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Fumigant Toxicity of Plant Essential Oils and Selected Monoterpenoid Components against the Adult German Cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae)

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Abstract: The fumigant toxicity of various essential oils and selected monoterpenoid components were tested against the adult German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). Responses varied according to plant material, concentration, and exposure time. High insecticidal activity against adult *B. germanica* specimens was achieved with allyl isothiocyanate (component of horseradish) and the essential oil of *Allium sativum* (L.) (Liliales: Liliaceae) at the concentration of 2.5 and 5 µl l^{-1} of air, respectively, while the essential oils of *Thymus vulgaris* (L.) (Lamiales: Lamiaceae), *Oregano dubium* (L.) (Lamiales: Lamiaceae), *Allium cepa* (L.) (Liliales: Liliaceae), and *Rosemarinus officinalis* (L.) (Lamiales: Lamiaceae), and the monoterpenoid components eugenol, carvacrol, and citronella at the concentration of 5 µl l^{-1} of air did not show any insecticidal activity. Allyl isothiocyanate at 2.5 µl l^{-1} of air caused 100% mortality within 18 h, whereas over 95% mortality within 48 h was achieved with the essential oil of *A. sativum* at 5 µl l^{-1} of air. Allyl isothiocyanate was the most toxic compound, followed by *A. sativum* oil. Concentration- and time-mortality tests were conducted for these 2 chemicals. Estimated LC_{50} and LT_{50} values for allyl isothiocyanate were 0.68 µl l^{-1} of air and 6.51 h, respectively. The findings show that allyl isothiocyanate and *A. sativum* had high insecticidal activity, and that they have potential as fumigants for use against the German cockroach. The essential oils and monoterpenoid components studied herein require further investigation to determine their potential as fumigants for cockroach control.

Key Words: Essential oils, fumigant toxicity, allyl isothiocyanate, *A. sativum*, *B. germanica*

Bitki Uçucu Yağlarının ve Bazı Monoterpenoid Bileşenlerin Alman Hamamböceği, *Blattella germanica*, Erginlerine Fumigant Toksisiteleri

Özet: Çeşitli uçucu yağların ve bazı bileşenlerin Alman hamamböceği, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), erginlerine fumigant toksisiteleri test edilmiştir. Elde edilen ölüm oranları bitki materyali, konsantrasyon ve maruz bırakma süresine göre farklılık göstermiştir. *B. germanica* erginlerine karşı *Thymus vulgaris*, *Oregano dubium*, *Allium cepa* ve *Rosemarinus officinalis*'in uçucu yağlarının, ve monoterpenoid bileşenler olan eugenol, carvacrol ve citronellanın 5 µl l^{-1} konsantrasyonunda her hangi bir insektisit aktivite göstermez iken, yüksek insektisit aktivitesi, allyl isothiocyanate (karaturp bileşeni) ve *Allium sativum*'un uçucu yağının sırasıyla 2.5 ve 5 µl l^{-1} konsantrasyonları ile elde edilmiştir. 2.5 µl l^{-1} konsantrasyonunda allyl isothiocyanate 18 saatte %100 ölüme neden olurken *A. sativum*'un uçucu yağının 5 µl l^{-1} konsantrasyonunda 48 saatte %95'in üzerinde ölüm görülmüştür. Allyl isothiocyanate en toksik bileşen olup bunu *A. sativum* yağı takip etmiştir. Bu iki kimyasal için konsantrasyon- ve zaman-ölüm testleri yürütülmüştür. Allyl isothiocyanate için tahmin edilen LC_{50} ve LT_{50} değerleri sırasıyla 0.68 µl l^{-1} ve 6.51 saattir. Bulgular allyl isothiocyanate ve *A. sativum*'un yüksek insektisit aktivitelerinin bulunduğunu ve Alman hamamböceğine karşı fumigant olarak potansiyele sahip olduklarını belirtmektedir. Bununla birlikte, burada kullanılan uçucu yağlar ve monoterpenoid bileşenleri üzerinde hamamböceği mücadelesinde fumigant potansiyeli bakımından daha fazla incelemenin yapılmasına gereksinim bulunmaktadır.

Anahtar Sözcükler: Uçucu yağlar, fumigant toksisite, allyl isothiocyanate, *A. sativum*, *B. germanica*

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Introduction

The German cockroach, *Blattella germanica* (L.), which is found in homes and other buildings, is a major carrier of pathogens and a main source of allergens. The German cockroach is also associated with swine and has been observed on pig manure and on other animals (Waldvogel et al., 1999); The German cockroach is an important primary medical and economic insect pest (Roberts, 1996). The cockroach is controlled primarily by chlorinated hydrocarbons, organophosphates, pyrethroid compounds, and carbamates (Rust et al., 1993); however, this cockroach has developed resistance to these insecticides (Rust and Reierson, 1991; Dong et al., 1998; Holbrook et al., 1999; Jialin et al., 2007). Because of human and animal safety, the disruption of natural biological control systems, and insecticide resistance, development of new types of alternative selective cockroach controls is needed.

Plant essential oils and monoterpenoid components are considered insect-control agents because their bioactive chemicals are selective and have little or no harmful effect on the environment and non-target organisms (Arnason et al., 1989; Hedin et al., 1997). Many essential oils and monoterpenoid components degrade quickly, do not accumulate in the body or environment, and some are very pest specific. Hence, essential oils from various plants and monoterpenoids may be potential alternatives to presently used *B. germanica* control agents, as they are a rich source of bioactive chemical compounds. Although the insecticidal activity of a large number of essential oils and other plant extracts has been demonstrated against several major agricultural and stored product pests (Regnault-Roger et al., 1993; Weaver and Subramanyam, 2000; Lee et al., 2001; Papachristos and Stamopoulos, 2002; Kim et al., 2003; Park et al., 2006; Rozman et al., 2007), few researchers have studied the control of *B. germanica* by using plant essential oils and monoterpenoid components (Chang and Ahn, 2001; Peterson et al., 2002; Lee et al., 2003).

Therefore, the purpose of the present study was to investigate the fumigant activity of plant essential oils derived from *Allium sativum* (garlic), *Thymus vulgaris* (thyme), *Oregano dubium* (oregano), *Allium cepa* (onion), and *Rosemarinus officinalis* (rosemary), and selected monoterpenoid components (allyl isothiocyanate, eugenol, carvacrol, and citronella) against the German cockroach. To the best of our knowledge this is the first report of the fumigation activity of these essential oils and monoterpenoid components against the German cockroach.

Materials and Methods

Insect

Colonies of *B. germanica* were reared in 60-l plastic containers and maintained at room temperature. The cockroaches were provided with water in glass tubes fitted with cotton stoppers, and dry dog food. Each container was provided with paper egg cartons as shelter. The adult cockroaches (5-10 days old) were tested for each bioassays at 25 ± 2 °C and 50 ± 5% relative humidity.

Chemicals

The main compounds present in the essential oils of the plants and the monoterpenoids (Table 1) were obtained from Sigma-Aldrich (Deisenhofen, Germany), Fluka (Buchs, Switzerland), Merck (Merck Schuchardt OHG Honenbrunn, Germany), ATL (Pure essential Inc. East London, Ontario, Canada), Mountain Rose Herbs (Eugene, Oregon, USA), and Liberty Natural Products (Oregon city, Oregon, USA). These products were tested via fumigant bioassays.

Table 1. The essentials oils and monoterpenoid components purchased from Sigma, Merck, Mountain Rose Herbs, Liberty Natural Products, and Fluka.

Essential oils and monoterpenoid components	Plant part	Produced by
Allium sativum	Bulb	Liberty Natural Products
Allium cepa	Bulb	Liberty Natural Products
Thymus vulgaris	Leaf	Mountain Rose Herbs
Oregano dubium	Leaf	ATL
Rosemarinus officinalis	Flower/Leaf	Liberty Natural Products
Allyl isothiocyanate		Merck
Eugenol		Fluka
Carvacrol		Merck
Citronella		Sigma

Fumigant Activity

Fumigation bioassays were used to evaluate insecticidal activity. Test insects were placed in a 1-l glass jar. Ten 5-10-day-old adult German cockroaches were used in each replicate. About 1 g of dry dog food was supplied as food for the test adults. A thin layer of petroleum jelly was applied 2 cm from the top of the jar. This prevented direct contact of the adult cockroaches with the tested essential oils and components. A piece of filter paper (No. 20, 40 mm in diameter) glued to the underside of the lid of each 1-l glass jar was treated with essential oils (*Allium sativum, Thymus vulgaris, Oregano dubium, Allium cepa*, and *Rosemarinus officinalis*), or the monoterpenoid components (allyl isothiocyanate, eugenol, carvacrol, and citronella) at 5 μl l^{-1} of air), and then the lid was tightly sealed on the jar. The control insects were placed in a jar with untreated filter paper. Insects treated with essential oils or monoterpenoid components were maintained at room temperature. Mortality was assessed 1 or 2 days after treatment. Mortality was defined as inability to move when placed on the dorsal side and inability to respond to prodding. Each test was replicated 3 times and 10 (5-10 days old) adult German cockroaches were used for each replicate. Concentrations were calculated assuming that all the applied oils or monoterpenoid components volatilized off the filter paper.

Concentration-Mortality Test with Allyl Isothiocyanate and *A. sativum*

For concentration-mortality studies, allyl isothiocyanate and *A. sativum* oil were chosen based on their significant insecticidal effects on *B. germanica* adults in the initial mortality experiment. In order to demonstrate the fumigant activity of allyl isothiocyanate and *A. sativum* oil against *B. germanica* adults, they were treated with various concentrations of allyl isothiocyanate and *A. sativum* oil, as described above. The concentrations were 0.5, 0.75, 1.0, 1.25, 1.5, 2.5, and 5.0 μl l^{-1} of air for the allyl isothiocyanate, and 1.0, 2.0, 3.0, 4.0, and 5.0 μl l^{-1} of air for *A. sativum*. Mortality was assessed 24 h after treatment with allyl isothiocyanate and 48 h after treatment with *A. sativum*.

Exposure Time-Mortality Response of *B. germanica* Adults to Allyl Isothiocyanate and *A. sativum*

In order to demonstrate the effects of exposure time to allyl isothiocyanate and *A. sativum* oil on mortality in *B. germanica* adults, the insects were treated with 2.5 μl l^{-1} of air of the allyl isothiocyanate and 5 μl l^{-1} of air of *A. sativum* as described above. Different control units were set for each time. Mortality was assessed 3, 6, 9, 12, 18, and 24 h after treatment with allyl isothiocyanate, and 18, 24, 30, 42, and 48 h after treatment with *A. sativum*.

Statistical Analysis

The percentage of mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Data were analyzed using the general linear models procedure, and mean comparisons were made using the least significant difference (LSD) test ($P \leq 0.01$) (SAS Institute Inc., 1989). Concentration-mortality data, corrected according to Abbott's formula (Abbott 1925), were recorded after 24 h for allyl isothiocyanate and after 48 h for *A. sativum*, and were then subjected to probit analysis using POLO-PC statistics software (LeOra 1987) to estimate LC_{50} and the slope of the regression line. Exposure time-mortality data, corrected according to Abbott's formula (Abbott, 1925), recorded for allyl isothiocyanate at 2.5 μl l^{-1} of air and for *A. sativum* oil at 5 μl l^{-1} of air were also subjected to probit analysis using POLO-PC statistics software (LeOra 1987) in order to estimate LT_{50} and the slope of the regression line.

Results

Fumigant Activity of Essentials Oils and Selected Monoterpenoid Components

The 9 tested commercial essential oils and monoterpenoid components varied in their toxicity to adult German cockroaches (Table 2). Among these, allyl isothiocyanate caused 100% mortality to the adult cockroaches 24 h after treatment at the concentration of 5 μl l^{-1} of air and *A. sativum* oil caused over 95% mortality to the adult cockroaches 48 h after treatment at the concentration of 5 μl l^{-1} of air (Table 2). On the other hand, the essential oils of *T. vulgaris, O. dubium, A. cepa*, and *R. officinalis*, and the monoterpenoid components eugenol, carvacrol, and citronella at 5 μl l^{-1} of air did not have any fumigant activity against the adult cockroaches, either 24 h after treatment or 48 h after treatment, as compared with the control treatment (Table 2) (LSD, $P \leq 0.0001$).

Concentration-Mortality Curves for Allyl Isothiocyanate and *A. sativum* Oil

Figure 1 indicates that mortality due to the fumigant activity of allyl isothiocyanate was concentration

Table 2. Fumigant toxicity of the essential oils and selected monoterpenoid components against the adult German cockroach, *Blattella germanica*.

Essential oils and the monoterpenoid components (5 µl l^{-1} of air)	% Mortality ± SEM	
	24 h	48 h
Allium sativum	33.3 ± 5.39[b]	93.3 ± 6.39[a]
Allium cepa	0 ± 0[c]	3.3 ± 3.3[b]
Thymus vulgaris	3.3 ± 3.3[c]	3.3 ± 3.3[b]
Oregano dubium	0 ± 0[c]	0 ± 0[b]
Rosemarinus officinalis	0 ± 0[c]	13.3 ± 13.03[b]
Allyl isothiocyanate	100 ± 0[a]	100 ± 0[a]
Eugenol	0 ± 0[c]	0 ± 0[b]
Carvacrol	0 ± 0[c]	0 ± 0[b]
Citronella	0 ± 0[c]	0 ± 0[b]
Untreated	0 ± 0[c]	3.3 ± 3.3[b]

*Means in the same column followed by the same letters are not significantly different ($P < 0.0001$), as determined by the LSD-test

dependent. At 2.5 and 5 µl l^{-1} of air, mortality was 100% 24 h after treatment. Concentrations of 0.75, 1.0, 1.25, and 1.5 µl l^{-1} of air caused 70%-90% mortality 24 h after treatment. This was followed by the application of 0.5 µl l^{-1} of air 24 h after treatment. Figure 2 indicates that mortality due to the fumigant activity of *A. sativum* oil was also concentration dependent. Mortality was 96% 48 h after treatment with 5 µl l^{-1} of air. Concentrations of 3 and 4 µl l^{-1} of air caused 70%-75% mortality 48 h after treatment. This was followed by the application of 1 and 2 µl l^{-1} of air 48 h after treatment.

According to the goodness of fit test, deviation of the obtained data was low enough to perform a probit analysis for allyl isothiocyanate ($X^2 = 25.56$, df = 19), and a linear relation between concentration and mortality was evident from the t value of the slope ($t = 6.68$). The index of significance of the potency estimation ($g = 0.13$) was also low enough to estimate LC values with 95% confidence. The intercept and slope (± SE) of the probit line were 0.87 and 5.10 ± 0.76, respectively. The estimated LC_{50} (95% confidence limits) was 0.68 (0.55-0.78) µl l^{-1} of air. According to the goodness of fit test ($X^2 = 56.64$, df = 13), the data for *A. sativum* oil was not suitable for performing probit analysis.

The Effect of Exposure Time on Mortality in Adult German Cockroaches

Figure 3 shows the effect of exposure time on mortality in 2 groups of adult *B. germanica*—experimental (allyl isothiocyanate) and control. Allyl isothiocyanate treatment (2.5 µl l^{-1} of air) caused over 40% mortality 6 h after treatment, which increased to 100% mortality 18 h after treatment, whereas the control treatment produced no mortality at either time. Similarly, Figure 4 shows the effect of exposure time on mortality in 2 groups of adult *B. germanica*—experimental (*A. sativum* oil) and control. Treatment with *A. sativum* (5 µl l^{-1} of air) caused about 30% mortality 24 h after treatment, which increased to 93% mortality 48 h after treatment, whereas the control treatment produced significantly less mortality at both times.

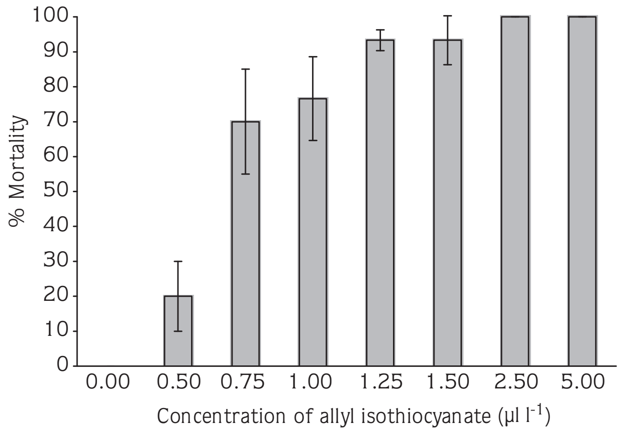

Figure 1. The influence of allyl isothiocyanate concentration on mortality in *B. germanica* adults. The adults were treated with the indicated concentrations of allyl isothiocyanate and mortality was recorded 24 h after treatment.

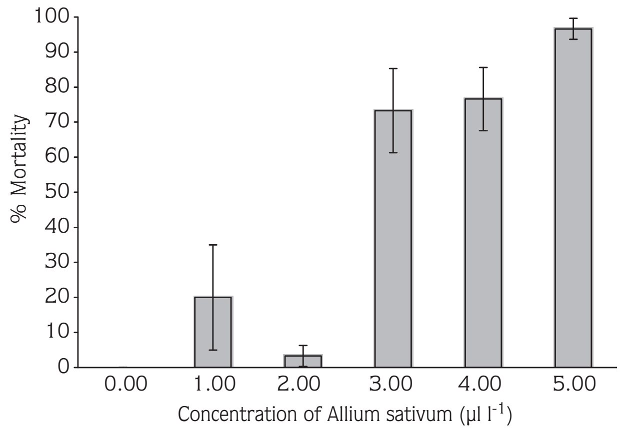

Figure 2. The influence of *Allium sativum* oil concentration on mortality in adult *B. germanica* adults. The adults were treated with the indicated concentrations of *A. sativum* oil solution and mortality was recorded 48 h after treatment.

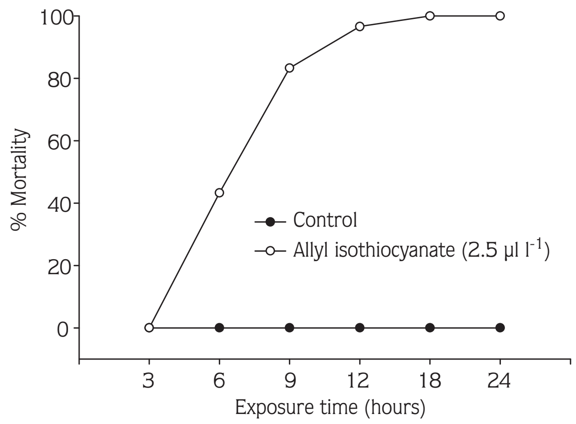

Figure 3. Mortality in *B. germanica* adults exposed to allyl isothiocyanate (2.5 µl l^{-1} of air) for various periods of time.

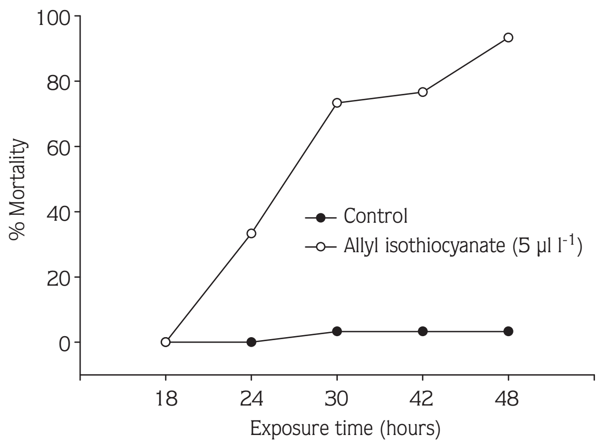

Figure 4. Mortality in *B. germanica* adults exposed to *Allium sativum* oil (5 µl l^{-1} of air) for various periods of time.

According to the goodness of fit test, deviation of the obtained data was low enough to perform a probit analysis for allyl isothiocyanate (X^2 = 4.55, df = 16), and a linear relationship between exposure time and mortality was evident from the t value of the slope (t = 6.17). The index of significance of the potency estimation (g = 0.10) was also low enough to estimate LT values with 95% confidence. The intercept and slope (± SE) of the probit line were –5.86 and 7.20 ± 1.17, respectively. The estimated LT_{50} (95% confidence limits) was 6.51 h (range: 5.72-7.20 h) after treatment. Again, according to the goodness of fit test (X^2 = 31.10, df = 13), the data for *A. sativum* oil was not suitable for performing probit analysis.

Discussion

The results of the present study strongly support the idea that 2 of the tested monoterpenoid components and essential oils significantly increased adult cockroach mortality due to their fumigant activity. First, relative to control adults, allyl isothiocyanate and *A. sativum* oil caused significantly higher rates of adult mortality. Second, mortality due to allyl isothiocyanate and *A. sativum* was expressed in a concentration-dependent manner. Finally, treating *B. germanica* with allyl isothiocyanate and *A. sativum* oil significantly caused mortality at all time points in the exposure time experiments. We infer from these data that plant essential oils and the monoterpenoid components could be used as alternatives for *B. germanica* control. Moreover, we conclude that allyl isothiocyanate and *A. sativum* oil were very effective fumigants, and as the mode of delivery of these oils was by vapor action their effects were likely to have been via the respiratory system.

Although many plant essential oils are reported to have insecticidal or fumigant activity against insect pests (Kim and Ahn, 2001; Kim et al., 2003; Choi et al., 2006; Park et al., 2006; Sahaf et al., 2007), control of *B. germanica* has mostly depended on synthetic pesticides (Rust et al., 1993; Agrawal et al., 2005; Tilak et al., 2005). Few studies report that essentials oils and monoterpenoid components have insecticidal activity against *B. germanica* (Appel et al., 2001; Chang and Ahn, 2001; Peterson et al., 2002). Chang and Ahn (2001) showed that *illicium* fruit-derived materials had potent insecticidal activity against *B. germanica.* They indicated that (E)-anethole from *Illicum verum* fruit caused 80.3% mortality at 0.159 mg cm^{-2} 1 and 3 days after treatment. Similarly, Appel et al. (2001) observed that when fumigated with 50 µl of 100% mint oil, 100% of *B. germanica* were killed after 24 h. In the present study, among the 9 tested commercial essential oils and monoterpenoid components, allyl isothiocyanate at the concentration of 5 µl l^{-1} of air caused 100% mortality in adult cockroaches 24 h after treatment and *A. sativum* oil at 5 µl l^{-1} of air caused over 95% mortality in adult cockroaches 48 h after treatment, which is comparable to the results of similar experiments reported by Chang and Ahn (2001), and Appel et al. (2001).

Ally isothiocyanate is known to be a principal pungent constituent of horseradish essential oil and has been shown to have strong antimicrobial activity in its vapor form (Delaquis and Sholberg, 1997; Li et al., 2007). In the present study allyl isothiocyanate had greater and quicker fumigant activity against *B. germanica*, as compared with the other tested essential oils and monoterpenoid components. The LC_{50} value of allyl isothiocyanate in the present study was 0.68 μl l^{-1} of air. This finding is in agreement with the outcome of a similar experiment with *Lycoriella ingenua* (Park et al., 2006), in which allyl isothiocyanate was the most toxic compound to *L. ingenua*, as compared with other essential oils (such as, *A. sativum*, o-anisaldehyde, and diallyl disulfide). The LC_{50} value of allyl isothiocyanate in that study was 0.15 μl l^{-1} of air.

Results of the present study indicate that allyl isothiocyanate from horseradish essential oil and *A. sativum* oil could be useful as fumigants against *B. germanica*. For the practical use of this essential oil and monoterpenoid component as novel fumigants, additional study is required on their safety for mammals and on the development of formulations to improve their effectiveness and stability.

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