Chemical constituents and toxic, repellent, and oviposition-deterrent effects of ethanol-extracted *Myristica fragrans* (Myristicaceae) oil on *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract

Most studies on the essential oil of nutmeg (*Myristica fragrans* Houtt,; Myristicaceae) have been laboratory based. To our knowledge, this is the first study describing the practical application of the essential oil of this species in the greenhouse for controlling the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Three concentrations (10, 5, and 2.5 mg/mL) of the ethanol-extracted essential oil of *M. fragrans* were prepared. In laboratory experiments, fumigation toxicity was evaluated by applying 0.1 mL of extracted oil onto a filter paper (6 cm diameter) that was attached to the inside of the cap of a 100 mL glass jar containing 20 whiteflies; mortality was recorded 1, 2, 4, and 8 h after application. For assessment of contact toxicity, a tomato leaf was treated with 0.1 mL of the essential oil extract solutions and placed in cages containing 20 whiteflies each; the mortality was recorded at 3, 6, 12, and 24 h of the bioassay. For determination of the repellency effect, 2 leaves, 1 treated with extracted oil and another with the control solution, were placed in cages, and 20 insects were released into each cage; repellency was observed after 24, 48, and 72 h. In the greenhouse, 2 potted plants were placed in a cage; 1 was treated with extracted oil and the other was treated with a control solution; 100 whiteflies were released into the cage, and repellency and anti-oviposition effects were observed at 24 and 48 h of the bioassay. Maximum fumigation toxicity (79.17 ± 3.00%), contact toxicity (72.50 ± 4.23%), and repellency (76.67 ± 7.15%) were observed at 10 mg/mL in the laboratory experiments. The maximum repellency (58.33 ± 3.50%) and anti-oviposition (46.11 ± 5.38%) effects were observed at 24 h of exposure in greenhouse tests. These results suggest that the essential oil of *M. fragrans* was toxic, repellent, and prevented whitefly oviposition in laboratory and greenhouse tests. Further studies are recommended to assess the bioactivity of the chemical components of the essential oil on other insect species.

Key Words: nutmeg; GC-MS; bioactivity; whitefly

Resumen

La mayoría de los estudios sobre el aceite esencial de nuez moscada (*Myristica fragrans* Houtt.; Myristicaceae) han sido hachos en el laboratorio. A nuestro entender, este es el primer estudio que describe la aplicación práctica del aceite esencial de esta especie en el invernadero para el control de la mosca blanca *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Se prepararon tres concentraciones (10, 5 y 2,5 mg/ml) del aceite esencial de *M. fragrans* extraído con etanol. En experimentos de laboratorio, se evaluó la toxicidad de la fumigación aplicando 0,1 ml de aceite extraído sobre un papel de filtro (6 cm de diámetro) que se unió al interior de la tapa de un frasco de vidrio de 100 ml que tenía 20 moscas blancas; se registró la mortalidad a los 1, 2, 4 y 8 horas después de la aplicación. Para la evaluación de la toxicidad de contacto, se trató una hoja de tomate con 0,1 ml de la solución de extracto de aceite esencial y se colocaron en jaulas que tenían 20 moscas blancas cada una; se registró la mortalidad a las 3, 6, 12 y 24 horas del bioensayo. Para la determinación del efecto de repelencia, 2 hojas, 1 tratada con aceite extraído y otra con la solución de control, fueron colocadas en las jaulas, y se liberaron 20 insectos en cada jaula; se observó la repelencia a las 24, 48 y 72 horas. En el invernadero, 2 plantas en macetas fueron colocadas en una jaula; 1 se trató con aceite extraído y el otro se trató con una solución de control; se liberaron 100 moscas blancas en la jaula y se observaron los efectos de la repelencia y anti-oviposición a las 24 y 48 horas del bioensayo. En los experimentos de laboratorio se observaron la toxicidad máxima de fumigación (79,17 ± 3,00%), toxicidad de contacto (72,50 ± 4,23%) y repelencia (76,67 ± 7,15%) a 10 mg / ml. Se observaron los efectos de la repelencia máxima (58,33 ± 3,50%) y anti-oviposición (46,11 ± 5,38%) a las 24 horas de exposición en los ensayos en invernadero. Estos resultados sugieren que el aceite esencial de *M. fragrans* mostró toxicidad, repelencia y actividades anti-oviposición en el laboratorio y en el invernadero. Se recomiendan estudios adicionales para evaluar la bioactividad de los componentes químicos del aceite esencial en otras especies de insectos.

Palabras Clave: nuez moscada; CG-SM, bioactividad; mosca blanca

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a notorious insect pest of agricultural and horticultural plants in greenhouses as well as in open fields (Barro et al. 1998; Lapidot & Polston 2006; Bográn & Heinz 2016). It is known to suck plant cell sap, release honeydew that causes sooty mold, and is responsible for the transmission of several kinds of plant viruses. The management of this pest is challenging once its population builds up. Scientists are increasingly refraining from the use of synthetic chemicals and are now focus-

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ing on new environmentally safe methods such as using plants, and their essential oils, that repel insects and have a lethal effect. Essential oils are responsible for protecting plants from many endogenous and exogenous infections. They provide defense against insect pests, herbivores, and microorganisms, and are responsible for attracting insects that facilitate pollination (Pichersky & Gershenzon 2002; Bakkali et al. 2008). Essential oils also have applications in food preservation, agronomy, and in the manufacture of pharmaceutical and perfumery products (Buchbauer 2000).

Nutmeg (*Myristica fragrans* Houtt.; Myristicaceae) is an evergreen tree that typically grows up to 9.12 m in height; it is indigenous to Indonesia, India, and Sri Lanka, and is now being cultivated in tropical regions of the world, including South Africa (Krishnamoorthy et al. 2001; Pal et al. 2011). The seed of this plant has 2 major parts—the seed itself, commonly referred to as "nutmeg" and the outer layer of the seed called "mace." Both these products are used as spices (Krishnamoorthy & Rema 2001). Nutmeg plays an important role in plant defense, protecting against many infections, infestations, and diseases. Its uses in traditional medicine have been reported (Latha et al. 2005). Essential oils from the nutmeg seed have applications in controlling harmful organisms, and they have been shown to be toxic to insects such as cockroaches (Krishnamoorthy et al. 2001) and termites *Microcerotermes beesoni* Snyder (Isoptera: Hodotermitidae), with an LC_{50} value of 28.6 mg/g (Pal et al. 2011). Nutmeg essential oil showed nematicidal activity against the southern root-knot nematode *Meloidogyne incognita* (Tylenchida: Heteroderidae) (Gotke & Maheswari 1990) and antimicrobial activity against *Salmonella typhi* Kauffmann & Edwards (Enterobacteriaceae) at a concentration of 12.5 mg/mL (Rani & Khullar 2004).

The present study was planned to determine the fumigant and contact toxicity effects as well as repellency effects of different concentrations of ethanol-extracted *M. fragrans* essential oil in the laboratory and repellency and anti-oviposition activities in the greenhouse. We identified the chemical components of the essential oil using gas chromatography–mass spectrometry. We believe that the results of this study will be useful in the safe and organic control of whitefly.

Materials and Methods

INSECT AND PLANT CULTURE

Whiteflies were reared on tomato and sweet potato year-round as alternative hosts in a greenhouse under 60 ± 10% relative humidity (RH) and 20 to 30 °C temperature conditions, without any application of chemicals on the plants. The experiments were performed in the early winter during Oct and Nov 2016 at Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, Huazhong Agricultural University, Wuhan, China. Tomato seeds (variety 'Xian Zao Hong') were sown in plastic pots (15 cm diameter) containing organic matter. For experimental use, the tomato plants were transplanted to pots (12 cm diameter) containing an equal ratio of clay loamy soil and organic matter. The potted plants bearing between 30 and 40 leaves were used for the repellency experiment in the greenhouse. In addition, 1 leaf was used for the contact toxicity experiment, and 2 opposite leaves on a stem were used for repellency tests in the laboratory.

ETHANOL EXTRACTION OF ESSENTIAL OILS

The fruits of *M. fragrans* were purchased from a seed shop registered with Beijing Tongrentang Group, China. The extraction of essential oil was based on the method described by Su et al. (2009). The seeds were cleaned and dried in a microwave oven for 2 d; they were then ground in an electric grinder and sieved through a 40 mm mesh. The powder was extracted with 95% ethanol (1 g powder with 5 mL ethanol) in the dark for 7 d at 20 to 25 °C; the bottles in which the extraction was performed were rotated twice a day for proper mixing. Thereafter, the solvent extract was filtered through a filter paper into a conical flask, and the residue was extracted again with 2.5 mL ethanol per gram of powder under the same conditions. Both the 1st and 2nd filtrates were pooled and dried in a rotary evaporator; the oil was thus extracted along with a residue of bulk solids on the bottom. We obtained 88.70 g of crude oil from 516.44 g of nutmeg seeds.

For dissolution and preparing the test solution, we dissolved 0.05 g of the crude oil in 0.3 mL of dimethyl sulfoxide (DMSO) (Sinopharm Chemical Reagent Co., Ltd., Beijing, China) and added 1% Tween-20 (Sinopharm Chemical Reagent Co., Ltd., Beijing, China). Finally, double-distilled water was added to bring the volume to 5 mL for a final concentration of 10 mg/mL (10,000 ppm). For dilution, 1 mL of 10 mg/mL oil was added to 1 and 3 mL of double-distilled water to obtain final concentrations of 5 mg/mL (5,000 ppm) and 2.5 mg/mL (2,500 ppm), respectively. For the control solution, 0.3 mL of DMSO, 1% Tween-20, were mixed and double-distilled water was added to achieve the volume of 5 mL.

LABORATORY EXPERIMENTS

Fumigant Toxicity

For this experiment, 0.1 mL of 2.5 mg/mL extract was applied with a micropipette on 6-cm-diameter filter paper discs, and the same volume of control solution was applied to a control filter paper disc. After 1 h of application, when the liquids had dried from the filter paper, the discs were attached to the underside of the cap on a 100 mL glass jar. Thereafter, twenty 5-d-old adult whiteflies were aspirated into the jars, which were covered with a cap and wrapped with polythene strips to seal the jar. The jars were then incubated in the laboratory at 25 ± 2 °C, 50 ± 5% RH, and a photoperiod of 10:14 h L:D. Throughout the experiment, no contact of insects with the filter paper was observed. Insect mortality was recorded at 1, 2, 4, and 8 h from the start of the bioassay. The experiment was repeated with 0.1 mL of the 5 mg/mL and 0.1 mL of the 10 mg/mL extracts; and 8 replications were performed for each treatment.

Contact Toxicity

Two opposite leaves on the stem of a tomato plant that already had whitefly nymphs were used in this test. The test solution (0.1 mL of 2.5 mg/mL) of the extracted essential oil was applied to a single leaf with a cotton wick; the same volume of control solution was applied to a control leaf. The leaves were placed in cages and the leaf petiole was dipped in water placed in a container underneath the cage. Nymphal mortality was recorded at 6, 12, 24, and 48 h from the beginning of the bioassay. The experiment was repeated with 0.1 mL of the 5 mg/mL and 0.1 mL of the 10 mg/mL extracts; and 8 replications were performed for each treatment.

Repellency Test

Two opposite leaves of tomato were used for the experiment; 1 leaf was treated with 0.1 mL (2.5 mg/mL) of the test solution and the other leaf was treated with the same volume of the control solution. The leaves were placed in a round cage (12 cm diameter, 20 cm height) and their petioles dipped in water provided in a 150 mL bowl on the bottom of the cage; the bowl was covered to prevent the drowning of the flies. Twenty 5-d-old whiteflies were aspirated from a colony and released into the cage. Repellency was observed at 24, 48, and 72 h after insect release. The experiment was re-

peated with 0.1 mL of the 5 mg/mL extract and 0.1 mL of the 10 mg/mL extract; and 8 replications were performed for each treatment.

GREENHOUSE EXPERIMENT

Repellency and Oviposition Deterrence

Potted tomato plants with 30 to 40 leaves were sprayed with 10 mL (2.5 mg/mL) of the test solution with a hand sprayer; the same volume of the control solution was sprayed on the control plants. Thirty minutes after the spray, the pots with both the treated and control plants were covered with a thin cloth-made cage (80 cm height, 60 cm width, and 60 cm length). One hundred 5-d-old female adult whiteflies were aspirated from the colony and released into the cages. The response of the insects with respect to their choice was observed 24 and 48 h after release into the cage in the early morning when the insects were not active. Ten leaves were collected randomly from each plant after every observation, and these were microscopically examined to count deposited eggs. The experiment was repeated with 10 mL of the 5 mg/mL solution and 10 mL of the 10 mg/mL extract; and 8 replications were performed for each treatment.

IDENTIFICATION OF THE CHEMICAL COMPONENTS OF THE ESSENTIAL OIL

The chemical components of the essential oil were identified by gas chromatography–mass spectrometry on a Varian 450-GC/320-MS (Varian, Inc., Walnut Creek, California). An HP-5MS capillary column (film thickness: 30 m length × 0.25 mm inner diameter) was used, and the compounds were detected with a flame ionization detector. For gas chromatography, the injector oven temperature was initially maintained at 60 °C for 3 min, ramped at 10 °C/min to 180 °C and maintained for 1 min, and ramped again at 20 °C/min to 280 °C and maintained for 15 min. One microliter of the samples diluted with 1% hexane was injected with a split ratio of 1:10. The column pressure was maintained at 100 kPa. Helium gas, passed at a rate of 1.0 mL/min, was used as the sample carrier. The temperatures of the MS quadrupole, ion source, and transmission line were 150, 230, and 250 °C, respectively. Chemical constituents were identified from the gas chromatogram with the online libraries of Wiley, REPLIB, MANLIB, and PMWTox3N (NIST 2011).

STATISTICAL ANALYSES

Insect repellency percentage was calculated according to Liu et al. (2013), based on the formula: PR (%) = [(C − T)/(C + T)] × 100. A chi-squared test was used to evaluate the comparison between the treatment and control repellencies. A paired *t*-test was used to compare the number of eggs in the treated and control samples. One-way analysis of variance (ANOVA) and the Tukey test were used to analyze the differences in the mean percentages of toxicity, repellency, and oviposition with SPSS® 20.0 for Windows 2007 (SPSS, Inc., Chicago, Illinois). The percentage was subjected to an arcsine square-root transformation before ANOVA and Tukey test. All the figures were drawn with SigmaPlot® version 10.0 for Windows 2007 (Systat Software, Inc., San Jose, California).

Results

LABORATORY EXPERIMENT

Fumigant Toxicity

Different concentrations of the essential oil from *M. fragrans* showed fumigant toxicity against whitefly adults. We observed significant differences at 1 h (F = 25.12; df = 2, 15; P < 0.01), 2 (F = 46.75; df = 2, 15; P < 0.01), 4 h (F = 81.14; df = 2, 15; P < 0.01), and 8 h (F = 241.57; df = 2, 15; P < 0.01) between the tested concentrations (Fig. 1). Tests with the 10 mg/mL concentration of the essential oil showed the maximum toxicity (22.50 ± 2.14, 32.50 ± 3.35, 45.83 ± 3.00, and 79.17 ± 3.00%, respectively) at 1, 2, 4, and 8 h of the bioassay, followed by concentrations of 5 mg/mL and 2.5 mg/mL. The toxicities increased with increase in time, and maximum mortality was observed in the final observation at 8 h (Fig. 1).

Contact Toxicity

Significant differences in contact toxicity were obtained between the concentrations at 6 h (F = 9.09; df = 2, 15; P < 0.01), 12 h (F = 24.75; df = 2, 15; P < 0.01), 24 h (F = 23.63; df = 2, 15; P < 0.01), and 48 h (F = 72.43; df = 2, 15; P < 0.01). Maximum concentration resulted in high mortality and minimum concentration resulted in the lowest mortality. The maximum toxicity (72.50 ± 4.23%) was observed at 48 h in the bioassay with the highest concentration (10 mg/mL) of the essential oil, followed by the toxicities at 5 mg/mL and 2.5 mg/mL (Fig. 2).

Repellency

The results from ANOVA showed significant differences in repellency for all concentrations at 24 h (F =14.12; df = 2, 15; P < 0.01), 48 h (F = 15.59; df = 2, 15; P < 0.01), and 72 h (F =13.07; df = 2, 15; P < 0.01) of the bioassay. The maximum repellency was observed with 10 mg/mL at 24, 48, and 72 h with mean values of 76.67 ± 7.15, 61.67 ± 4.77, and 56.67 ± 7.60%, respectively, followed by the repellencies at 5 mg/mL and 2.5 mg/mL (Fig. 3). The chi-squared test showed a strong and significant difference between the treatment with 10 mg/mL and the control at 24 h (χ^2 = 41.34; df = 1; P < 0.01), 48 h (χ^2 = 25.21; df = 1; P < 0.01), and 72 h (χ^2 = 20.95; df = 1; P < 0.01). We also observed significant differences between 5 mg/mL and the control at 24 h (χ^2 = 17.16; df = 1; P < 0.01), 48 h (χ^2 = 8.35; df = 1; P < 0.01), and 72 h (χ^2 = 6.17; df = 1; P < 0.01). However, poor repellency was observed at 2.5 mg/mL with the difference between treatment and control being significant only at 24 h (χ^2 = 4.92; df = 1; P < 0.01) (Fig. 4).

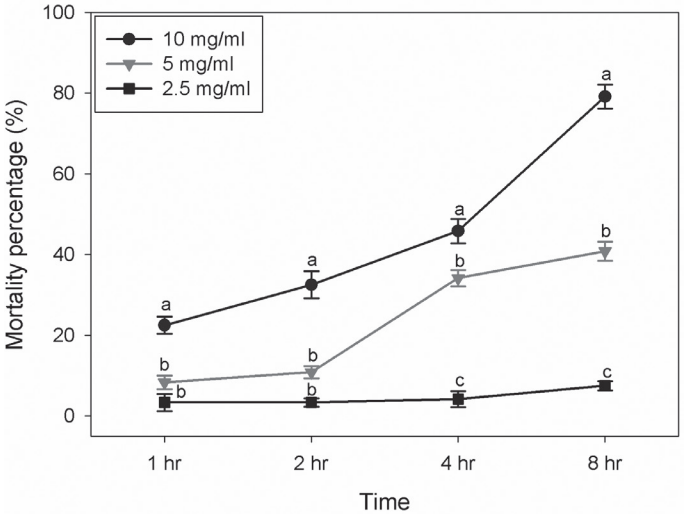

Fig. 1. Fumigant toxicity of nutmeg essential oil to whitefly adults in laboratory experiments at concentrations of 10, 5, and 2.5 mg/mL. Values are the means of 8 replications. The mean numbers of adults were analyzed by 1-way ANOVA, with a Tukey HSD post-hoc test at a significance level of P < 0.05; means topped by the same letter are not significantly different.

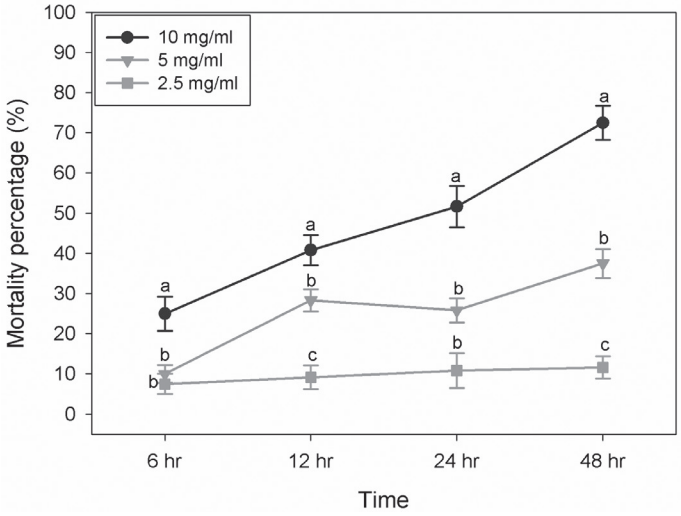

Fig. 2. Contact toxicity of nutmeg essential oil to whitefly nymphs in laboratory experiments at concentrations of 10, 5, and 2.5 mg/mL. Values are the means of 8 replications. The mean numbers of nymphs were analyzed by 1-way ANOVA, with a Tukey HSD post-hoc test at a significance level of $P < 0.05$; means topped by the same letter are not significantly different.

GREENHOUSE EXPERIMENT

Adult Repellency

The highest concentration of essential oil resulted in escape of the maximum number of adult whiteflies from the treated plants. The maximum repellency was observed at 10 mg/mL of the essential oil at 24 h (58.33 ± 3.50%) and 48 h (45.67 ± 5.18%) of exposure, followed by that at 5 mg/mL and 2.5 mg/mL; there were significant differences between the repellency at all the concentrations at 24 h ($F = 65.37$; df = 2, 15; $P < 0.01$) and 48 h ($F = 22.65$; df = 2, 15; $P < 0.01$) of the bioassay (Fig. 5). The chi-squared analysis of adult repellency showed strong and significant differences between the control and the 10 mg/mL concentration at 24 h ($\chi^2 = 62.99$; df = 1; $P < 0.01$) and 48 h ($\chi^2 = 40.18$; df = 1; $P < 0.01$). The difference in repellency between 5 mg/mL and the control was significant only at 24 h ($\chi^2 = 7.10$; df = 1; $P < 0.01$) but not at 48 h ($\chi^2 = 2.62$; df = 1; $P > 0.06$), and it was significant between the control and 2.5 mg/mL at 24 h ($\chi^2 = 0.85$; df = 1; $P < 0.19$) and 48 h ($\chi^2 = 0.05$; df = 1; $P < 0.43$) of exposure (Fig. 6).

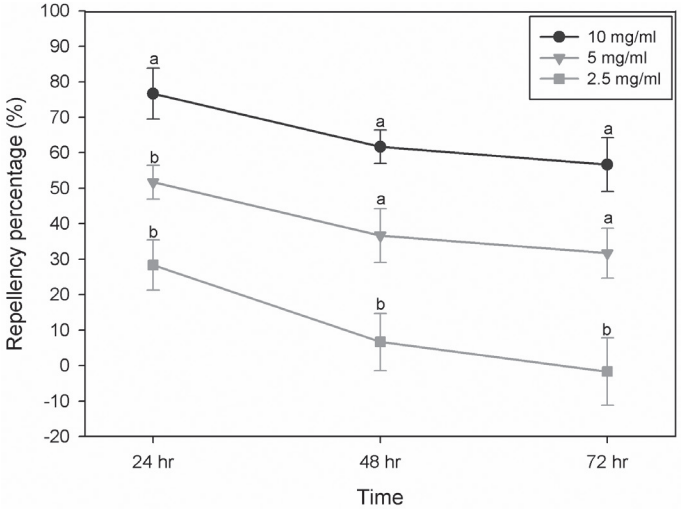

Fig. 3. Repellency of nutmeg essential oil to whitefly adults in laboratory experiments at 24, 48, and 72 h of exposure to concentrations of 10, 5, and 2.5 mg/mL. Values are the means of 8 replications. The mean numbers of adults were analyzed by 1-way ANOVA, with a Tukey HSD post-hoc test at a significance level of $P < 0.05$; means topped by the same letter are not significantly different.

Oviposition Deterrence

The 10 mg/mL concentration had the strongest anti-oviposition activity among all the concentrations tested at 24 and 48 h of the bioassay, with an anti-oviposition index (i.e., reduction of oviposition compared with the control, based on numbers of eggs) of 46.11 ± 5.38% and 35.86 ± 4.05%, respectively, followed by the values obtained for the 5 mg/mL and 2.5 mg/mL concentrations. There were significant differences among all the concentrations at 24 h ($F = 16.40$; df = 2, 15; $P < 0.01$) and 48 h ($F = 17.27$; df = 2, 15; $P < 0.01$) of exposure (Fig. 5). The results of *t*-test analysis showed significant differences between the highest concentration, i.e., 10 mg/mL and the control at 24 h ($t = 5.13$; df = 5; $P < 0.01$) and 48 h ($t = 6.91$; df = 5; $P < 0.01$), whereas at 5 mg/mL, significant repellency compared to the control was observed only at 24 h ($t = 3.60$; df = 5; $P < 0.02$); no significant difference was observed at 48 h ($t = 2.45$; df = 5; $P > 0.06$). Similarly, no significant difference was observed with the 2.5 mg/mL concentration at 24 h ($t = 0.58$; df = 5; $P > 0.59$) and 48 h ($t = 4.27$; df = 5; $P < 0.01$) of the exposure (Fig. 6).

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL

Analysis by gas chromatography–mass spectrometry revealed the chemical composition of the ethanol-extracted essential oil from the seeds of *M. fragrans*. Twenty components were identified (Table 1). Of these, sulfonylbismethane, alpha-cubebene, 1,3-benzodioxole, 4-methoxy-1,3-benzodioxole , 1-methoxybenzene, tetradecanoic acid, 4,5-dimethoxyphthalide, desmethylnomifensine, agarospirol, and ethanol were the major constituents of the oil.

Discussion

LABORATORY EXPERIMENT

The essential oil extracted from *M. fragrans* showed fumigant action against whitefly adults and contact toxicity against whitefly nymphs. The maximum toxicity was obtained with the highest concentration of 10 mg/mL, and was followed by the toxicities at the lower concentrations. The toxic effects of the essential oil have been previously described by Jung et al. (2007) and Jaiswal et al. (2009). The compounds in the seed of *M. fragrans* were toxic against adult female German cockroaches, *Blattella germanica* L. (Blattodea: Ectobiidae). *Myristica fragrans* essential oil was toxic to the immature stage of *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), and *Lycoriella ingenua* Dufour (Diptera: Sciaridae) (Chaubey 2008; Park et al. 2008). The results of this and previous studies demonstrate that *M. fragrans* is toxic to several insect species.

We observed maximum repellency up to 72 h in the laboratory with concentrations of 10 mg/mL and 5 mg/mL; however, the lower concentration of 2.5 mg/mL had no effective repelling activity towards the whitefly females. The ethanol-extracted essential oil from the seeds of *M. fragrans* was reported to repel the malarial vector mosquito, *Anopheles stephensi* Liston (Diptera: Culicidae), from the test person until 210 min of exposure, with no allergy observed in the test person (Subramaniam & Murugan 2013). Hydrodistilled essential oil from the seeds of *M. fragrans* and the major components

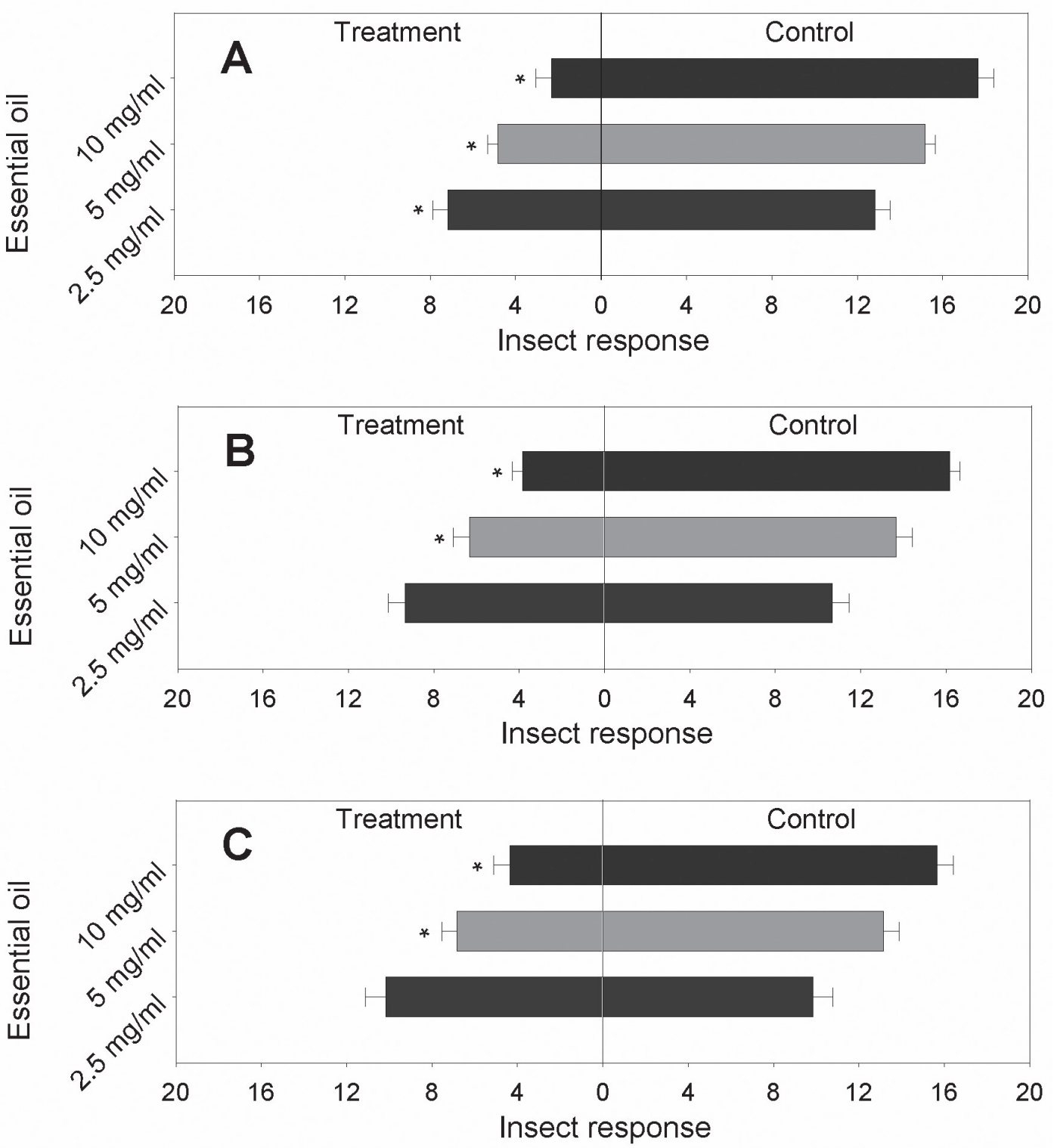

Fig. 4. Repellency of nutmeg essential oil to whitefly adults in laboratory experiments at A. 24 h, B. 48 h, and C. 72 h of exposure to concentrations of 10, 5, and 2.5 mg/mL. Values are means of 8 replications. The mean numbers of adults were compared by paired *t*-tests at a significance level of $P \leq 0.05$. Asterisk indicates a significant difference between control and treatment.

showed strong repellency to the cigarette beetle, *Lasioderma sericorne* F. (Coleoptera: Anobiidae), in an area preference test with 9 cm Petri dishes in the laboratory (Du et al. 2014). In the present study, we observed that *M. fragrans* repelled the whiteflies wherever they had a choice available.

GREENHOUSE EXPERIMENT

Whitefly is a greenhouse pest, and in our experiments, the application of essential oils in the greenhouse represented a model for controlling the pest. We observed that the highest concentration of

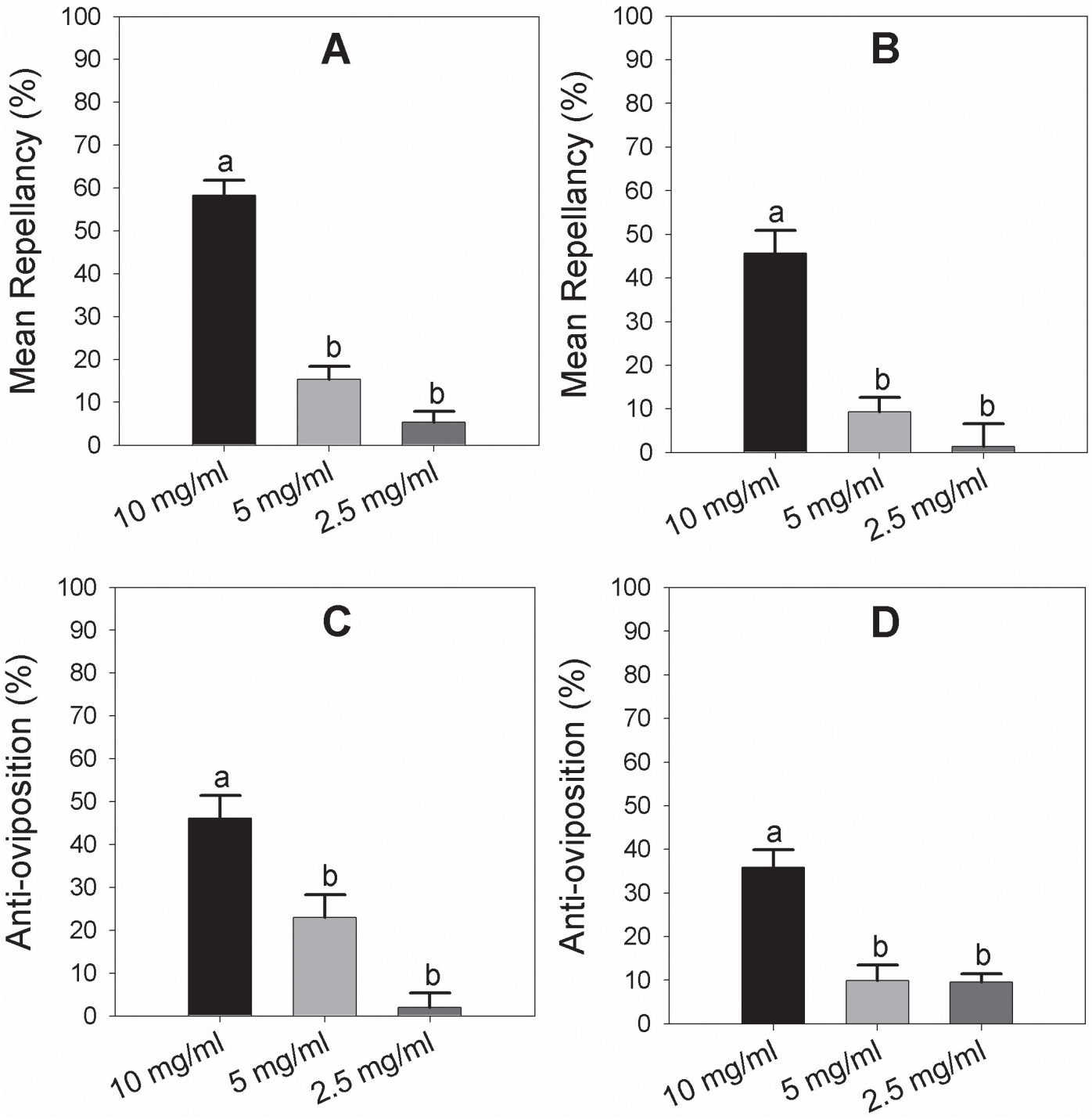

Fig. 5. Greenhouse experiments with whitefly adults, testing nutmeg essential oil at concentrations of 10, 5, and 2.5 mg/mL. A. Repellency at 24 h, B. repellency at 48 h, C. oviposition at 24 h, D. oviposition at 48 h of exposure. Values are means of 8 replications. The mean numbers of adults and eggs were analyzed by 1-way ANOVA, with a Tukey HSD post-hoc test at a significance level of $P < 0.05$; means topped by the same letter are not significantly different.

essential oil resulted in the escape of the maximum number of whiteflies from the treated plants for the maximum period of time, confirming the repellent activity of *M. fragrans* essential oil we had observed under the laboratory conditions. When applied on humans, the oil protected against 4 mosquito species, namely *Aedes albopictus* Skuse (Diptera: Culicidae), *Aedes aegypti* (L.) (Diptera: Culicidae), *Anopheles dirus* Peyton & Harrison (Diptera: Culicidae), and *Culex quinquefasciatus* Say (Diptera: Culicidae), with varying durations of repellence observed (range: 2.8–8 h) (Tawatsin et al. 2006). Most of the studies on essential oil of *M. fragrans* have been laboratory based, but our study is the first to propose practical applications of this oil. We observed that in the greenhouse, the highest concentration of the essential oil showed maximum repellency but its effect did not last longer than 48 h and was shorter than the repellency in the laboratory experiment, which lasted up to 72 h of the exposure.

In our study, the essential oil was not only toxic and repellent to the whitefly females, but it also prevented oviposition up to 48 h of exposure in the greenhouse trial. The results agree with those of Su

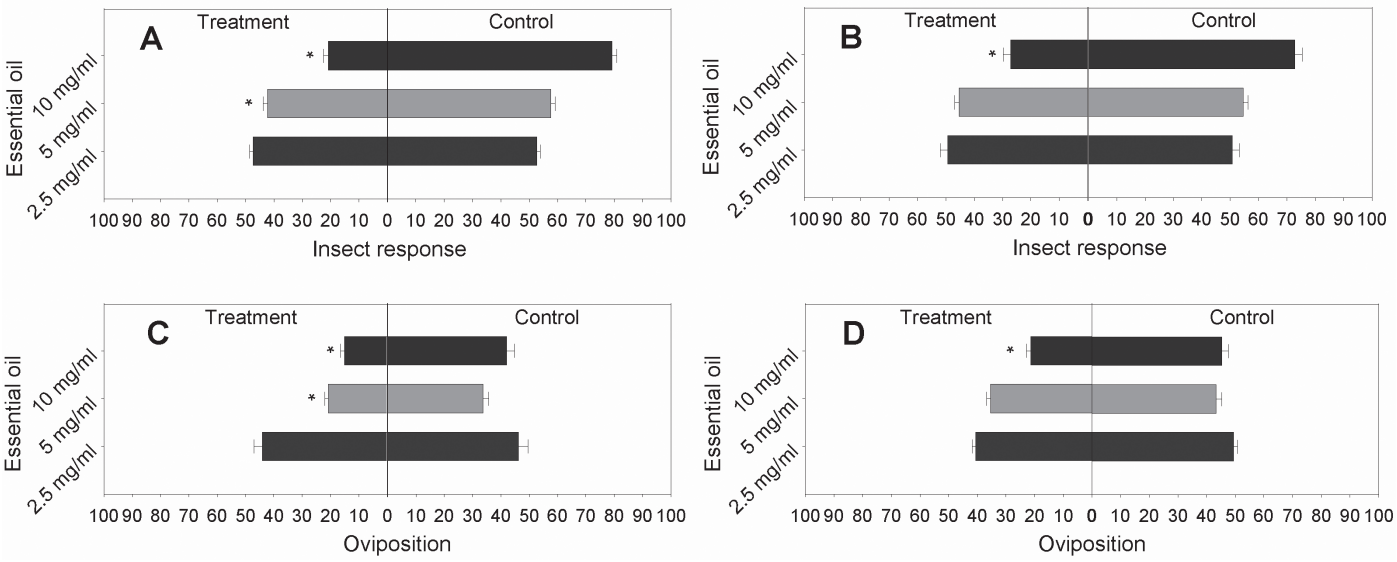

Fig. 6. Greenhouse experiments testing repellency and oviposition deterrence of nutmeg essential oil against whiteflies. A. Repellency at 24 h, B. repellency at 48 h, C. oviposition at 24 h, D. oviposition at 48 h of exposure. Values are means of 8 replications. The mean numbers of adults or eggs were compared by paired *t*-tests at a significance level of $P \leq 0.05$. Asterisk indicates a significant difference between control and treatment.

et al. (2009), where the ethanol-extracted *M. fragrans* essential oil had anti-oviposition activity of 83.40% against the brown planthopper *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) at 24 h of exposure. The essential oil from *M. fragrans* deterred oviposition in the mosquito *Ae. aegypti* when compared with 2 synthetic insecticides (Tawatsin et al. 2006). From our observations, and based on previous results, we can suggest that the essential oil of *M. fragrans* shows oviposition-deterrent activity against some harmful insect species.

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL

To our knowledge, no previous complete analysis of the chemical components in the essential oil of nutmeg has been published. However, previous studies with some of the chemical components that we have found in *M. fragrans* essential oil showed that they exert bioactivity against many organisms. For example, alpha-phellandrene is a volatile chemical and is harmful to human when it comes in contact with the human body upon evaporation in air at high temperature (Urben 2007). Delta 3-carene can cause neuro-depression and skin irritation when it is used at high concentrations (Wikipedia 2016). Sabinene has antimicrobial properties against fungi and bacteria (Arunkumar et al. 2014). Beta-pinene has toxic properties against a dimorphic fungus, *Candida albicans* Berkhout (Saccharomycetaceae), and can cause death of 100% of the inoculum within 1 h (Rivas da Silva et al. 2012). 1,3-Benzodioxole is a colorless organic liquid containing methylenedioxyphenyl, which is found in pharmaceuticals and pesticides (Murray 2000). Tetradecanoic acid, also called myristic acid, is used as a flavoring agent. It has been shown to possess low acute toxicity in rodent

Table 1. Chemical constituents of *Myristica fragrans* essential oil based on gas chromatography–mass spectrometry analysis.

Component	Percentage of Total	Retention Time
sulfonylbismethane	27.22	5.15
alpha-phellandrene	0.13	5.92
delta-3-carene	0.03	6.03
sabinene	0.53	6.47
beta-pinene	0.12	6.55
gamma-terpinene	0.33	7.33
trans-sabinene hydrate	0.28	7.48
4-oxo-beta-isodamascol	0.31	7.95
phenyl-cis-3,3a,4,5,6,6a-hexahydro-4,5,6-methenocyclopentapyrazole	0.95	8.45
1,3-benzodioxole	0.78	9.30
alpha-cubebene	4.84	9.99
1-[4-hydroxybenzyl]-1,2,3,4-tetrahydro-7-methoxy-isoquinolin-6-ol	1.86	10.62
4-methoxy-1,3-benzodioxole	7.29	10.84
1-methoxybenzene	4.67	10.92
agarospirol	0.04	11.02
tetradecanoic acid	2.25	12.18
5,5-dimethyl-2,2′-bithienyl	0.73	11.27
4,5-dimethoxyphthalide	1.27	17.8
desmethylnomifensine	1.97	18.81
allyl-3-phenyl-3-trimethylstannybutanoate	0.72	18.71

species and may cause irritation in skin and eyes if used in pure form (Burdock & Carabin 2007). Our observations show that the essential oil from nutmeg seed has toxic, repellent, and oviposition deterrent action against whitefly, possibly due to one or more of its chemical components.

The ethanol-extracted essential oil of *M. fragrans* showed bioactivity against adult female whiteflies, with different concentrations exerting significant activities for different durations. The maximum activity (toxic, repellent, and oviposition-deterrent), as well as the activity for the maximum duration, was observed at the highest concentration in both the laboratory and greenhouse experiments.

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