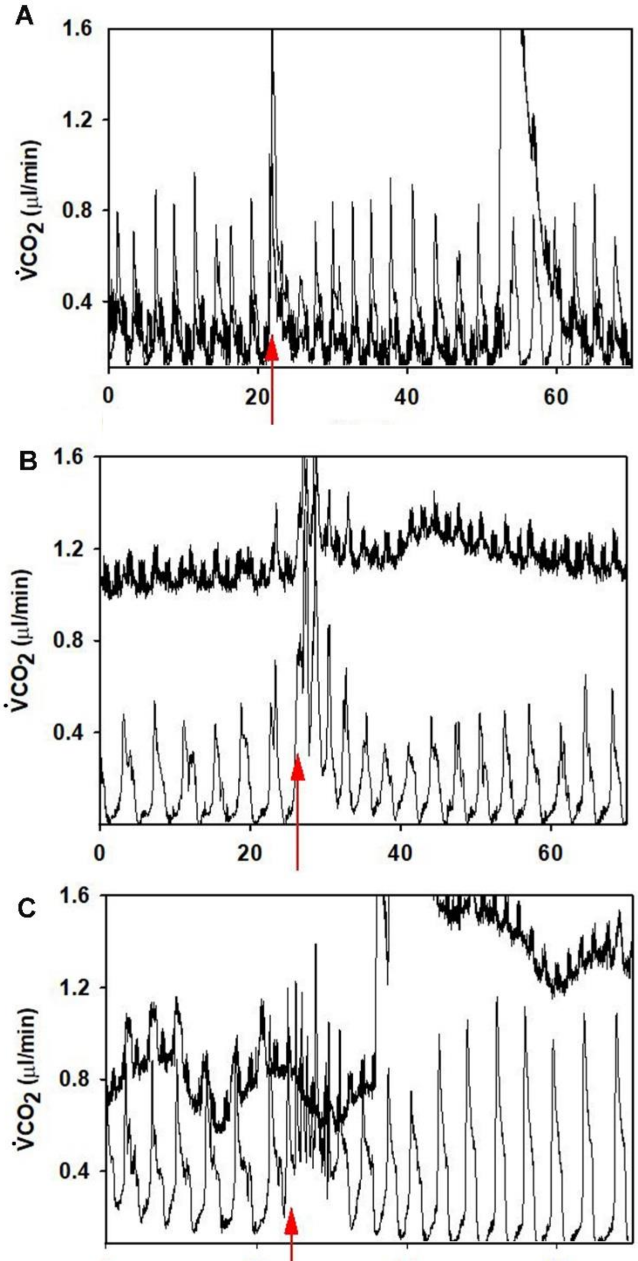

Fig. 3. Typical recording of DGC in a male (A) susceptible and (B-C) multi-resistant *B. germanica* strains measured at 25 °C. The red arrow indicates the topical application of 1 µl of acetone

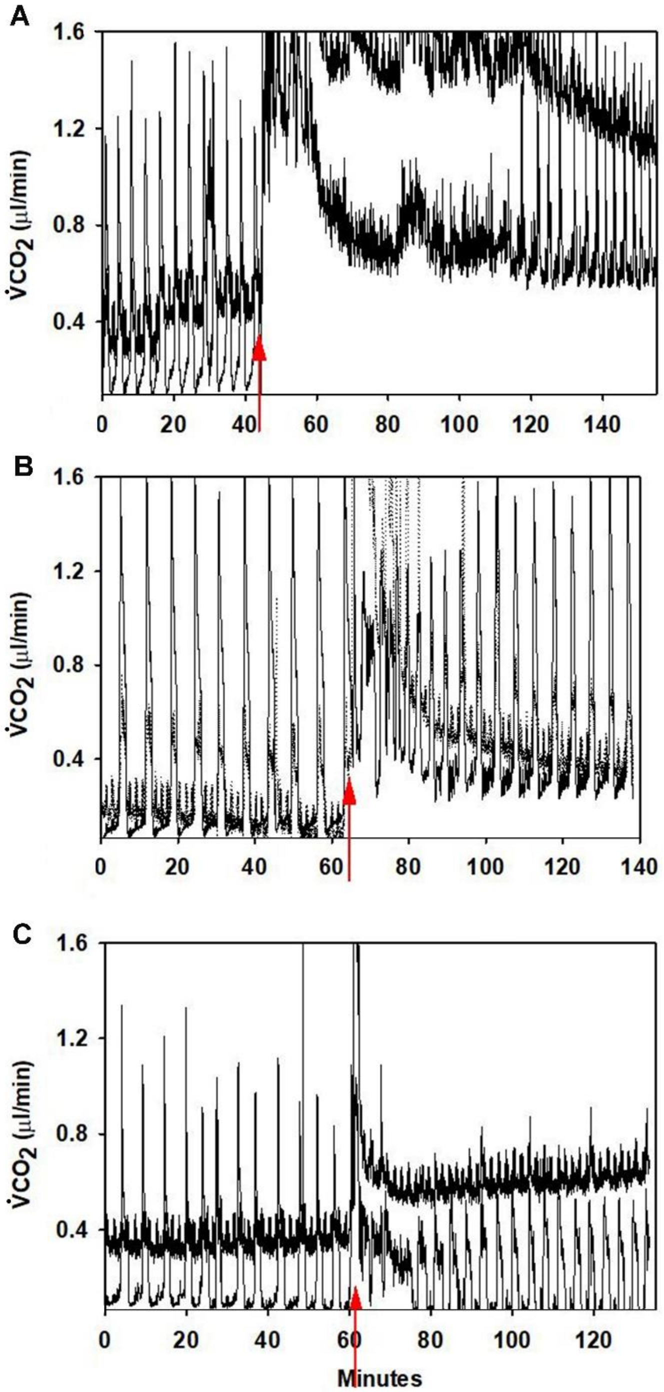

Fig. 4. Effects of topical application of limonene (LD_{50}) on DGC of male (A) susceptible and (B-C) multi-resistant *B. germanica* strains measured at 25 °C. The red arrow indicates the topical application of 1 µl of limonene

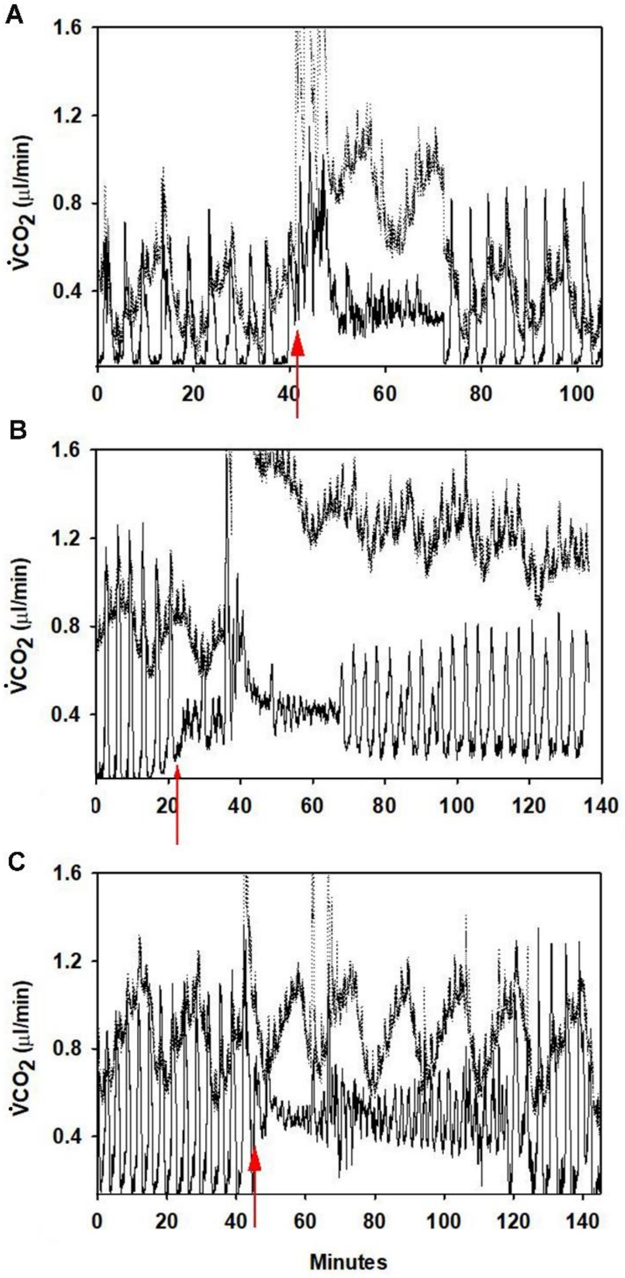

Fig. 5. Effects of topical application of carvacrol (LD_{50}) on DGC of male (A) susceptible and (B-C) multi-resistant *B. germanica* strains measured at 25 °C. The red arrow indicates the topical application of 1 µl of carvacrol

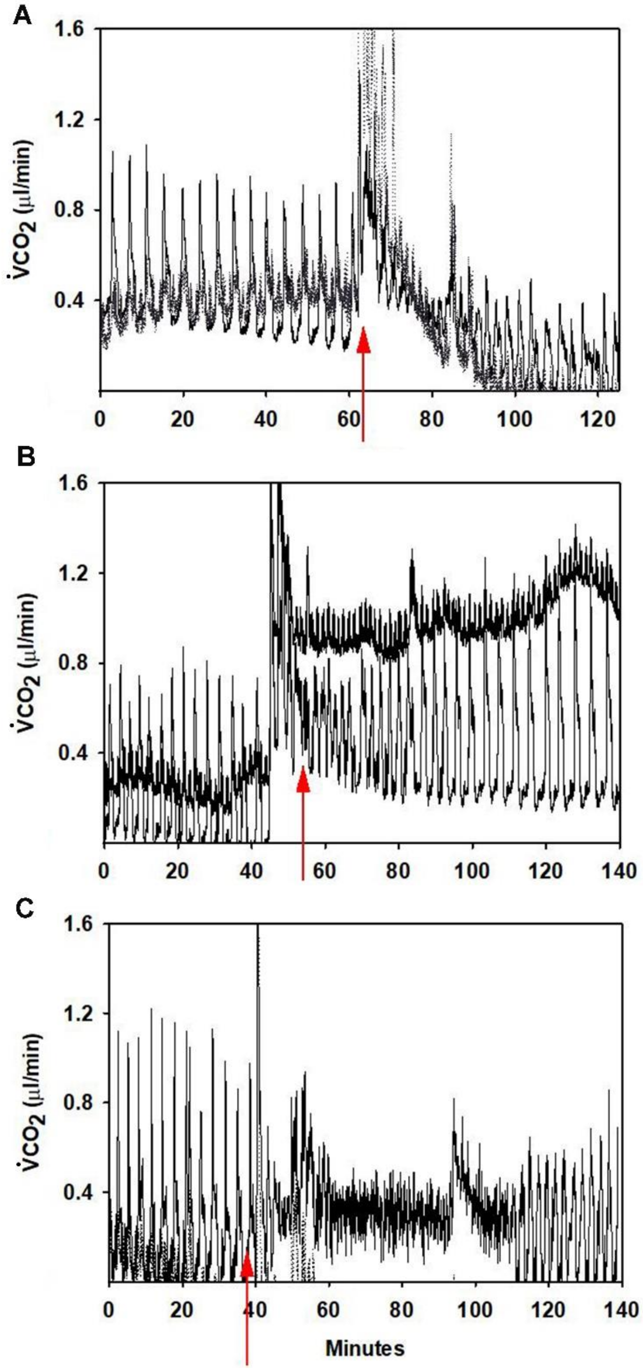

Fig. 6. Effects of topical application of β-thujaplicin (LD50) on DGC of male (A) susceptible and (B-C) multi-resistant *B. germanica* strains measured at 25 °C. The red arrow indicates the topical application of 1 µl of β-thujaplicin

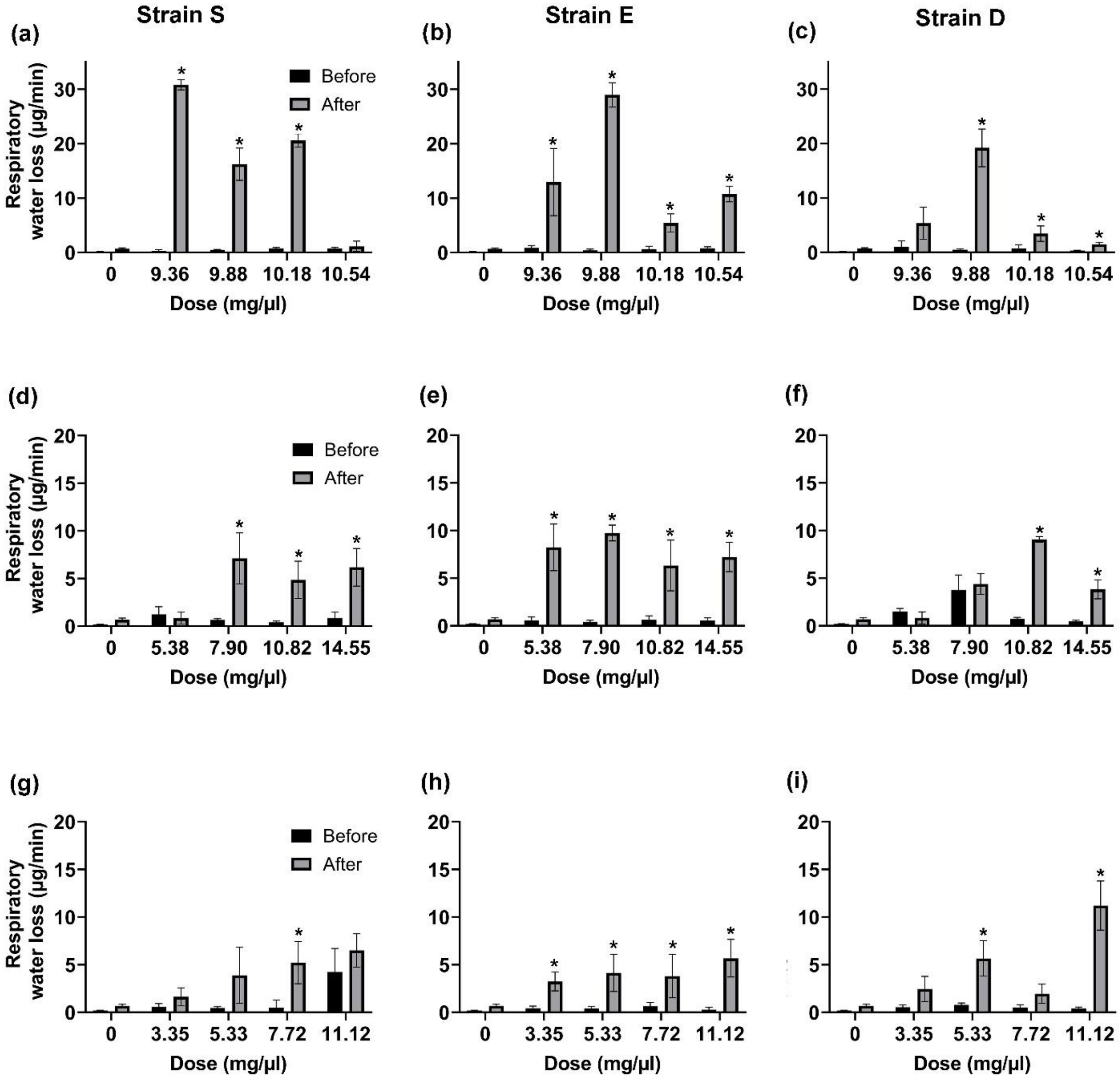

Fig. 7. Effects of topical application of (a-c) Limonene, (d-f) Carvacrol, and (g-i) β-thujaplicin on respiratory water loss (µg/min) during DGC expressed by susceptible and multi-resistant *B. germanica* strains measured at 25 °C. Values are mean ± standard error. * indicates statistical significance at $P < 0.05$

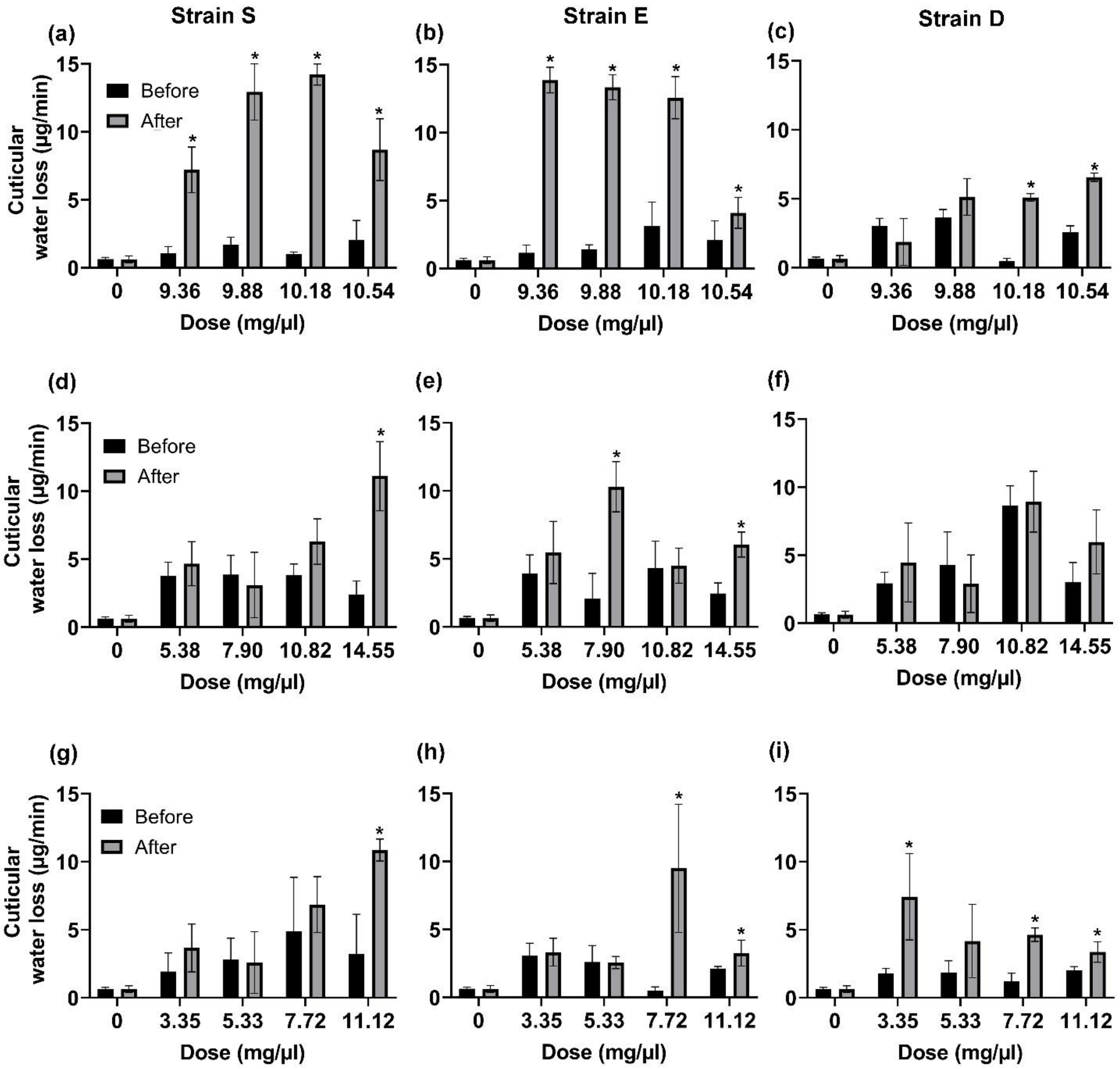

Fig. 8. Effects of topical application of (a-c) Limonene, (d-f) Carvacrol, and (g-i) β-thujaplicin on cuticular water loss (μg/min) during DGC expressed by susceptible and multi-resistant *B. germanica* strains measured at 25 °C. Values are mean ± standard error. * indicates statistical significance at $P < 0.05$

Chapter Six

Why Do Insects Close their Spiracles? A Meta-analytic Evaluation of the Adaptive Hypothesis of Discontinuous Gas Exchange in Insect

6.1 Abstract

The earliest description of the discontinuous gas exchange cycle (DGC) in lepidopterous insects supported the hypothesis that the DGC serves to reduce water loss (hygric hypothesis) and facilitate gaseous exchange in hyperoxia/hypoxia (chthonic hypothesis). With technological advances, other insect orders were investigated, and both hypotheses were questioned. Thus, we conducted a meta-analysis to evaluate the merit of both hypotheses. This included 46 insect species in 24 families across nine orders. We also quantified the percent change in metabolic rates per °C change of temperature during the DGC. The DGC reduced water loss (−3.27 ± 0.88; estimate ± 95% confidence limits [95% CI]; $p < 0.0001$) in insects. However, the DGC does not favor gaseous exchange in hyperoxia (0.21 ± 0.25 [estimate ± 95% CI]; $p = 0.12$) nor hypoxia but did favor gaseous exchange in normoxia (0.27 ± 0.26 [estimate ± 95% CI]; $p = 0.04$). After accounting for variation associated with order, family, and species, a phylogenetic model reflected that metabolic rate exhibited a significant, non-zero increase of 8.13% (± 3.48 95% CI; $p < 0.0001$) per °C increase in temperature. These data represent the first meta-analytic attempt to resolve the controversies surrounding the merit of adaptive hypotheses in insects.

6.2 Introduction

Insects modulate (Oxygen) O_2 and (carbon dioxide) CO_2 by a means of internal air-filled tracheae. The tracheal system ramifies into progressively thinner branches to deliver O_2 and remove CO_2 as a waste product of cellular respiration from every cell in the insect body (Wigglesworth 1990, Gullan 2000). Externally, the tracheae open out as spiracles that are positioned laterally along the insect body. Typically, insects have zero or one pair of spiracles per segment, with a maximum number of 10 pairs on an adult insect (Wigglesworth 1972, Gullan 2000). Some basic variations abound in the number, role, and sophistication of spiracles in insects depending on the species and stage of development (Wigglesworth 1972) For example, adult cockroaches have simple tracheae with valve spiracles located laterally along the abdomen, mosquito larvae have one functional terminal spiracle, and most endoparasitic larvae have a closed tracheal system with cutaneous gas exchange (Gullan 2000). Regardless of the organism and the respiratory medium, gaseous exchange is always through either convection (i.e., bulk flow) and/or diffusion (i.e., movement from a higher concentration to a lower concentration region) (Woods and Smith 2010) This is true because while atmospheric pressure at sea level is 760 mmHg (101.33 kPa), the atmospheric volume of O_2 (~21%) and CO_2 (~0.04%) creates a partial pressure gradient between the atmosphere and an organism's interior. In other words, the partial pressure (PO_2) of oxygen and carbon dioxide (PCO_2) is 1590 mm Hg (21.28 kPa) and 0.30 mmHg (0.04 kPa), respectively. Based on these calculations and the partial pressure difference in insects, it is easy to see why O_2 readily diffuses in and CO_2 diffuses out of any living system. However, insect respiration is not that straightforward. This is because gaseous exchange can be influenced by the environment and metabolic demands. Consequently, insects may employ more than one type of gas exchange pattern. Interestingly, it is not uncommon to have the same insect employ more than one gas exchange pattern over a period (Marias et al., 2005). This begs the question: what advantage could there be to the choice of one pattern and abandonment of another?

To date, most measures of gas exchange patterns in insects rely on the measurement of CO_2 and not O_2, because the latter is more difficult to measure, whereas CO_2 can be measured much more accurately (Quinlan and Gibbs 2006). As such, unsurprisingly, the flow-through respirometry is optimized for CO_2 readings. Hence, the gas patterns in insects are described based on the state (efflux/influx) of CO_2. In any case, there is a consensus on three gas patterns in insects (Fig. 1). A continuous gas exchange in which there is an irregular efflux of CO_2, a discontinuous gas exchange (DGC) in which there is a periodic burst of CO_2 separated by intervals of low/negligible CO_2 release, and a cyclic gas exchange pattern in which there is a regular increase of CO_2 but separated intervals with minimal CO_2 emission are lacking (Kestler 1985, Shelton and Appel 2000, Dingha et al. 2005, Chown 2011, Groenewald et al. 2013). Gaseous exchange in insects is established by inward diffusion (or in some cases convection) of O_2 via the spiracles to the tracheae and cells, and the outward net movement of CO_2 (and water vapor molecules) is often regarded as uncoupling of O_2 consumption and CO_2 emission. Thus, it is the characteristic of the spiracles at a given period that creates the three distinct gas exchange patterns: a discontinuous gas exchange cycle (DGC), cyclic gas exchange, and continuous gas exchange (Duncan 2002)

Of these gas exchange patterns, the DGC has received the most attention, possibly due to the presence of an F-phase and its implications (Contreras et al. 2014) Classical experiments involving the diapausing pupae of the *Cecropia* moth revealed the phases of a DGC (Levy and Schneiderman 1966) It starts with a closed phase (i.e., C-phase), where O_2 consumption by the tissues lowers its endotracheal O_2 partial pressure (i.e., PO_2) such that the organism's total endotracheal pressure is lowered, and the extracellular fluid buffers CO_2 (Figure 1c). When the O_2 level in the organism substantially drops, the spiracles flutter, rapidly opening and closing, allowing the flow of O_2 in the air into the organism. This is the flutter phase (i.e., the F-phase). This continues until the level of CO_2 becomes high in the tracheal system and triggers the spiracular valves to open widely to allow for CO_2 emission and additional O_2 uptake (Marias et al. 2006, Quinlan and Gibbs 2006) This is the open phase (O-phase). The cyclic gas exchange consists of a regular burst of gas exchange and no prolonged C-phase, while an O-phase dominates a continuous gas exchange pattern producing irregular curves (Terblanche and Woods 2018)

As highlighted earlier, the questions surrounding the significance of the DGC are centered on the understanding of the F-phase. For example, the hygric hypothesis argues that the DGC serves to reduce respiratory water loss (Kestler 1985, Levy and Schneiderman 1966). This hypothesis is strengthened by the water and CO_2 retention during the F-phase. It assumes that during the F-phase, in which the spiracles flutter to allow for gaseous exchange, the flow of air is convectional, and thus, only O_2 uptake occurs. If otherwise (i.e., bidirectional), the simultaneous intake of O_2 and emission of CO_2 would exhibit concomitant water loss. This is true because tracheole gases are saturated with water vapor; thus, emitted CO_2 would have incurred a water-loss penalty. Another hypothesis argues that the DGC serves to facilitate gaseous exchange in hypercapnic and hypoxic conditions (Lighton and Berrigan 1995) Again, this hypothesis relies on the F-phase. In this case, it is believed that some insects might find themselves or might predominantly occupy a habitat with high CO_2 (hypercapnic)—as is the case with subterranean termites and ants with nests underground—or low O_2 (hypoxic). Thus, by buffering CO_2, for an extended period, the internal PCO_2 can be high enough so that external PCO_2 and CO_2 emission can follow the concentration gradient by diffusion away from the insect. Similarly, by consumption of O_2 internally, the O_2 becomes lower than external PO_2 and O_2 can easily flow in by diffusion. Interestingly, for this hypothesis to be also true, the F-phase has to be convectional; otherwise, the hypothesis becomes weakened.

Importantly, the three phases of the DGC provide a mechanistic way of comparing and evaluating hypotheses. For example, by comparing cuticular (interburst; CF-phases) to respiratory (burst; O-phase) water loss, the DGC is argued to minimize respiratory water loss (Buck et al 1953, Kestler 1985, Hadley 1994). By modulating spiracular closure sessions, the DGC is thought to enhance gaseous exchange in hyperoxic/hypoxic environments (Lighton and Berrigan 1995) or prevent O_2 toxicity (Hetz and Bradley 2005). Additionally, it is often argued that the DGC is expressed to regulate metabolic demand (Contreras and Bradley 2010, Contreras et al. 2014) but there is no quantification of how temperature influences metabolic rate during the DGC beyond two temperatures. However, we know that metabolic and temperature rates can be driving forces of the DGC (Moerbitz and Hetz 2010)

It is noteworthy to add that other adaptive (e.g., the strolling arthropod hypothesis and oxidative damage hypothesis) and non-adaptive (e.g., the emergent properties hypothesis and neural hypothesis) hypotheses have been posited to explain the occurrence of the DGC (see review by Contreras et al. 2014, Terblanche and Woods 2018). Nevertheless, the focus of this meta-analytic study is on hypotheses that measure CO_2, O_2, and water from rhythmic spiracular closure and opening during the DGC. To date, there is a controversy surrounding the acceptance of these hypotheses from one insect clade to another (see the extensive and excellent reviews by Chown et al. 2006, Schilman 2017, Terblanche and Woods 2018) Such contention may be because quantitative comparisons across clades (i.e., diverse taxa) to allow for a conclusion are lacking. To compare, it might be helpful to utilize an approach robust enough to allow for the synthesis of results across taxa (and/or studies) while maintaining not just the statistical significance but the biological relevance of individual studies. Statistically speaking, such an approach would highlight the magnitude of the finding(s) from each study (regardless of the inference drawn from such data) and resolve to find an overarching theme across studies. Indeed, such a comparison would require the synthesis of results across life stages, species (and possibly geographically isolated species), families, orders, habitats, and experimental conditions. In short, by synthesizing results from published data on the DGC across taxa, one might be able to resolve the conundrum surrounding the adaptive significance of the DGC on a broader scale.

Synthesizing results across studies is not a new approach; narrative reviews essentially do that. However, narrative reviews, at best, gather evidence together and fail to synthesize the evidence transparently and objectively. Thus, it follows that most narrative reviews are not reproducible and bias-laden. To circumvent this, a meta-analytic approach evaluates the estimates of magnitude or effects of interest (i.e., effect sizes) from each study beyond significance testing (as done with a *p*-value) using a quantitative approach (Glass 1976). In other words, a meta-analysis goes beyond the dichotomy of a *p*-value (i.e., reject or fail to reject the null) to determine the magnitude and precision of the estimate (i.e., effect size) from each study and converts this estimate to a standardized metric (Nakagawa and Cuthill 2007, Gurevitch et al 2018). This conversion is critical given the variability in study designs and outcomes towards a given question, and thus, creates a metric to facilitate comparison of outcomes across studies. This makes a meta-analytical approach transparent, reproducible, and updatable. The reproducibility of a meta-analytical study is well established by the reporting guidelines of

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses; http://www.prisma-statement.org/ last accessed 15/12/2022). PRISMA is "an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses" and includes a checklist and a template flowchart to reflect the path an investigator employs to arrive at study selection. In short, a meta-analysis provides a more powerful and less biased means for clarifying, quantifying, synthesizing, and disproving (or confirming) assumed wisdom than do conventional approaches. Moreover, it is an index of the biological importance of individual study as opposed to statistical importance from null-hypothesis testing (Nakagawa and Cuthill 2007). Importantly, a meta-analysis is a powerful tool for evidence appraisal especially when controversies exist.

The meta-analytic design of this study is an attempt to create a broad-scale comparison to evaluate the merit of some of the adaptive hypotheses described to account for the significance of the DGC in insects. We propose that for a given DGC, the metabolic rate ($\dot{V}_{CO_2}$) is most likely an intrinsic component that gives the relevance of one DGC phase to another across different taxa. That is, this component may help answer the question of why insects close their spiracles under a given condition. Additionally, it is pertinent to ask further questions by taking a closer look at insects in general exhibiting a DGC. For example, does rhythmic spiracular closure maintained to reduce respiratory water loss in insects? Does a DGC facilitate gas exchange in hypoxia and hyperoxia? How much does the metabolic rate change with a degree change in temperature for insects breathing during a DGC?

Consequently, the objectives of this study were to (1) evaluate if the DGC serves to reduce respiratory water loss in insects, (2) determine if the DGC facilitates gaseous exchange under chthonic conditions, and (3) quantify the percent change in metabolic rates per °C increase in temperature during the DGC. This study is the first attempt to employ a quantitative meta-analytic approach to evaluate the merit of adaptive hypotheses associated with the DGC across taxa.

6.3 Materials and methods

Study selection for this meta-analysis was done according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA; http://prisma-statement.org/PRISMAStatement/CitingAndUsingPRISMA last accessed 15/12/2022) (Figure 2).

6.3.1 Search strategy

Literature searches were conducted in the English language on 10 January 2020. Searches were conducted using Web of Science and PubMed with the following terms: “gas patterns*”, “discontinuous gas exchange”, “DGC*”, “DGE” “cuticular water loss”, “respiratory water loss”, and “gas exchange patterns”. The title and abstract for each paper (in both search engines) were screened for relevancy. Duplicates and papers that were not DGC- or DGE-specific were removed. A study was selected if it satisfied the inclusion criteria for at least one objective.

6.3.2 Inclusion criteria and data extraction

One criticism of meta-analyses is the number of stringent rules regarding paper selection; thus, we were less stringent with the exclusion criterion, as demonstrated by the variability in treatment types of the selected studies. Papers were selected based on the assessment of the gas pattern by the author as either a DGC or DGE. In order of perceived significance, the coefficient of variation CO_2 emission over the entire DGC, $\dot{V}_{CO_2}$ across DGC phases, the volume of burst CO_2 emissions, the duration of burst CO_2 emissions, the duration of interburst CO_2 interval, and the DGC frequency are the relevant indices (Lighton, personal communication). Given that we were able to find more datasets with $\dot{V}_{CO_2}$, we decided to use this index. Additionally, all selected papers reported the flow rate, experimental temperature—either at a specific temperature or over a range of temperatures (if corresponding metabolic rates were recorded)—mean mass (±SD or SE), and the number of insects investigated (n). To be included in objective 1 (does the DGC reduce respiratory water loss in insects?), a study needed to report mean cuticular and respiratory water loss and some form of measurement variance (SD or SE). Consequently, a comparison was made between water loss during the cuticular phase (evaporative water loss) and the respiratory as a metric to evaluate the water-saving hypothesis of DGC. For objective 2 (what is the role of the DGC in chthonic conditions?), a study needed to investigate the DGC in normal and either hypoxia/hyperoxia/hypercapnia conditions and report the metabolic rate and some form of variance. Where the flutter (F) and closed (C) phases were analyzed separately, the data were combined to generate the interburst.

For objective 3 (how much does the metabolic rate change with a degree change in temperature for insects during the DGC?), a study needed to report the mean metabolic rate, at a minimum, across two experimental temperatures under normoxia. Where applicable, mean metabolic rates and standard errors (SE) were converted to ml g^{-1} h^{-1} and standard deviations (SD), respectively, to allow for comparisons across studies. Metabolic rate data presented as ml h^{-1} were converted to ml g^{-1} h^{-1} by dividing the mean metabolic rate by the mean body mass of insects (grams), while estimates (SE/SD) were generated using a Taylor series expression (see below):

$$\text{Taylor series expression: SE} = \frac{\text{Mean A}}{\text{Mean B}}\sqrt{\frac{V_A}{(\text{Mean A})^2} + \frac{V_A}{(\text{Mean B})^2}}$$

where SE = standard error, Mean A = metabolic rate at ml h^{-1}, Mean B = body mass of insects in grams, and V_A and V_B = variance at A and B, respectively.

Similarly, water loss data were converted to mg h^{-1} to facilitate comparisons across all studies. All data were extracted independently by two researchers (S.O.O. and K.O.O.—see acknowledgments). Data from figures were extracted in R (R Core Team 2018) using the *metaDigitize* package (1.0.0). When dovetailing studies provided insufficient data for inclusion in the meta-analysis (five studies), the corresponding authors were contacted via email for the possibility of providing data; however, only one corresponding author responded, and no additional data were sent.

6.3.3 Statistical analyses

Meta-analysis was conducted in R using the *metafor* (2.1–0) and *meta* (4.11–0) packages. The random-effects model was preferred to a fixed-effect model because of the variability in experimental parameters between studies. The type of data provided in selected studies always influences the choice of effect size. Such data must be computable right from the study and should be easy to interpret. Here, because of the availability of categorical sets of data (i.e., means of groups), variances (i.e., standard deviations/standard errors), and sampling distribution supplied in each study, the Hedges' *g* effect size metric was pertinent. Statistically, the Hedges' *g* is a standardized mean difference that has the same meaning regardless of the study design. Therefore, we can compute the effect size and variance from each study using the appropriate formula, and then include all studies in the same analysis. For objective 1, the Hedges' *g* effect size was used to compare cuticular and respiratory water loss (mg h^{-1}). For objective 2, metabolic rates (CO_2: ml g^{-1} h^{-1}) were compared between normoxia and either hypoxia/hyperoxia using Hedges' g effect size. For objective 3, the effect size from each study was calculated as a function of change (slope; β_1) in respiration per °C increase in temperature. Since studies reported the mean metabolic rate (ml CO_2 g^{-1} h^{-1}) and standard error/deviation of mean rates (σ_M), the standard error of the log-linear model (σ_{LM}) was first calculated using the delta method (Ver Hoef 2012, Heine et al. 2019)

$$\sigma_{LM} = \sigma_M \cdot \text{mean}^{-1}$$

Then, σ_{LM} was used to calculate the sampling variance of the log-linear model slope:

$$\boldsymbol{SE^2} = \frac{(\boldsymbol{\sigma^2}_1 + \boldsymbol{\sigma^2}_2)/(\sum \mathbf{n} - \mathbf{2})}{\sum(\mathbf{n} \cdot (\mathbf{x} - \grave{}\mathbf{x})^2)}$$

where $\sigma^2{}_1$ is $\sum\left((n - 1) \cdot \sigma^2{}_{LM}\right)$, $\sigma^2{}_2$ is $\sum(n \cdot (\ln R - \ln f)^2)$, n is the number of individuals for each mean metabolic rate at a given temperature x, and $\bar{x}$ is $\sum(n \cdot x)/\sum n$. $\ln R$ is the natural logarithm of respiration rates and $\ln f$ is the fitted values of the log-linear model.

The effect size from each study was calculated as a function of change in respiration per °C increase in temperature using the following equation:

$$\text{ES} = (\boldsymbol{e^{\beta_1}} - \mathbf{1}) \cdot \mathbf{100}$$

Variance (V) in effect size was calculated using the delta method:

$$\text{V} = (\mathbf{100})^{\mathbf{2}} \cdot \boldsymbol{e^{2\beta_1}} \cdot (\boldsymbol{SE})^{\mathbf{2}}$$

Since comparisons were made between respiratory water loss and cuticular water loss (objective 1), a negative effect size estimate would support the conservatory role of the DGC, while a positive effect size estimate would support otherwise. Additionally, a negative effect size estimate between either normoxia vs. hypoxia or normoxia vs. hyperoxia (objective 2) would suggest that the DGC serves to facilitate gaseous exchange during chthonic conditions, while a positive effect size suggests no correlation between the DGC and chthonic conditions.

Phylogenetic meta-analyses were completed using the *MCMCglmm* (Hadfield 2010, Hadfield and Nakagawa 2010) and *ape* (Paradis et al. 2004) packages in R studio version 3.6.1. (R Core Team 2018). The *MCMCglmm* was used to create a generalized linear mixed model. *ape* was used for reading, writing, and plotting the phylogenetic tree. Sensitivity analyses were conducted by removing effect sizes that showed negative percent changes. For all objectives, we ran a subgroup analysis by order. The underlying assumptions are that studies within each subgroup (order) do not share a common effect size and that true between-studies variance (T^2) is not the same for all subgroups. Thus, T^2 within each subgroup was computed separately. Forest and funnel plots were either drawn in GraphPad prism (8.4.0), R studio version 3.6.1, or RevMan 5.3 (RevMan 2014). Publication bias was assessed with funnel plot asymmetry and Egger's regression test (Egger et al. 1997). A biased dataset would be asymmetrical (i.e., skewed), while an unbiased dataset would be symmetrical (Egger et al. 1997). In other words, the distribution of data points would be relatively even on either side of the plot. The concept of bias here draws on the adequacy of the sample size in making a reliable precision about the effect size estimate.

6.4 Results

6.4.1 Included studies

The search strategy yielded > 1500 studies on Web of Science and PubMed, respectively (Figure 2). The title and abstract for each paper (in each search engine) were screened for relevancy and 979 papers were downloaded from Web of Science (670) and PubMed (309). After reading each article's abstract, 179 duplicates and 569 papers that were not DGC- or DGE-specific were removed. Out of 231 papers left, 32 papers satisfied the inclusion criteria for at least one objective (see above for a list of objectives). The characteristics of the included studies are summarized in Supplementary Material S1 (hereafter referred to as **S**). Overall, 46 insect species in 24 families in nine orders are represented in the meta-analysis (Obj. 1: five families, three orders; Obj. 2: six families, four orders; and Obj.3: 13 families, nine orders).

6.4.2 Objectives

6.4.2.1 Objective 1: Does the DGC reduce water loss in insects?

Objective 1 incorporated seven studies with 42 sets of effect sizes between respiratory and cuticular water loss (mg h^{-1}) measured during the discontinuous gas exchange cycle (DGC) in insects. Among these studies, 17 species of six families in three orders (Blattodea, Coleoptera, and Orthoptera) were represented. Although there were studies on hymenopterans that estimated water loss, there were no comparable data based on this study inclusion criterion to rationalize inclusion into this meta-analysis. To reiterate, a study needed to report mean cuticular and respiratory water loss and some form of measurement variance (SD or SE) to satisfy inclusion. Our result showed that the DGC significantly reduce respiratory water loss (−3.27 ± 0.88; estimate ± 95% confidence interval [95% CI]; $p < 0.0001$) in insects (**S2**). Pooled effect sizes (Hedges' g) ranged from −4.15 to −2.38. When outliers were identified and removed, the DGC was still shown to reduce respiratory water loss (−3.80 ± 0.54; estimate ± 95% CI; $p < 0.0001$, $I^2 = 38.4\%$) (**S3**). Heterogeneity or between-studies variance in the model was high ($I^2 = 91\%$). To explain heterogeneity, a subgroup analysis was conducted, and between studies, variation was 43%, 43%, and 92% for Blattodea, Coleoptera, and Orthoptera, respectively (Fig. 3). The subgroup analyses showed that the DGC is extremely important for Coleoptera ($p = 0.02$) and

Orthoptera ($p = 0.01$) compared with Blattodea ($p = 0.19$). Publication bias was estimated using the funnel plot, Egger's test, and the trim-and-fill method. Funnel plot showed a slight skewness of data to the left, while Egger's test (intercept = −3.92; confidence interval = −1.37; t = −5.95; $p < 0.05$) showed bias, suggesting negative results may be under-reported (Fig. 4).

6.4.2.2 *Objective 2: What is the role of the DGC under chthonic conditions?*

Does the DGC facilitate a gaseous exchange under hyperoxia and hypoxia? This question was asked in studies where either normoxia (~21% O_2) vs. hyperoxia (~41% O_2) or normoxia vs. hypoxia (~10% O_2) was investigated in insects exhibiting a DGC. The normoxia vs. hyperoxia questions was found in seven studies, with 29 effect sizes distributed in seven species in six families of four orders. Overall, the DGC was not maintained under hyperoxia (0.21 ± 0.25; estimate ± 95% CI; $p = 0.12$) (**S4**). The between-study variance was low ($I^2 = 0\%$). However, a subgroup analysis (by order) indicated that the DGC facilitated gaseous exchange in dipterans (0.43 ± 0.34; estimate ± 95% CI; $p = 0.01$), but not in blattoids ($p = 0.89$) or orthopterans ($p = 0.57$) (Fig. 5a).

Similarly, the DGC does not facilitate gaseous exchange in hypoxia (Z = 2.05; 0.27 ± 0.26; estimate ± 95% CI; $p = 0.04$); rather, it favors normoxia in insects (**S5**). This estimate had a moderately low heterogeneity ($I^2 = 18\%$). Subgroup analysis suggested that during normoxia, the DGC was maintained in dipterans (0.34 ± 0.35; estimate ± 95% CI; $p = 0.05$) and blattoids (1.45 ± 01.26; estimate ± 95% CI; $p = 0.02$) (Fig. 5b). Funnel plot analysis showed fairly even symmetry in hyperoxia and hypoxia studies (Fig. 6).

6.4.2.3 Objective 3. How does the metabolic rate change with respect to temperature?

How does the metabolic rate change with respect to temperature? This was estimated in 18 studies with 30 effect sizes distributed in 23 species in 12 families of nine orders, estimating the metabolic rate across a minimum of two temperatures for a single species exhibiting a DGC. The effect size (slope) of the log-linear model gives an index of the percent change in the metabolic rate per °C increase in temperature [29]. With the inclusion of order phylogeny, family, and species as random effects, the model reflected that the metabolic rate exhibited a significant, non-zero increase of 8.13% (± 3.48% 95% CI; $p < 0.001$) per °C increase in temperature (S6). Order-level relatedness with corresponding meta-analytical means and 95% CIs are shown in Fig. 7.

6.5 Discussion

It was the early description of discontinuous gas exchange cycle in lepidopterous insects (particularly pupae) that underpinned the roles played by spiracles and the tracheal system as the sites for modulating the release of CO_2 (Buck et al. 1953, Schneiderman 1960, Levy and Schneiderman 1966). For example, a significant proportion of 90% of metabolic CO_2 accumulated within *Cecropia* pupae is expelled through the spiracles when they open briefly, and the rest is lost through the cuticle when the spiracles are closed (Levy and Schneiderman 1966). Further studies led to the conclusion that the regulated opening and closure of the spiracles also reduces respiratory water loss (i.e., the hygric hypothesis; (Buck et al. 1953, Kestler 1985) and enhances gaseous exchange in hyperoxia/hypoxia environments (i.e., the chthonic hypothesis; (Lighton and Berrigan 1995). As advances in technology appeared – from a shift in manometric technique, electronic microbalance, and mass loss technique to flow-through respirometry—and more insect orders were investigated, hygric and chthonic hypotheses were questioned (Dingha et al. 2005, Gibbs and Johnson 2004, Lighton and Turner 2008) and competing hypotheses arose (Chown et al. 2006, Schilman 2017). As pointed out by Marias et al. (2005), an idiosyncratic feature of the experiments from which competing hypotheses arose is that these studies are based on "small-scale manipulative experiments and closely related species". Hence, comparison across multi-order levels is pertinent to evaluate the broader merit of these hypotheses. Consequently, the goal of this meta-analysis was to evaluate hypotheses by accounting for the outlier effect and weighing the findings from each study to understand the dominating or prevailing role of the DGC across insect orders. After all, insects are uniquely different in lifestyle, biology, living habitats, etc., so the DGC roles will likely vary among species, families, or orders.

The first question asked by this meta-analytic study was "Does the DGC reduce (respiratory) water loss in insects?" The focus here was the direct comparison of respiratory water loss to cuticular water loss. The meta-result provided strong support that the DGC serves to reduce respiratory water loss in insects, especially in the orders Coleoptera and Orthoptera. This is interesting and informative because this is a result obtained from another broad-scale evaluation of the hygric hypothesis. Although not a meta-analytic evaluation, White et al. (2007) performed the first broad-scale phylogenetic experimental evaluation on the veracity of the hygric hypothesis. The authors found strong support for the water conservatory role of the DGC. Mechanistically, just before the burst phase, there is a build-up of CO_2 in the tracheal. Once the CO_2 reaches its maximum critical level, the spiracles open to allow for gaseous exchange with the environment (Quinlan and Gibbs 2006, Lighton 2007). A closer look at this process lends further credence to suggest that spiracular closure for a prolonged period is most likely adaptive to prevent water vapor loss (Lighton 1992).

Consequently, the question is why is the hygric hypothesis of the DGC unsupported by data from some few-species studies and/or insects inhabiting dry environments? A closer look at the effect size from each study from the overall forest plot (S2) showed that 74% of the effect sizes agree with the hygric hypotheses. Meanwhile, the subgroup analysis (Fig. 3) showed skewness in the available literature for insect orders. Intriguingly, when a modest random literature search (i.e., looking through random articles on PubMed and Web of Science) was conducted on hypotheses refuting the hygric hypothesis, most were on studies on hymenopterans—there were no comparable data based on the inclusion criterion of this study to rationalize inclusion into this meta-analysis. On the one hand, Lighton and Turner (2004) observed the correlation of events occurring in ants during DGC and DGC abolishment and outlined that the abolishment of the DGC does not influence water loss rates in ants. After all, water loss through the cuticle predominates total water loss in hymenopterans Lighton and Turner (2008). Moreover, cuticular water loss occurs during the interburst phase, which makes up more than 75% of a given ant’s DGC (Lighton 1996) and an ant’s cuticle is characterized by extremely low cuticular permeability, low spiracular conductance, and extremely low respiratory water loss rates (Lighton 1990, 1996). On the other hand, Zachariassen (1996) argued that even such a low water loss rate is an important “avoidable” cost to insects adapted to dry environments. Or perhaps, they have other “easy” ways to deal with water loss. Finally, there is another important question that no study, to the best of our knowledge, addresses; notably, how often does an insect exhibit a DGC during the day? Yes, the DGC serves to reduce respiratory water loss, but if an individual only does it a few minutes a day, how relevant would that be?

Beyond statistical bootstrapping, why does the hygric hypothesis fail for xeric insects and/or insects with low cuticular permeability? Perhaps this hypothesis fails because of the nature of the question being addressed and the simplistic view with which the objective is viewed. For example, arguments such as the abandonment of the DGC in conditions where water loss restriction is pertinent (Hadley and Quinlan 1993, Quinlan and Hadley, 1993, Rourke 2000) and the insignificant proportion of respiratory water loss to total water loss (Edney 1977, Lighton 1992, Hadley 1994, Chown 2002) are sometimes used to discredit the water conservatory role of the DGC. In arguments like these, what is sometimes not considered, as Chown (2002) put it, is the absence of the null hypothesis on what the proportion of respiratory water loss to total water loss should be? Now, even if respiratory water loss contributes a proportion to the total water loss of an insect, by coordinating the spiracles, the insect stands the chance to arguably minimize this trans-spiracular water loss rates (Lighton 1988) The cuticular water loss modulation may be beyond such an insect. Of course, this is not absolute. For example, the American cockroach, *Periplaneta americana* L., can rapidly reduce its cuticular water loss (Noble-Nesbitt et al. 1995) Interestingly, the DGC reduces water loss in comparison to other gas patterns, corroborating the assertion that the DGC is likely maintained to minimize “avoidable” water loss for xeric insects and/or insects with low cuticular permeability. Compared to mesic insects, xeric insects have cuticular permeabilities in half the range of those recorded in the former (Edney 1977, Lighton and Feener 1989). Conversely, mesic insects would lose water more rapidly than xeric insects. Thus, any physiological or behavioral mechanism to minimize respiratory water loss is likely to be adaptive to xeric insects, even if such an act is not entirely consistent for mesic insects. This is probably why ants and other xeric inhabitants would probably still exhibit a DGC. Moreover, insects adapted to different environments will show a remarked difference in their ability to tolerate (desiccation tolerance) and resist (desiccation resistance) water loss (Chown 2002) Taken together, these observations caution against discrediting the water conservatory role of the DGC as not a water-saving mechanism. More importantly, it serves as a guide to interpreting the role of the DGC for any given insect species, as this is likely to go beyond spiracular closure and openness, but correlated with body mass, habitat characteristics, cuticular permeability, insect taxa, and metabolic rate (Zachariassen 1996, Addo-Bediako et al 2001, Chown 2002).

The second question asked was “Does the DGC facilitate a gaseous exchange under hyperoxia or hypoxia?” In other words, is it safe to conclude that the DGC facilitates gaseous exchange in chthonic environments? The meta-result provided no support for this hypothesis; rather, it suggested that the DGC is only maintained during normoxia. This conclusion is unsurprising given the characteristics of the studies included in this objective. The selected articles included research conducted on mostly pupa and adult stages of insects. To establish the adaptive significance of spiracular closure during the respiratory gaseous exchange in insects, Schneiderman (1956) noted that oxygen enters the trachea at many times the rate of carbon dioxide (due to simple diffusion: the concentration of O_2 in the air is 20.95%, whereas the concentration of CO_2 is 0.04%) when the spiracles are closed in *Cecropia* pupae. Hence, both periods of spiracular closure and opening will offer little resistance to oxygen entry, if any, during hyperoxia (high oxygen) or hypoxia (low oxygen relative to normoxic conditions). Similarly, the argument is that diffusion of CO_2 away from the insect body can only occur if there is a diffusion gradient between “neat air” and “expelled air”. Therefore, insects “hold their breath” and build up a high concentration of CO_2. However, when the spiracles open, the CO_2 escapes from the body. Moreover, the chthonic hypotheses have been demonstrated not to lower the ratio of respiratory water loss to CO_2 release (Kainwisher 1966, Gibbs and Johnson 2004, Heinrich and Bradley 2014). In short, as suggested by this meta-result, the DGC might have no role in supporting the chthonic hypothesis.

Therefore, does the possibility exist that the hygric and chthonic hypotheses are mutually exclusive, or can the DGC serve two or more adaptive functions at the same time? Based on the available evidence, the DGC can, potentially, serve more than one adaptive function. Whether or not these functions can occur at the same time remains unclear. For example, Schilman et al. (2017) recorded a peak in respiratory water loss after ants were placed in anoxia conditions. Similarly, a substantial increase in water loss occurs after hypercapnia was used to induce a spiracular opening (Edney 1977, Lighton 1996, Chown 2002). Within the same colony, ant castes may exhibit different gas patterns depending on the habitat characteristics and caste roles (Lighton 1988, Lighton and Berrigan 1995, Vogt and Appel, 2000) For example, queen ants are reserved in underground chambers that are likely to have a low O_2/high CO_2 influx. The worker ants are not as restricted, constantly moving between the underground chamber and the outer surface (normoxia) for colony duties. In this type of scenario, Lighton and Berrigan (1995) noted that the gas patterns were remarkably different between queens and workers. Hence, for the queen, the DGC is most likely employed during anoxia to “firstly” facilitate gaseous exchange before “secondly” minimizing respiratory water loss (Schilman et al. 2005). For the worker that forages, the DGC would most likely be employed to reduce transpirational water loss rates (Lighton 1988) given external conditions. One cannot but wonder, could multiple “small adaptations” lead to or reinforce the DGC?

The third question asked by this meta-analytic study was “How much does the metabolic rate change with a degree change in temperature (°C) for insects breathing during the DGC?” Insect metabolic rates can be affected by several factors, including temperature, reproduction, and feeding (Waters and Harrison, 2012; Henrich and Bradley, 2014), but no attempt has been made to scale how metabolic rate changes per unit increase in temperature for insects during the DGC. Mechanistically, the DGC is initiated by the interburst phase, where uptake of O_2 occurs in endotracheal cells with simultaneous catabolic production of CO_2 that accumulates in the hemolymph. O_2 pressure in the endotracheal system reaches a critical setpoint and the insect's spiracular muscles become inactivated due to CO_2 build-up to allow for air outflow (i.e., burst phase) (Lighton 1996, Quinlan and Gibbs 2006) Thus, a scaling metabolic rate ($\dot{V}_{CO_2}$) with temperature may explain how O_2 uptake and CO_2 emission in insects change in response to temperature (Waters and Harrison 2012). Such information can explain how the metabolic rate scales to thermal sensitivity (Irlich et al. 2009) how the DGC controls the rate at which an insect transforms energy and materials (Gillooly et al. 2001) and how temperature influences the rate of CO_2 emission in insects. We made this scaling using slope instead of temperature coefficient (i.e., Q_{10}) values. The comparison of studies using slope is advantageous over common Q_{10} values in two ways. It can be used to compare metabolic rate across more than two temperatures and its interpretation does not require reference to other Q_{10} values (Heine et al. 2019). Irlich et al. (2009) conducted a meta-analytic evaluation of metabolic-rate temperature relationships on a global level (i.e., irrespective of the gas pattern) in insects. Effect sizes were calculated from 37 families distributed in nine orders. Like this meta-analytic study, Irlich et al. (2009) utilized the slope of the metabolic rate temperature but described their results in terms of mean activation energy of the respiratory complex (0.62 eV). Activation energy is an index of temperature dependence term of the metabolic theory of ecology (Gillooly et al. 2001) This study estimated effect sizes from 18 studies, with 30 effect sizes distributed in 23 species in 12 families of nine orders. The meta-result in this study indicates that metabolic rate exhibits a significant, non-zero increase of 8.13% per °C (a Q_{10} value of 2.02) increase in temperature during the DGC. Further understanding is required to establish the link between mean activation energy and slope. It may also be informative to consider the 8.13% per °C increase in temperature in the context of ecological implications. Schilman et al. (2017) discussed how scaling the metabolic rate with the temperature rate of an insect can be factored into mathematical models to predict the vectorial capacity in propagating diseases. For example, an increase in the metabolic rate could drive catabolism in insects, thereby accelerating the rate of feeding and development. Such an occurrence would increase the burden of agricultural pest insects on crops. Even so, using $\dot{V}_{CO_2}$ as a proxy for the metabolic rate across species has its limitations. First, $\dot{V}_{CO_2}$ is less accurately translated to energy metabolism units and subject to give false signals in the presence of an acid-base imbalance (Lighton 2008). Moreover, the energy equivalence of $\dot{V}_{CO_2}$ varies with respiratory quotient (RQ). Interestingly, RQ can also vary with temperature and from one species to another (Leis et al. 2016)

It is known that an increase in temperature can trigger a shift in gas exchange patterns (Dingha et al. 2005, Contreras and Bradley 2009). However, that is not the case here, as all data were retrieved from insects that breathe discontinuously over acute temperatures (i.e., a range of temperatures). As highlighted by Terblanche et al. (2010) an acute increase in temperature is directly proportional to the metabolic rate and a given DGC frequency. Thus, one can assume that such a change in the cycle frequency is likely to hold important implications for water balance. While an inverse relationship between the metabolic rate and temperature is well documented in insects across all gas patterns (see Terblanche et al. 2010 and references therein), our results show that metabolic rate exhibits an 8.13% per °C increase in temperature, at least for insects breathing discontinuously. Although not directly related, another meta-analytic study found that copepods respiration increases by 7% per °C increase in temperature (Heine et al. 2019). Thus, we suggest that this range reflects the general characteristics of arthropod ectothermic poikilotherms. Considering that the DGC is mostly exhibited by quiescent insects, and an increase in metabolic rates predates water loss (Duncan et al. 2001, Heinrich and Bradley 2014). Further clarity needs to be sought as to what the adaptive significance and or implication of this metabolic rate increase is to insects. On the one hand, Terblanche et al. (2010) evinced that water loss rates were reduced in response to the acclimatization of high temperatures. On the other hand, our result here may inflate that observation. Understandably, the work by Terblanche and colleagues Terblanche et al. 2010 was conducted on a single dung beetle species, while this is a realization from broad-scale studies.

Finally, and this goes for all the adaptive hypotheses suggested to explain the significance of DGC, the concept of adaptation, even though suggested/mentioned, is often overlooked. This is not the case within the Darwinian concept. The Darwinian concept of adaptive-*ism* argues that these traits must give or make the insect better able to survive and reproduce compared to others that lack those traits. Importantly, these traits must be heritable (Darwin 1859). First, no study has been conducted to investigate the genetic relevance (basis) of gas pattern respiration. After all, for it to be adaptive, there must be a genetic basis. Second, to the best of our knowledge, no study exists comparing the relevance of the DGC on a large scale to the biological fitness of insects (of course, this would need to be defined using a heuristic approach). Studies involving *Drosophila melanogaster* Meigen have demonstrated the capacity of desiccation-resistant populations to evolve and recover from the effects of desiccation at a rate more than non-desiccant-resistant populations (Folk and Bradley 2004). By extension, such a distinct advantage should be sought for insects that make use of the DGC and those that do not.

It is noteworthy that the three questions asked in this meta-analytic study combined data from insects with varying life stages, body masses, treatment types, number of spiracles, and habitats (S1). While the model for the third question accounted for these nuances, we exercise caution in interpreting these data as absolute. We have only presented a holistic approach to solving the significance of the adaptive hypotheses posited to explain DGC occurrence in insects. Understandably, there are a few ways to circumvent these inherent variabilities—all of which would require data that are lacking. One way would be to design small-scale experiments involving the same technique/protocol and environmental parameters for each insect order/group of species. Thereafter, a meta-analysis can be sought.

As pointed out earlier, the skewness of the available data in this meta-analytic study to include nine out of the possible 31 insect orders may represent one major limitation to the interpretations from this study. To compare across a phylogenetic broad-scale study, Marias et al. (2005) and White et al. (2007) had to provide new information by conducting experiments of orders unavailable in the literature. Such an approach is beyond the scope of any meta-analytic study. It is possible that upon the availability of data from other orders, the conclusions may change. This possibility remains yet unseen, and on the premise of the available literature, the meta-result indicates three conclusions; (1) DGC serves to minimize respiratory water loss, (2) DGC does not facilitate gaseous exchange in hyperoxia/hypoxia, and (3) the metabolic rate exhibits a significant, non-zero increase of 8.13% per °C increase in temperature during DGC. These data represent the first quantitative meta-analysis attempt to resolve the controversies surrounding the merit of adaptive hypotheses in insects.

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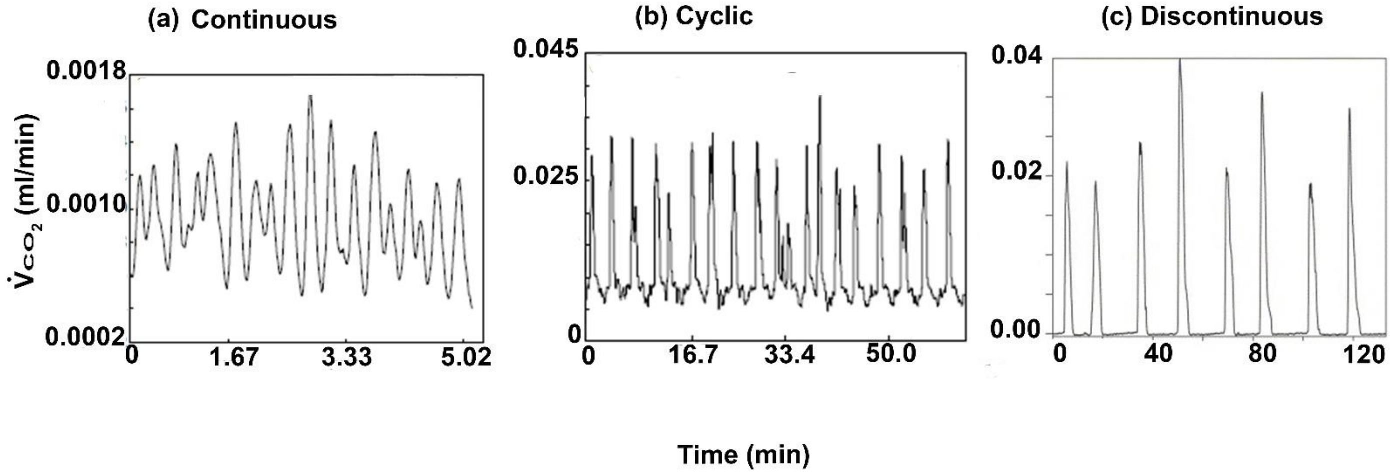


Fig. 1. An illustration of the consensus of the three types of gas patterns commonly observed in insects: (**a**) continuous (Marias et al. 2005), (**b**) cyclic (Marias et al. 2005), and (**c**) discontinuous gas exchange (Dingha et al. 2005)

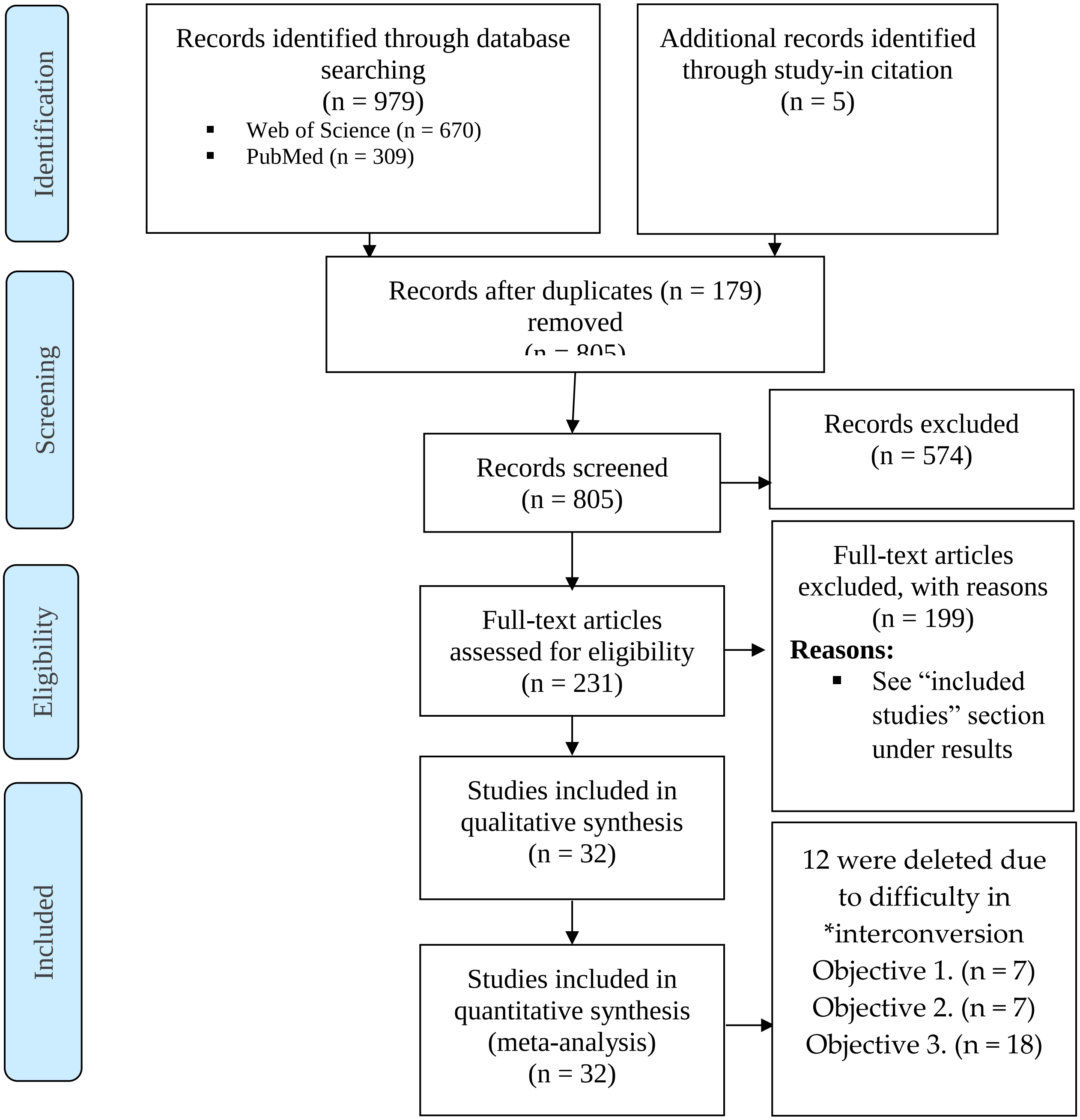


Fig. 2. PRISMA flow diagram of study identification, eligibility screening, and inclusion. * interconversion = the inability to convert units reported in those studies to units in this meta-analysis due to lack of requisite data (see "*inclusion criteria and data extraction"* section)

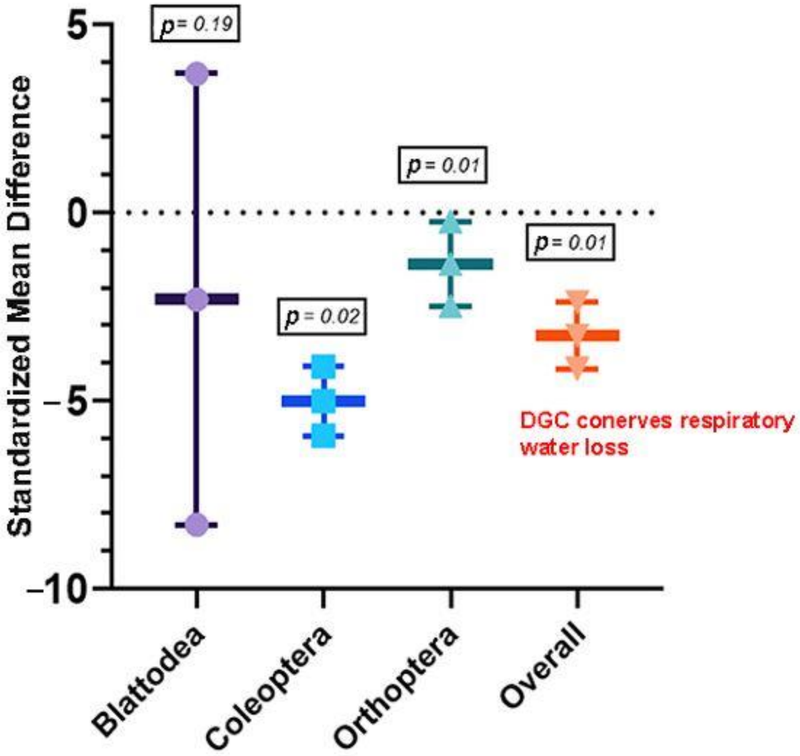


Fig. 3. Meta-analytical, subgroup estimate of water-saving hypothesis evaluation during the DGC with 95% CI in Blattodea, Coleoptera, and Orthoptera insect orders. The overall effect represents the collective effect of all three orders. Effect size estimates (measured as the standardized mean difference; Hedges' *g*) with 95% CIs that do not overlap zero are considered statistically significant at $p = 0.05$. *p*-values are provided in the textbox

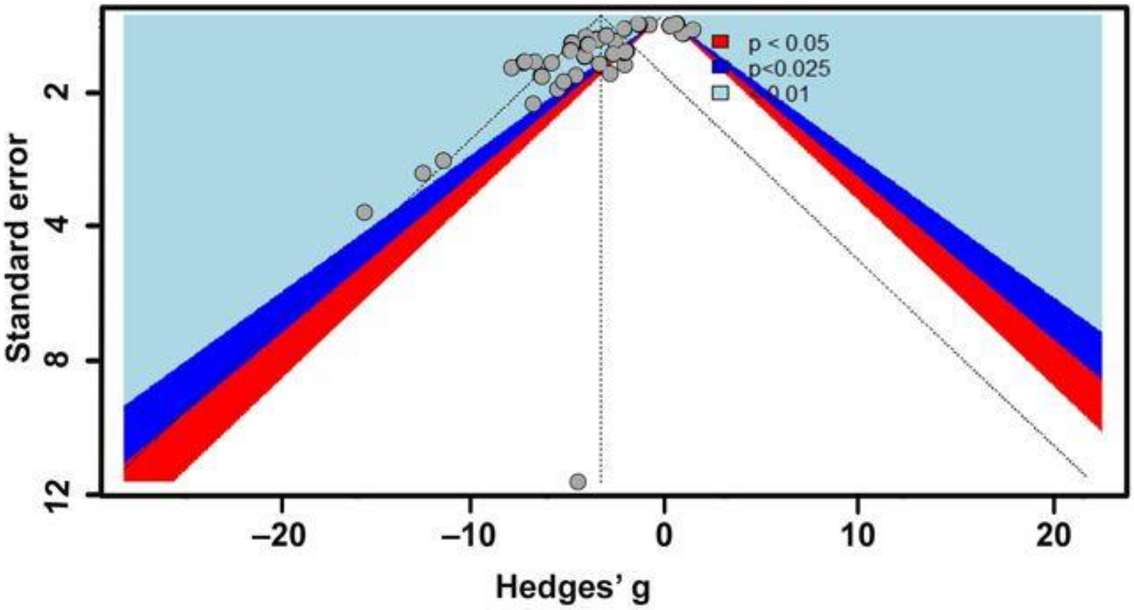
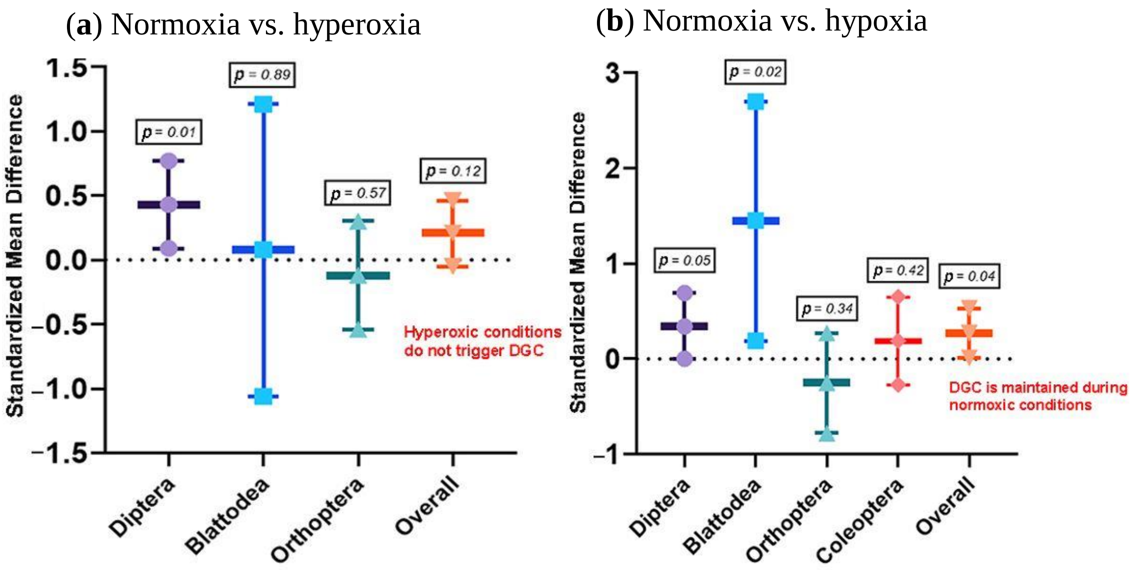


Fig. 4. Funnel plot for water-saving hypothesis studies (Objective 1: Does the DGC reduce water loss in insects?). The light blue, dark blue, and red areas correspond to 99%, 99.75%, and 95% confidence intervals, respectively. Asymmetric distribution of studies indicates publication bias



(**a**) Normoxia vs. hyperoxia

(**b**) Normoxia vs. hypoxia

Fig. 5. Meta-analytical, subgroup estimate of chthonic hypothesis in (**a**) normoxia vs. hyperoxia, and (**b**) normoxia vs. hypoxia conditions in insects during the DGC with 95% CI. The overall effect represents the collective effect of all three orders. Effect size estimates with CIs that do not overlap zero are considered statistically significant at $p = 0.05$. *p–values* are provided in the textbox

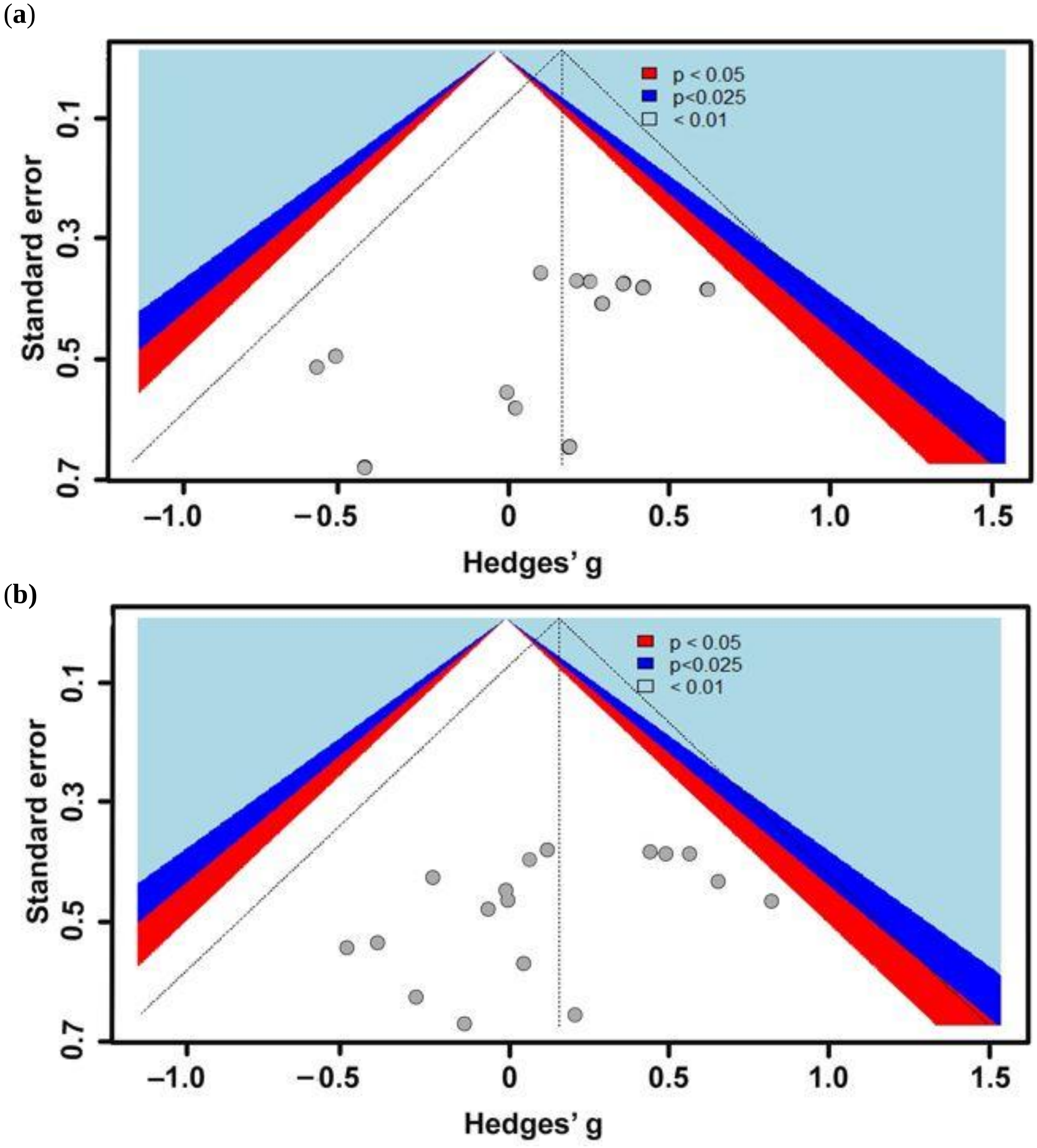


Figure 6. Funnel plot of (**a**) normoxia–hyperoxia and (**b**) normoxia–hypoxia discontinuous gas exchange studies. The dotted line represents a 95% confidence interval. The slightly skewed distribution of studies suggests a possible publication bias

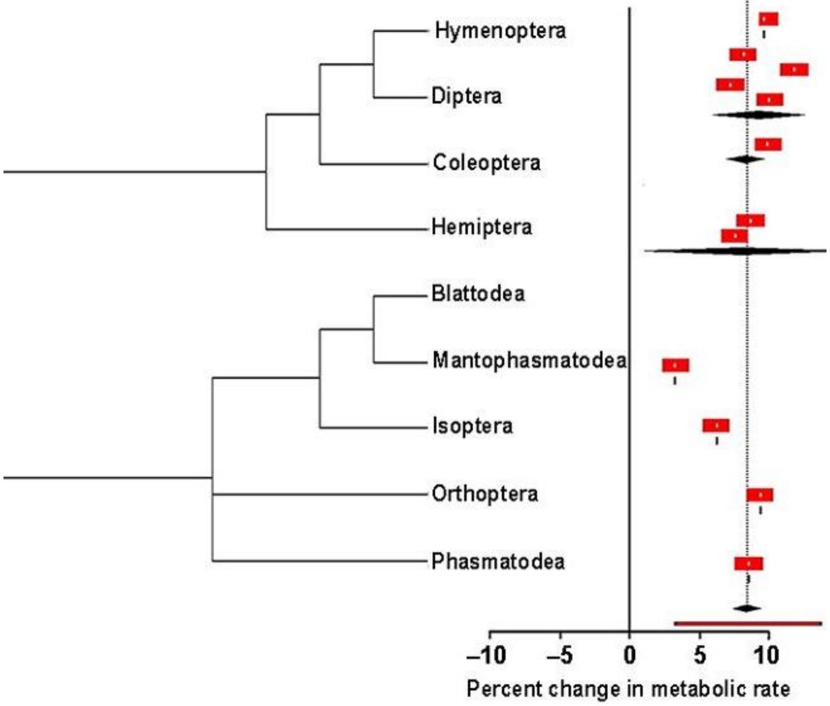


Figure 7. Meta-analytical, mean percent changes in metabolic rate of insects respiring through the DGC with 95% CIs of insect orders. Effect size estimates with CIs that do not overlap zero are considered statistically significant (*p* = 0.05). The phylogenetic tree is redrawn from Gullan and Cranston 2000

CHAPTER 7

Summary and Suggestions for Further Research

7.1 Summary and suggestions for further research

To date, the insecticide resistance action committee (IRAC) classification for botanicals such as essential oils components (EOCs) is the Group UNE i.e., unknown mode of action (IRAC, 2021). This is because the target gene(s) or protein(s) responsible for the observed insecticidal action is (are) unknown or uncharacterized. On one hand, it could be that the target sites are not well known because EOCs act non-specifically on multiple targets. On the other hand, it could be a reflection of the limited knowledge of the range of the physiological effects of EOCs. Either way, we would benefit by knowing more than we currently do. Understandably, there are several endpoints if the range of physiological effects of EOCs is sought. As a result, this work investigated the topical toxicity, oral toxicity, and respirometry effects of EOCs against the cosmopolitan urban insect pest, the German cockroach, *Blattella germanica* (L.). Additionally, I investigated the effect of EOCs on the reproductive biology of *B. germanica*. In Chapter One, I disentangled the myths surrounding the origin of *B. germanica*, expanded on its associated public health concerns, and reviewed its resistance mechanisms to conventional insecticides. Chapter Two presents essential oils (EOs) and EOCs employed in the management of urban insects including *B. germanica*. I also highlighted key EOCs with insecticidal activity against a broad range of urban insect pests, discussed knowledge gaps/conundrums, and offered probable insights into how laboratory/field-based investigations of EOs/EOCs should be approached if eventual integration into urban insect management is desired. Finally, I advocated the release of

EOs/EOCs in laboratory toxicity bioassays in a manner closest to potential field applications: ultra-low volume sprays.

In Chapter Three, I confirmed the topical toxicity of some aliphatic and aromatic EOCs against multi-resistant *B. germanica* strains collected from Franklin County, North Carolina. Specifically, limonene (aliphatic), carvacrol, eugenol, and tropolone (aromatic) were the most toxic against these strains. In an earlier study, these *B. germanica* strains were observed to have 31–52-fold resistance to permethrin (Wu and Appel 2017). Therefore, we predicted that the synergist, piperonyl butoxide (PBO), would most likely increase toxicity by inhibiting the *B. germanica* detoxifcation enzymes. The results show clear synergism with PBO for permethrin and EOCs. Testing the role of structural activity relationship in moderating toxicity of EOCs showed that molecular weight and vapor pressure are important properties that influence toxicity especially for aromatic EOCs. An increase in molecular weight and vapor pressure results in an increase in toxicity. Since the goal of EO research includes the delay of resistance development, future research should investigate how EOCs could be used to synergize currently developed insecticides.

Increased vapor pressure suggests that the EOCs could be more volatile, so I sought formulations that could increase the bioavailability of EOCs. I explored superabsorbent polymer (SAP) gels as a carrier to deliver EOCs to the German cockroach, *Blattella germanica* in Chapter Four. The EOCs in SAP gels significantly suppressed adult females' reproductive period, oothecal hatchability, and reduced fecundity. The results highlight the potential use of materials such as SAP gels to prolong the bioavailability of EOCs, allowing them to exert their insecticidal effects against the targeted pest, and solving the problem of high volatility. It also demonstrates the potential of incorporating EOCs as baits. SAP gels are hydrogels containing water so field applications of EOCs with hydrogel are likely to fail due to numerous competing water sources in homes. Future studies should investigate carriers that have a better chance of being effective in the field or formulations that could be used to synergize/deliver EOCs in homes. Chapter Five and Six give insights into how EOCs achieve kill effect against *B. germanica*. The logical explanation, supported by the data, is that EOCs such as limonene, carvacrol, and β-thujaplicin act to disrupt the discontinuous gas exchange cycle (DGC) of *B. germanica*. The resultant effect of this is an increased respiratory water loss (Oladipupo et al 2022). Taken together, the outcomes of this research work are poised to contribute to the pest management industry by providing alternatives to synthetic pesticides, creating environmentally conscious pest management tools, providing solutions for public health pests, and creating affordable options.

As existing cockroach control strategies are not always sufficient (Pai et al. 2005, Fardisi et al. 2019, Hou et al. 2021), and must either be bolstered by other techniques or methods (Hamilton et al 2020; Perry and Choe 2020) or replaced by new methods (Oladipupo et al., 2020a, Oladipupo et al. 2020b), future research should concentrate on three main aspects: (1) the release of EOs/EOCs in laboratory toxicity bioassays in a manner similar to field applications; for example as ultra-low volume sprays, (2) combinations to synergize currently employed synthetic insecticides or in combination with other integrated pest management strategies, and (3) formulations that can accentuate realistic field-based studies for EOs/EOCs. I argue that it is by doing these three things that the knowledge concerning EOs/EOCs can be extended, and the eventual commercialization of these effective, environmentally friendly, and sustainable natural products can be achieved.

7.2 References cited

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Supplementary materials

Chapter 5

S1: Lethal doses (LD)[*] of essential oil components used

	β-thujaplicin	Carvacrol	Limonene
Lethal Dose	(99)[^]	(98)	(≥95)
LD_{20}	3.35	5.38	9.36
LD_{30}	5.33	7.90	9.88
LD_{40}	7.72	10.82	10.18
LD_{50}	11.12	14.55	10.54

[*] values were computed from lethal dose values presented in Oladipupo et al., 2020a).

[^] values in parentheses are percentage purity values for each essential oil component.

S2A *F*-statistics, degrees of freedom, and *p*-values for mean $\dot{V}_{CO_2}$ (ml/min), volume (ml), and duration (min) for (A) interburst and (B) burst phases of DGC between male susceptible (Strain S) and multi-resistant *B. germanica* strains (Strains D and E) before topical application of an essential oil component

	(A) Interburst			**(B) Burst**		
	F	df	*p*-value	*F*	df	*p*-value
$\dot{V}_{CO_2}$	0.943	2, 33	0.4	1.475	2, 33	0.244
Volume	0.041	2, 33	0.959	0.937	2, 33	0.402
Duration	3.042	2, 33	0.061	0.101	2, 33	0.904

S2B *F*-statistics, degrees of freedom, and *p*-values for mean $\dot{V}_{H_2O}$ (mg), volume (ml), and duration (min) for (A) interburst and (B) burst phases of DGC between male susceptible (Strain S) and multi-resistant *B. germanica* strains (Strains D and E) before topical application of an essential oil component

	(A) Interburst			**(B) Burst**		
	F	df	*p*-value	*F*	df	*p*-value
$\dot{V}_{H_2O}$	0.792	2, 33	0.462	0.729	2, 33	0.490
Volume	0.141	2, 33	0.869	0.636	2, 33	0.536
Duration	2.875	2, 33	0.071	0.119	2, 33	0.888

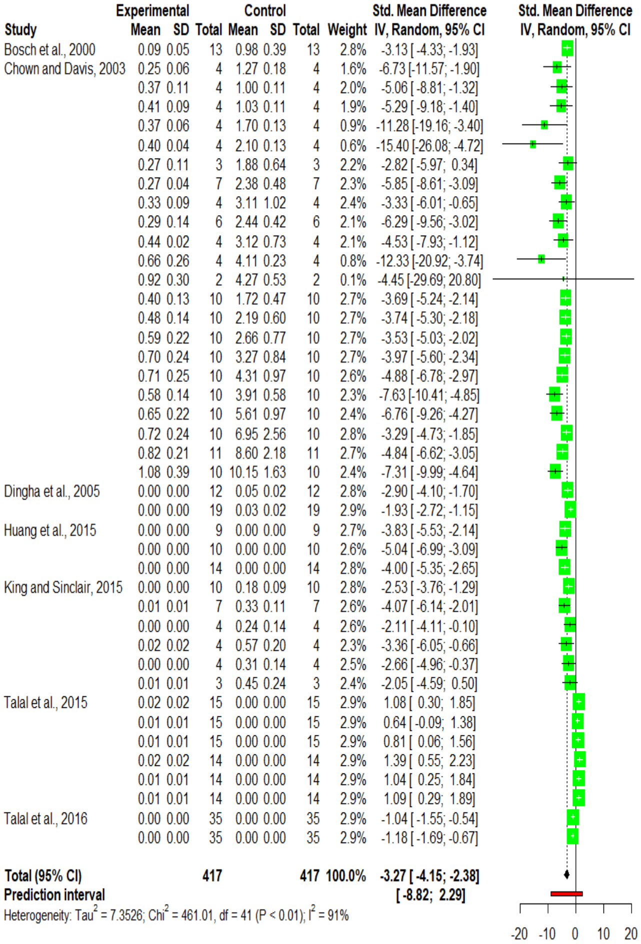
S3 *F*-statistics, degrees of freedom, and *p*-values for the rate of mass loss over time for susceptible (Strain S) and multi-resistant strains (Strains D and E) of *B. germanica* topically doused with essential oil components

Strain	***F***	**df**	***p*-value**
Strain S	0.410	13,40	0.957
Strain D	1.491	12, 43	0.165
Strain E	0.530	7, 53	0.808

Chapter 6

S1 All data files and R code used in this study is available at:

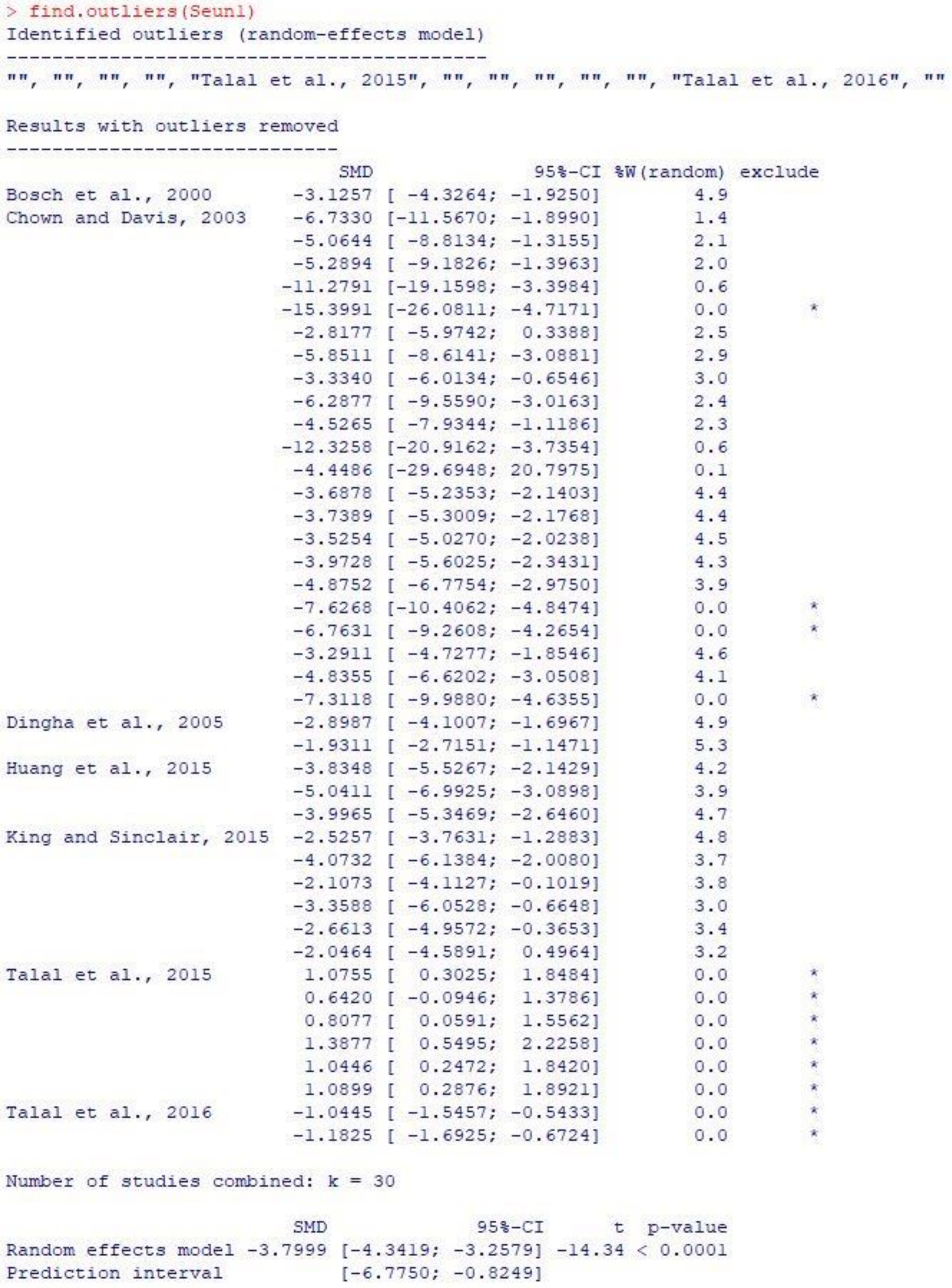
https://datadryad.org/stash/dataset/doi:10.5061/dryad.ht76hdrdz



Study	Experimental			Control				Std. Mean Difference
	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Bosch et al., 2000	0.09	0.05	13	0.98	0.39	13	2.8%	-3.13 [-4.33; -1.93]
Chown and Davis, 2003	0.25	0.06	4	1.27	0.18	4	1.6%	-6.73 [-11.57; -1.90]
	0.37	0.11	4	1.00	0.11	4	2.0%	-5.06 [-8.81; -1.32]
	0.41	0.09	4	1.03	0.11	4	1.9%	-5.29 [-9.18; -1.40]
	0.37	0.06	4	1.70	0.13	4	0.9%	-11.28 [-19.16; -3.40]
	0.40	0.04	4	2.10	0.13	4	0.6%	-15.40 [-26.08; -4.72]
	0.27	0.11	3	1.88	0.64	3	2.2%	-2.82 [-5.97; 0.34]
	0.27	0.04	7	2.38	0.48	7	2.3%	-5.85 [-8.61; -3.09]
	0.33	0.09	4	3.11	1.02	4	2.4%	-3.33 [-6.01; -0.65]
	0.29	0.14	6	2.44	0.42	6	2.1%	-6.29 [-9.56; -3.02]
	0.44	0.02	4	3.12	0.73	4	2.1%	-4.53 [-7.93; -1.12]
	0.66	0.26	4	4.11	0.23	4	0.8%	-12.33 [-20.92; -3.74]
	0.92	0.30	2	4.27	0.53	2	0.1%	-4.45 [-29.69; 20.80]
	0.40	0.13	10	1.72	0.47	10	2.7%	-3.69 [-5.24; -2.14]
	0.48	0.14	10	2.19	0.60	10	2.7%	-3.74 [-5.30; -2.18]
	0.59	0.22	10	2.66	0.77	10	2.7%	-3.53 [-5.03; -2.02]
	0.70	0.24	10	3.27	0.84	10	2.7%	-3.97 [-5.60; -2.34]
	0.71	0.25	10	4.31	0.97	10	2.6%	-4.88 [-6.78; -2.97]
	0.58	0.14	10	3.91	0.58	10	2.3%	-7.63 [-10.41; -4.85]
	0.65	0.22	10	5.61	0.97	10	2.4%	-6.76 [-9.26; -4.27]
	0.72	0.24	10	6.95	2.56	10	2.8%	-3.29 [-4.73; -1.85]
	0.82	0.21	11	8.60	2.18	11	2.7%	-4.84 [-6.62; -3.05]
	1.08	0.39	10	10.15	1.63	10	2.4%	-7.31 [-9.99; -4.64]
Dingha et al., 2005	0.00	0.00	12	0.05	0.02	12	2.8%	-2.90 [-4.10; -1.70]
	0.00	0.00	19	0.03	0.02	19	2.9%	-1.93 [-2.72; -1.15]
Huang et al., 2015	0.00	0.00	9	0.00	0.00	9	2.7%	-3.83 [-5.53; -2.14]
	0.00	0.00	10	0.00	0.00	10	2.6%	-5.04 [-6.99; -3.09]
	0.00	0.00	14	0.00	0.00	14	2.8%	-4.00 [-5.35; -2.65]
King and Sinclair, 2015	0.00	0.00	10	0.18	0.09	10	2.8%	-2.53 [-3.76; -1.29]
	0.01	0.01	7	0.33	0.11	7	2.6%	-4.07 [-6.14; -2.01]
	0.00	0.00	4	0.24	0.14	4	2.6%	-2.11 [-4.11; -0.10]
	0.02	0.02	4	0.57	0.20	4	2.3%	-3.36 [-6.05; -0.66]
	0.00	0.00	4	0.31	0.14	4	2.5%	-2.66 [-4.96; -0.37]
	0.01	0.01	3	0.45	0.24	3	2.4%	-2.05 [-4.59; 0.50]
Talal et al., 2015	0.02	0.02	15	0.00	0.00	15	2.9%	1.08 [0.30; 1.85]
	0.01	0.01	15	0.00	0.00	15	2.9%	0.64 [-0.09; 1.38]
	0.01	0.01	15	0.00	0.00	15	2.9%	0.81 [0.06; 1.56]
	0.02	0.02	14	0.00	0.00	14	2.9%	1.39 [0.55; 2.23]
	0.01	0.01	14	0.00	0.00	14	2.9%	1.04 [0.25; 1.84]
	0.01	0.01	14	0.00	0.00	14	2.9%	1.09 [0.29; 1.89]
Talal et al., 2016	0.00	0.00	35	0.00	0.00	35	2.9%	-1.04 [-1.55; -0.54]
	0.00	0.00	35	0.00	0.00	35	2.9%	-1.18 [-1.69; -0.67]
Total (95% CI)			**417**			**417**	**100.0%**	**-3.27 [-4.15; -2.38]**
Prediction interval								**[-8.82; 2.29]**

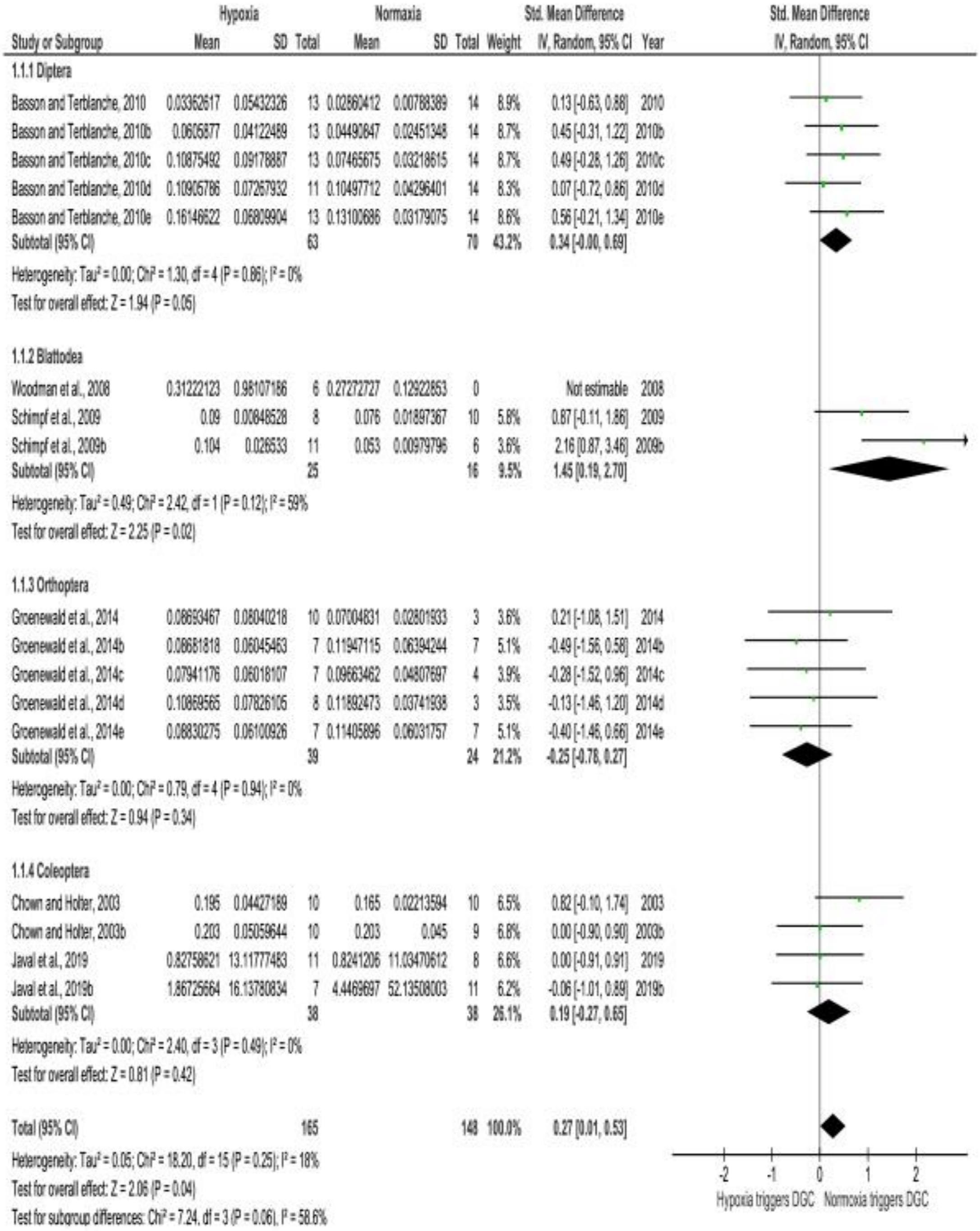
Heterogeneity: $Tau^2 = 7.3526$; $Chi^2 = 461.01$, df = 41 ($P < 0.01$); $I^2 = 91\%$

S2 Overall forest plot for Objective 1



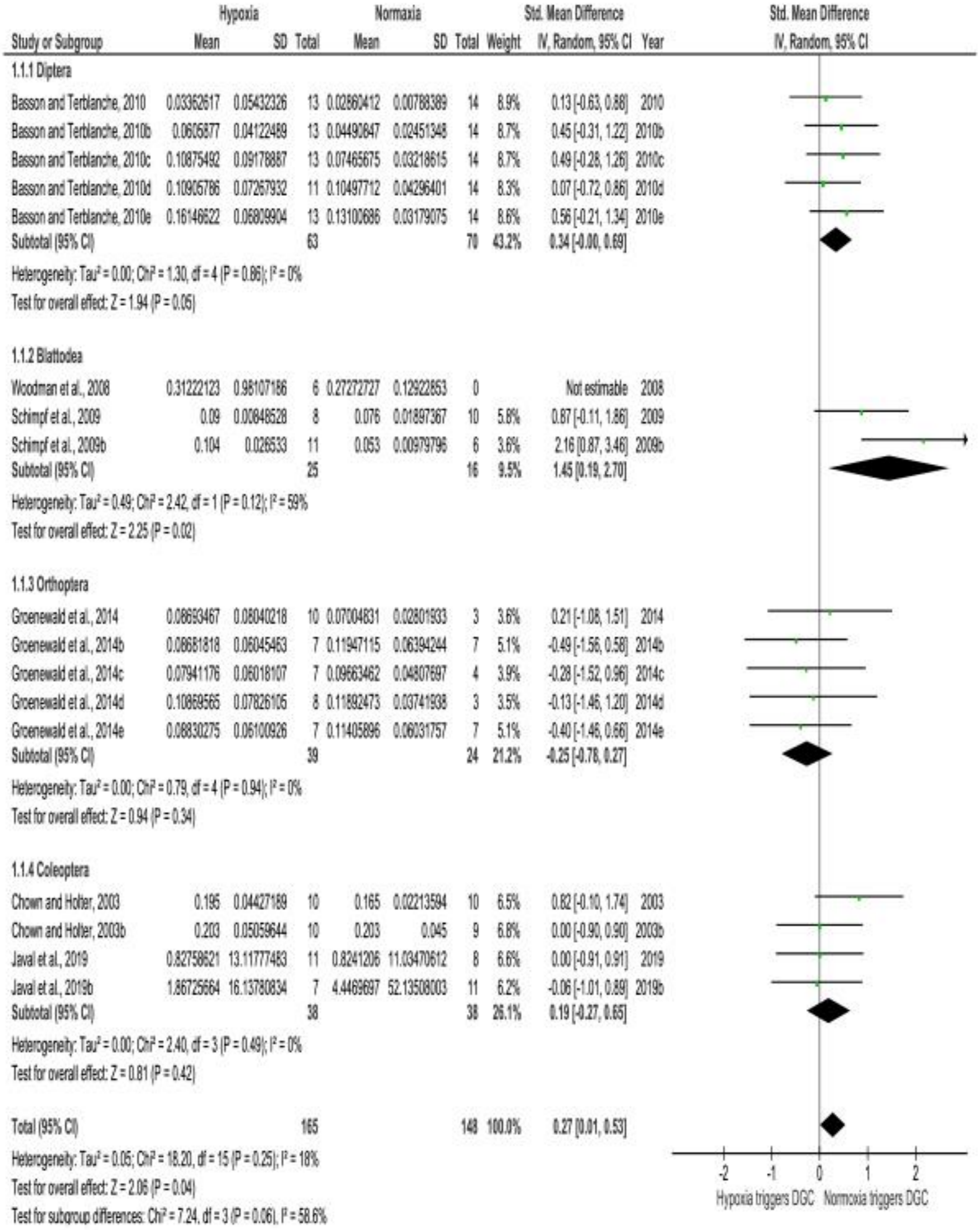
```
> find.outliers(Seun1)
Identified outliers (random-effects model)
------------------------------------------
"", "", "", "", "Talal et al., 2015", "", "", "", "", "", "Talal et al., 2016", ""

Results with outliers removed
-----------------------------
                             SMD              95%-CI %W(random) exclude
Bosch et al., 2000       -3.1257 [ -4.3264; -1.9250]        4.9        
Chown and Davis, 2003    -6.7330 [-11.5670; -1.8990]        1.4        
                         -5.0644 [ -8.8134; -1.3155]        2.1        
                         -5.2894 [ -9.1826; -1.3963]        2.0        
                        -11.2791 [-19.1598; -3.3984]        0.6        
                        -15.3991 [-26.0811; -4.7171]        0.0       *
                         -2.8177 [ -5.9742;  0.3388]        2.5        
                         -5.8511 [ -8.6141; -3.0881]        2.9        
                         -3.3340 [ -6.0134; -0.6546]        3.0        
                         -6.2877 [ -9.5590; -3.0163]        2.4        
                         -4.5265 [ -7.9344; -1.1186]        2.3        
                        -12.3258 [-20.9162; -3.7354]        0.6        
                         -4.4486 [-29.6948; 20.7975]        0.1        
                         -3.6878 [ -5.2353; -2.1403]        4.4        
                         -3.7389 [ -5.3009; -2.1768]        4.4        
                         -3.5254 [ -5.0270; -2.0238]        4.5        
                         -3.9728 [ -5.6025; -2.3431]        4.3        
                         -4.8752 [ -6.7754; -2.9750]        3.9        
                         -7.6268 [-10.4062; -4.8474]        0.0       *
                         -6.7631 [ -9.2608; -4.2654]        0.0       *
                         -3.2911 [ -4.7277; -1.8546]        4.6        
                         -4.8355 [ -6.6202; -3.0508]        4.1        
                         -7.3118 [ -9.9880; -4.6355]        0.0       *
Dingha et al., 2005      -2.8987 [ -4.1007; -1.6967]        4.9        
                         -1.9311 [ -2.7151; -1.1471]        5.3        
Huang et al., 2015       -3.8348 [ -5.5267; -2.1429]        4.2        
                         -5.0411 [ -6.9925; -3.0898]        3.9        
                         -3.9965 [ -5.3469; -2.6460]        4.7        
King and Sinclair, 2015  -2.5257 [ -3.7631; -1.2883]        4.8        
                         -4.0732 [ -6.1384; -2.0080]        3.7        
                         -2.1073 [ -4.1127; -0.1019]        3.8        
                         -3.3588 [ -6.0528; -0.6648]        3.0        
                         -2.6613 [ -4.9572; -0.3653]        3.4        
                         -2.0464 [ -4.5891;  0.4964]        3.2        
Talal et al., 2015        1.0755 [  0.3025;  1.8484]        0.0       *
                          0.6420 [ -0.0946;  1.3786]        0.0       *
                          0.8077 [  0.0591;  1.5562]        0.0       *
                          1.3877 [  0.5495;  2.2258]        0.0       *
                          1.0446 [  0.2472;  1.8420]        0.0       *
                          1.0899 [  0.2876;  1.8921]        0.0       *
Talal et al., 2016       -1.0445 [ -1.5457; -0.5433]        0.0       *
                         -1.1825 [ -1.6925; -0.6724]        0.0       *

Number of studies combined: k = 30

                         SMD             95%-CI      t  p-value
Random effects model -3.7999 [-4.3419; -3.2579] -14.34 < 0.0001
Prediction interval          [-6.7750; -0.8249]
```

S3 Forest plot when outliers were removed



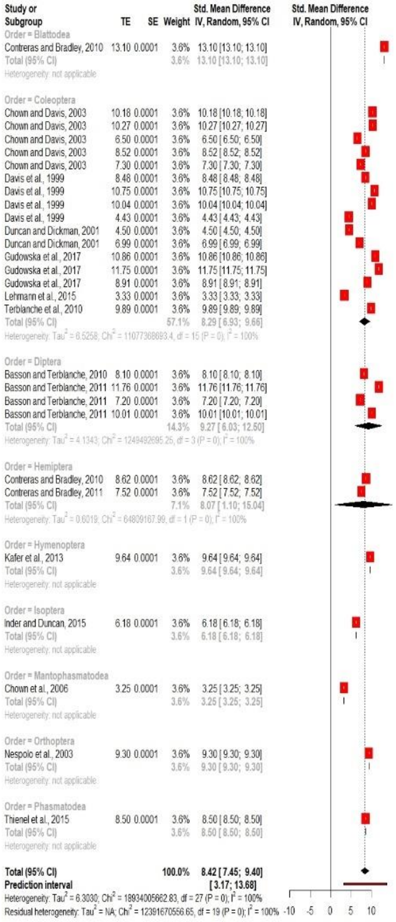
	Hypoxia			Normaxia				Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	Year
1.1.1 Diptera									
Basson and Terblanche, 2010	0.03362617	0.05432326	13	0.02860412	0.00788389	14	8.9%	0.13 [-0.63, 0.88]	2010
Basson and Terblanche, 2010b	0.0605877	0.04122489	13	0.04490847	0.02451348	14	8.7%	0.45 [-0.31, 1.22]	2010b
Basson and Terblanche, 2010c	0.10875492	0.09178887	13	0.07465675	0.03218615	14	8.7%	0.49 [-0.28, 1.26]	2010c
Basson and Terblanche, 2010d	0.10905786	0.07267932	11	0.10497712	0.04296401	14	8.3%	0.07 [-0.72, 0.86]	2010d
Basson and Terblanche, 2010e	0.16146622	0.06809904	13	0.13100686	0.03179075	14	8.6%	0.56 [-0.21, 1.34]	2010e
Subtotal (95% CI)			63			70	43.2%	0.34 [-0.00, 0.69]	
Heterogeneity: Tau² = 0.00; Chi² = 1.30, df = 4 (P = 0.86); I² = 0%									
Test for overall effect: Z = 1.94 (P = 0.05)									
1.1.2 Blattodea									
Woodman et al., 2008	0.31222123	0.98107186	6	0.27272727	0.12922853	0		Not estimable	2008
Schimpf et al., 2009	0.09	0.00848528	8	0.076	0.01897367	10	5.8%	0.87 [-0.11, 1.86]	2009
Schimpf et al., 2009b	0.104	0.026533	11	0.053	0.00979796	6	3.6%	2.16 [0.87, 3.46]	2009b
Subtotal (95% CI)			25			16	9.5%	1.45 [0.19, 2.70]	
Heterogeneity: Tau² = 0.49; Chi² = 2.42, df = 1 (P = 0.12); I² = 59%									
Test for overall effect: Z = 2.25 (P = 0.02)									
1.1.3 Orthoptera									
Groenewald et al., 2014	0.08693467	0.08040218	10	0.07004831	0.02801933	3	3.6%	0.21 [-1.08, 1.51]	2014
Groenewald et al., 2014b	0.08681818	0.06045463	7	0.11947115	0.06394244	7	5.1%	-0.49 [-1.56, 0.58]	2014b
Groenewald et al., 2014c	0.07941176	0.06018107	7	0.09663462	0.04807697	4	3.9%	-0.28 [-1.52, 0.96]	2014c
Groenewald et al., 2014d	0.10869565	0.07826105	8	0.11892473	0.03741938	3	3.5%	-0.13 [-1.46, 1.20]	2014d
Groenewald et al., 2014e	0.08830275	0.06100926	7	0.11405896	0.06031757	7	5.1%	-0.40 [-1.46, 0.66]	2014e
Subtotal (95% CI)			39			24	21.2%	-0.25 [-0.78, 0.27]	
Heterogeneity: Tau² = 0.00; Chi² = 0.79, df = 4 (P = 0.94); I² = 0%									
Test for overall effect: Z = 0.94 (P = 0.34)									
1.1.4 Coleoptera									
Chown and Holter, 2003	0.195	0.04427189	10	0.165	0.02213594	10	6.5%	0.82 [-0.10, 1.74]	2003
Chown and Holter, 2003b	0.203	0.05059644	10	0.203	0.045	9	6.8%	0.00 [-0.90, 0.90]	2003b
Javal et al., 2019	0.82758621	13.11777483	11	0.8241206	11.03470612	8	6.6%	0.00 [-0.91, 0.91]	2019
Javal et al., 2019b	1.86725664	16.13780834	7	4.4469697	52.13508003	11	6.2%	-0.06 [-1.01, 0.89]	2019b
Subtotal (95% CI)			38			38	26.1%	0.19 [-0.27, 0.65]	
Heterogeneity: Tau² = 0.00; Chi² = 2.40, df = 3 (P = 0.49); I² = 0%									
Test for overall effect: Z = 0.81 (P = 0.42)									
Total (95% CI)			165			148	100.0%	0.27 [0.01, 0.53]	
Heterogeneity: Tau² = 0.05; Chi² = 18.20, df = 15 (P = 0.25); I² = 18%									
Test for overall effect: Z = 2.06 (P = 0.04)									
Test for subgroup differences: Chi² = 7.24, df = 3 (P = 0.06), I² = 58.6%									

S4 Sub-group forest plot for hyperoxia



	Hypoxia			Normaxia				Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	Year
1.1.1 Diptera									
Basson and Terblanche, 2010	0.03362617	0.05432326	13	0.02860412	0.00788389	14	8.9%	0.13 [-0.63, 0.88]	2010
Basson and Terblanche, 2010b	0.0605877	0.04122489	13	0.04490847	0.02451348	14	8.7%	0.45 [-0.31, 1.22]	2010b
Basson and Terblanche, 2010c	0.10875492	0.09178887	13	0.07465675	0.03218615	14	8.7%	0.49 [-0.28, 1.26]	2010c
Basson and Terblanche, 2010d	0.10905786	0.07267932	11	0.10497712	0.04296401	14	8.3%	0.07 [-0.72, 0.86]	2010d
Basson and Terblanche, 2010e	0.16146622	0.06809904	13	0.13100686	0.03179075	14	8.6%	0.56 [-0.21, 1.34]	2010e
Subtotal (95% CI)			**63**			**70**	**43.2%**	**0.34 [-0.00, 0.69]**	
Heterogeneity: Tau² = 0.00; Chi² = 1.30, df = 4 (P = 0.86); I² = 0%									
Test for overall effect: Z = 1.94 (P = 0.05)									
1.1.2 Blattodea									
Woodman et al., 2008	0.31222123	0.98107186	6	0.27272727	0.12922853	0		Not estimable	2008
Schimpf et al., 2009	0.09	0.00848528	8	0.076	0.01897367	10	5.8%	0.87 [-0.11, 1.86]	2009
Schimpf et al., 2009b	0.104	0.026533	11	0.053	0.00979796	6	3.6%	2.16 [0.87, 3.46]	2009b
Subtotal (95% CI)			**25**			**16**	**9.5%**	**1.45 [0.19, 2.70]**	
Heterogeneity: Tau² = 0.49; Chi² = 2.42, df = 1 (P = 0.12); I² = 59%									
Test for overall effect: Z = 2.25 (P = 0.02)									
1.1.3 Orthoptera									
Groenewald et al., 2014	0.08693467	0.08040218	10	0.07004831	0.02801933	3	3.6%	0.21 [-1.08, 1.51]	2014
Groenewald et al., 2014b	0.08681818	0.06045463	7	0.11947115	0.06394244	7	5.1%	-0.49 [-1.56, 0.58]	2014b
Groenewald et al., 2014c	0.07941176	0.06018107	7	0.09663462	0.04807697	4	3.9%	-0.28 [-1.52, 0.96]	2014c
Groenewald et al., 2014d	0.10869565	0.07826105	8	0.11892473	0.03741938	3	3.5%	-0.13 [-1.46, 1.20]	2014d
Groenewald et al., 2014e	0.08830275	0.06100926	7	0.11405896	0.06031757	7	5.1%	-0.40 [-1.46, 0.66]	2014e
Subtotal (95% CI)			**39**			**24**	**21.2%**	**-0.25 [-0.78, 0.27]**	
Heterogeneity: Tau² = 0.00; Chi² = 0.79, df = 4 (P = 0.94); I² = 0%									
Test for overall effect: Z = 0.94 (P = 0.34)									
1.1.4 Coleoptera									
Chown and Holter, 2003	0.195	0.04427189	10	0.165	0.02213594	10	6.5%	0.82 [-0.10, 1.74]	2003
Chown and Holter, 2003b	0.203	0.05059644	10	0.203	0.045	9	6.8%	0.00 [-0.90, 0.90]	2003b
Javal et al., 2019	0.82758621	13.11777483	11	0.8241206	11.03470612	8	6.6%	0.00 [-0.91, 0.91]	2019
Javal et al., 2019b	1.86725664	16.13780834	7	4.4469697	52.13508003	11	6.2%	-0.06 [-1.01, 0.89]	2019b
Subtotal (95% CI)			**38**			**38**	**26.1%**	**0.19 [-0.27, 0.65]**	
Heterogeneity: Tau² = 0.00; Chi² = 2.40, df = 3 (P = 0.49); I² = 0%									
Test for overall effect: Z = 0.81 (P = 0.42)									
Total (95% CI)			**165**			**148**	**100.0%**	**0.27 [0.01, 0.53]**	
Heterogeneity: Tau² = 0.05; Chi² = 18.20, df = 15 (P = 0.25); I² = 18%									
Test for overall effect: Z = 2.06 (P = 0.04)									
Test for subgroup differences: Chi² = 7.24, df = 3 (P = 0.06), I² = 58.6%									

S5 Sub-group forest plot for hyporoxia



S6 Overall forest plot for Objective 3