Repellency of Vetiver Oils from Different Biogenetic and Geographical Origins against Formosan Subterranean Termites (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Twelve vetiver oils from different biogenetic and geographical origin were tested for their repellency against *Coptotermes formosanus* Shiraki, (Formosan subterranean termite). Despite the variety of the constituents from different origins, all vetiver oils, except one, showed repellency. The possible termite-repelling components from the different origins are discussed.

Keywords: Vetiver oil, biogenetic and geographical origin, *Coptotermes formosanus*, repellency.

INTRODUCTION

Vetiver grass (*Vetiveria zizanioides*, Linn Nash) is a fast growing plant with a huge spongy mass of roots. The plants are used against soil erosion (National Research Council 1993). Adams and Dafforn (1998) examined 121 accessions of vetiver and found that 86% appeared to be from a single clone with no variation in the DNA examined. That clone was named Sunshine, after its collection site in Sunshine, Louisiana, USA. Cultivated vetiver from Haiti and Reunion were identified as Sunshine. This work was expanded by Adams *et al.* (1998) to include closely related genera (*Chrysopogon* and *Sorghum*). Based on this and morphological data, Veldkamp (1999) combined *Vetiveria* and *Chrysopogon* under *Chrysopogon*. Although this led to the recognition of *Chrysopogon zizanioides* (L.) Roberty as the correct name for *Vetiveria zizanioides* (L.) Nash, in this paper we will continue to use both names for clarity. Analysis of additional cultigens from Bangkok (Adams *et al.* 1999) revealed that Sunshine and its allied cultigens form the bulk of the cultigens in the world.

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Based on these results, thirteen different DNA types were cloned and planted in test plots (Adams *et al.* 2003) with distinctly different environments: Florida USA, Kathmandu Nepal, Algarve Portugal and Nigeria. In addition to the typical commercial 'Sunshine' vetiver oil, additional oils sold as either 'vetiver oil' or 'khus oil' was used in this study.

The roots diffuse an odor that is much appreciated in the perfumery and flavor industry (Arctander 1960). The origin of this odor is the essential oil, oil of vetiver, which is distilled from the roots and used in perfume and soap production (Robbins 1982). The oil also has repellent effects against cockroaches and flies (Jain *et al.* 1982). In recent years there has been great interest in the development of botanical natural products for use in pest control because of their low mammalian toxicity and environmental soundness. It has been shown (Zhu *et al.* 2001a, 2001b, Maistrello *et al.* 2001) that vetiver oil from Haitian origin has repellency against Formosan subterranean termites, *Coptotermes formosanus* Shiraki, and disrupts termite tunneling behavior. Vetiver grass can be found in at least 70 countries around the world, and the major geographical origins of vetiver grass are from Haiti, Java, China, India and Congo (Adams & Dafforn 1998, Andersen 1970, Lemberg & Hale 1978). Among them, Java and Haiti are the world's largest suppliers (National Research Council 1993).

In the past ten years, much effort has been spent on the investigation of its composition and the elucidation of the chemical structure of the components of vetiver oil. The composition of the oil is dependent not only upon the country of origin, but also the age of the plants (Dool 1982). The vetiver oil from Haiti is one of the most complex, consisting of more than 300 sesquiterpenoids (Weyerstahl *et al.* 1996), of which 155 constituents have been identified (Weyerstahl *et al.* 2000a). Vetiver oil from Haiti has been exhaustively studied (most GC peaks contained 2-4 components) recently (Weyerstahl *et al.* 1996, 1997, 2000a, 2000b), but the composition is so complex, most GC peaks contained 2-4 components, that general, routine analyses of vetiver oils are probably not possible. The most recent review of the composition of commercial vetiver oils is by Adams *et al.* (2003). Vetivones (α- and β-) and khusimol always occur in the Haitian and Java oils in amounts up to 35% (Ohloff 1994). The oil of North Indian origin (Khus oil) is distinctly different chemically and represents a distinct race of *V. zizanioides* or perhaps it is a different species (Andersen 1970). The oil is chemically unique due to the presence of a large amount of khusinol and khusilal and other antipodal sesquiterpenoids and the absence of α-, β- vetivones (Andersen 1970).

It is difficult to test each chemical of vetiver oil or combinations there of against termites because only a few of the individual compounds are commercially available. Searching among vetiver oils from different origins, finding additional unique constituents and testing their repellency against termites will help identify active components in the oils. In our research we isolated and tested vetivone (α-, β-), the main components in Haitian vetiver oil, and found that they are repellents to termites. We chemically characterized 12 vetiver oils from different origin and tested their repellency to Formosan subterranean termites. Most of the oils exhibited termite-repelling activity.

METHODS AND MATERIALS

Composition and source of the 12 vetiver oils

Vetiver oils were obtained from both scientists and commercial sources. The following is a list of accession numbers and the alleged composition of these oils: 6477, Haitian vetiver oil, Texarome Corp., TX, USA; 8059, Indonesian vetiver oil, Berje, Inc., NJ, USA; 8060, "Vetiver", Aura Cacia, CA, USA; 9281, Java vetiver oil, Djasula Wangi pt., Jakarta, Indonesia; 9584, "Vetivert", St. John's Herb Garden, MD, USA; 9590, "Vetiver wild super India", Alternative Therapies Laboratory, ON, Canada; 9591, "Vetiver, France", Alternative Therapies Laboratory, ON, Canada; 9592, "Vetiver, Haiti", Alternative Therapies Laboratory, ON, Canada; 9595, wild vetiver (Khus) oil, Lakhan Singh, Kathmandu, Nepal; 9667, "Ruh Khus", Gulab Singh Johri Mal, Delhi, India; 9690, wild vetiver, Xia Hanping, Guang Dong, China; Sunshine vetiver oil, Xia Hanping, cultivated, Guang Dong, China.

The vetiver root samples from Guang Dong, China are air-dried in the shade for 4-5 days, then sent to the lab where they were kept at -20°C until distilled. The roots were steam distilled for 24 h using a circulatory Clevenger-type apparatus (Adams 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted roots were oven dried (48 h, 100°C) for determination of oil yields.

The essential oils were analyzed on a Hewlett-Packard 5973 MSD, directly coupled to a HP5980 gas chromatograph. EI mass spectra were collected at 70 eV ionization voltages over the mass range m/z 41-425. Oil samples of 0.1 µl (5% concentration) were injected and split 1/10. Analytical conditions: Column: J & W DB-5, (0.26 mm x 30 m, 0.25 mm film thickness); carrier gas: Helium at 1 ml/min; injector temperature 220°C; split ratio: 10:1; oven programming: initial temperature: 60°C, gradient 3°C/min., final temperature: 246°C. The percentages of each compound are TIC values. Identifications were made by library searches of our volatile oil library (Adams 2001) coupled with retention time data of known reference compounds.

Isolation of α-, β- vetivone

Vetivones (α-, β-) were isolated by silica gel chromatography. One gram of Haitian vetiver oil (Good Scents Company, Oak Creek, WI, 53154) was applied on a silica column (2 cm x 20 cm) and the compounds were eluted with $CHCl_3$. Each fraction was concentrated and α-,β- vetivone were identified by GC-MS. The crude α-, β- vetivones were further purified by preparative TLC. The purified vetivones were removed from the plate and tested by GC-MS. The purity of α-and β-vetivone was > 95%.

Termites

A carton nest (colony C) of Formosan subterranean termites was collected from an infested tree in Lake Charles, LA in 2001, and was held in a 250-liter polypropylene garbage can with pine wood as a food source at 24-26°C. Moistened corrugated cardboard rolls were used to retrieve termites from the can (La Fage *et al.* 1983). Termites were gently knocked from the cardboard rolls into clean plastic trays (40 cm x 50 cm) and separated from debris by allowing them to climb on moistened paper towels. Workers of at least the third instar were used in the tests.

Repellency test

The testing procedure used was similar to Lewis *et al.* (1978) with some modification. One ml of hot agar solution (1.0 g agar in 100 ml H_2O) was spread evenly in the bottom of a Petri dish (1 x 5 cm, 20 ml air) and allowed to cool. Blasting sand (fine, #4) was used after having been autoclaved for 30 min and oven dried. One half of the Petri dish was covered with 1 g of treated sand. The other half was covered with 1 g of untreated sand. The sand used was enough to cover the agar completely but thin enough not to conceal the termites once introduced. The agar ensured adequate moisture for the termites and helped to hold the sand in place. The oils and vetivones were dissolved in ethyl alcohol in a series of dilutions. For each chemical, 6 concentrations (2.5, 5.0, 12.5, 25.0, 50.0 and 100.0 μg/dish) and a control (ethyl alcohol treated sand) with 5 replicates (dishes) were prepared for testing. The lowest effective concentration (threshold value) of the vetiver oils was calculated as μg of oil per Petri dish.

Ten worker termites were introduced into each Petri dish, and all Petri dishes were covered with a sheet of aluminum foil between observations to reduce possible effect of light. The number of termites on the untreated sand side was recorded at 0.5, 1, 2, 3, 4, 5 and 24 h.

Data analysis

A repellent effect was recorded when the percent of termites on treated side was significantly less than the percent of termites on untreated side ($p < 0.05$). A binomial test with a null proportion of 50% was performed to determine the maximum number of termites needed to be on the treated side for the presence of repellency. If the observed number exceeded the calculated maximum number, repellency is considered absent. To evaluate the effect of some known components on the repellency, a multiple logistic regression model procedure, PROC GENMOD (SAS, Version 8) was applied with binomial distribution and logit link function. The dependent variable of the model was the proportion of the termites that stayed at the treated side in a dish. The explanatory variables were the natural log transformation of the concentrations of each of the 5 components, calculated by multiplying the concentration of the oil with the relative percentage of the component. Both linear and quadratic terms were included in the model.

RESULTS AND DISCUSSION

Identification of the major components in the vetiver oils

The constitution of the tested vetiver oils from different origins varied and the number of components was from a few to hundreds as shown in gas chromatograms (Fig. 1 A-D). The Haitian oil, (6477) was the most complicated vetiver oil with more than a hundred peaks (Fig. 1A). An aged Haitian oil from St. John's herbs (9584) contained small amounts of α-and β- vetivones and unknown higher boiling point components as shown in Fig. 1 B. The gas chromatogram of wild vetiver (Khus) oil from Nepal (9595) is shown in Fig. 1C. A peak of 35.83 minutes was identified as khusinol and the peak at 40.59 minutes was khusilal which are common components in "North Indian type" vetiver or "Khus" oils. A few components in wild type "vetiver super wild India" oil from India Aura Cacia (9590) are shown in Fig. 1D, and a major peak (83.6%) at 32.68 minutes was identified as diethyl phthalate which is an industrial plasticizer and may be an adulterant. The vetivones (α-and β-), vetiselinol, khusimol, isovelencenol and vetivenic acid were found in oils 6477, 8059, 9281, 9591, 9592, 8060 and 9691. We defined the oils as "Haitian type" oil and the composition of the oil is shown in Table 1. Oils contained khusilal and/or khusinol and absence of vetivones were defined as "North Indian type" oil (Table 2). The compositions of the wild type "Ruh Khus"oils were from Gulab Singh, India (9667) and the wild vetiver from Guang Dong, China (9690). Their composition are shown in Table 2. The structures of major components from different origins were shown in Fig. 2.

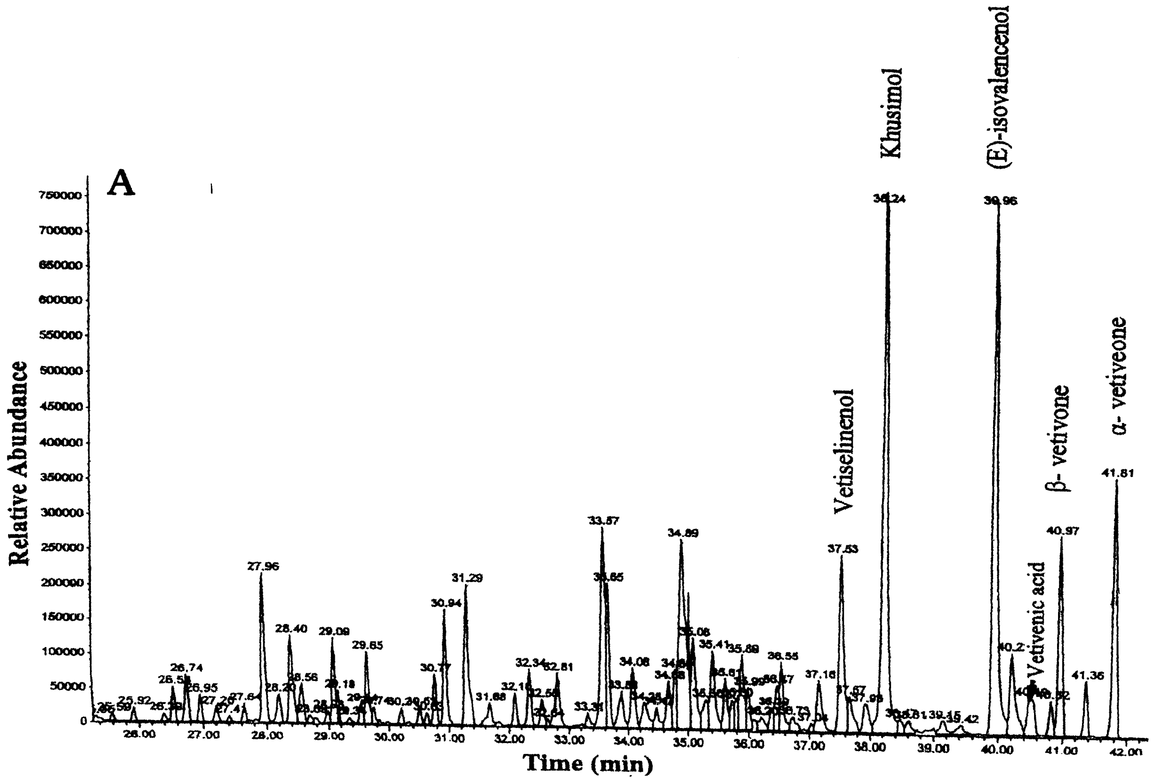


Fig. 1A-D. Gas chromatography of Haitian vetiver oil 6477 (Fig. 1A) from Texarome, Haiti; the major peaks were identified as khusimol (38.24 min); (E)-isovalencenol (39.96 min); β-vetivone (40.97 min) and α-vetivone (41.81min). Aged Haitian oil from St. John's herbs (9584) was shown in Fig. 1B in which peak 1 was β-vetivone and peak 2 was α-vetivone. Two major peaks were identified as khusinol (35.83 min) and khusilal (40.59 min) in "North Indian type Khus" vetiver oil from Nepal (9595) (Fig. 1C). Three components (diethyl phthalate, α-humulene and caryphyllene) were found in the imitation vetiver oil from "Vetiver, wild super, India" (9590) as shown in Fig. 1D.

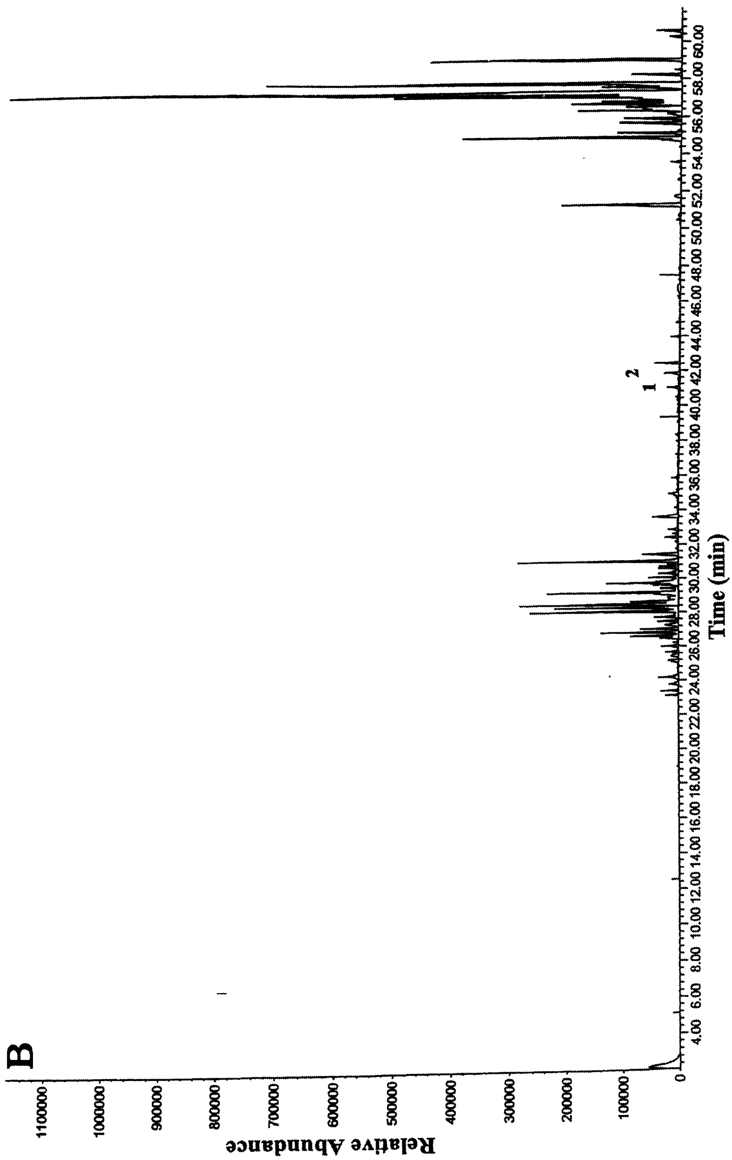
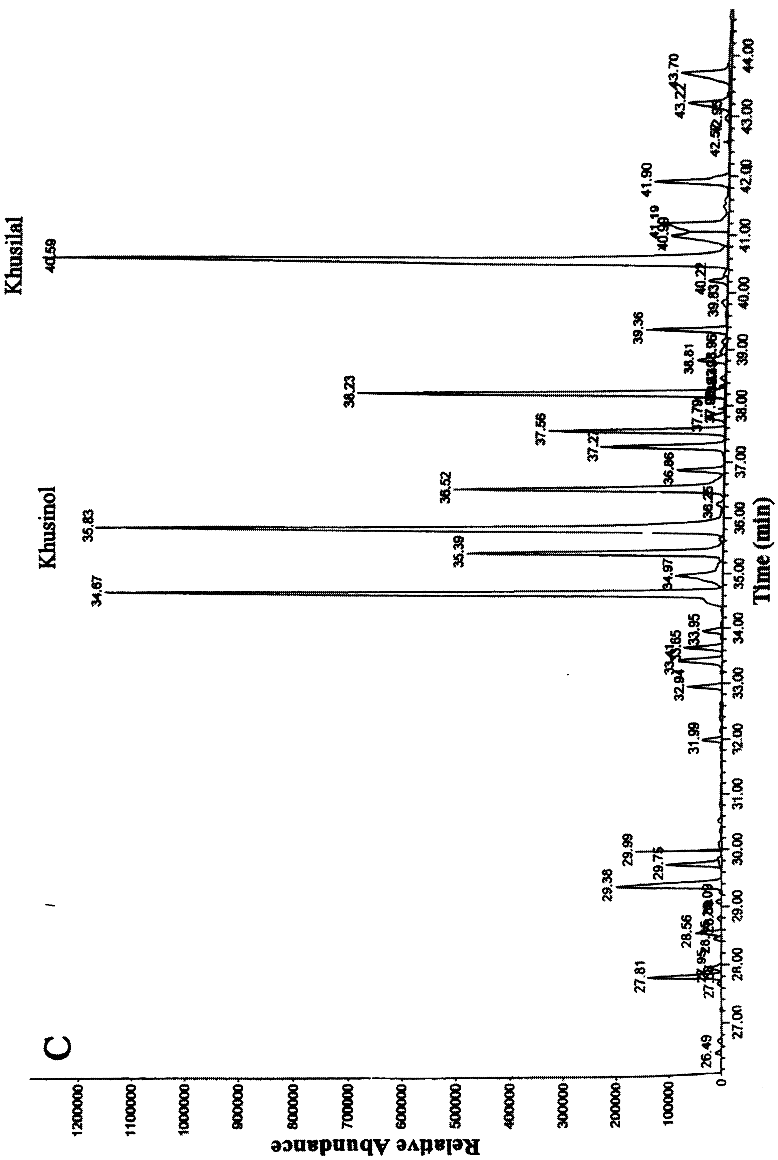


Fig. 1A-D. Legend in Fig. 1.



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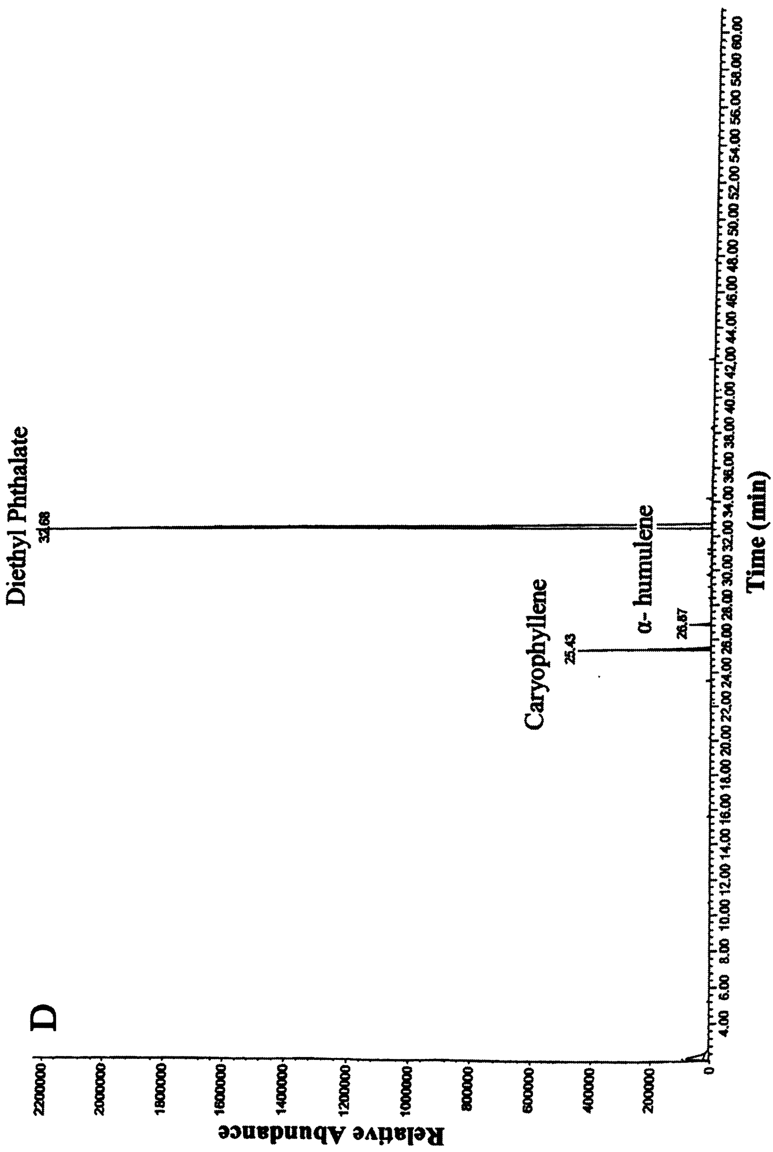

Fig. 1A-D. Legend in Fig. 1.

Table 1. Composition of "Haitian type" Vetiver Oil.

Stock number #	Source of the Vetiver oil	Relative % of the component in vetiver oil					
		Vetivenic linenol	Vetise-mol	Khusi-velence-nol	iso-vetivone	β-vetivone	α-acid
6477	Haiti, Texarome	5.2	22.9	16.1	4.8	6.6	none
8059	Indonisia, Berje	2.2	7.9	2.4	4.9	3.5	1.8
9281	Java, Djasula	2.0	7.3	2.1	4.8	4.0	1.5
9591	Aromatherapy, France	3.8	4.5	4.7	3.7	6.6	7.6
9592	Aromatherapy, Haiti	4.1	12.5	11.8	3.5	4.8	none
8060	Aura Cacia	3.5	14.4	8.2	3.2	4.2	1.0
9691	Cultivated, Sunshine, Guang Dong, China	1.7	8.7	7.7	4.4	4.4	7.0
9584	St. John's herbs	none	none	none	0.2	0.3	none

Table 2. Composition of "North Indian type" and Wild Type Vetiver Oil.

Stork Number #	Source of vetiver oil	Composition and percentage
9590	Aromatherapy., India, wild	83.6% diethyl phthalate; 9% caryopyllene ; 1.4% α-humulene
9595	Khus oil, Nepal	15.7% Khusinol; 18.3% Khisilal
9667	Ruh Khus oil, Gulab Singh, Indin	2.2% vetiselinenol; 23.2% khusimol; 2.1% isovalencenol; 0.4% α-vetivone; 5.2% khusimone; 20.6% khusinol
9690	Wild, Guang Dong, China	6.2% khusimol, 73.2% vetivenic acid

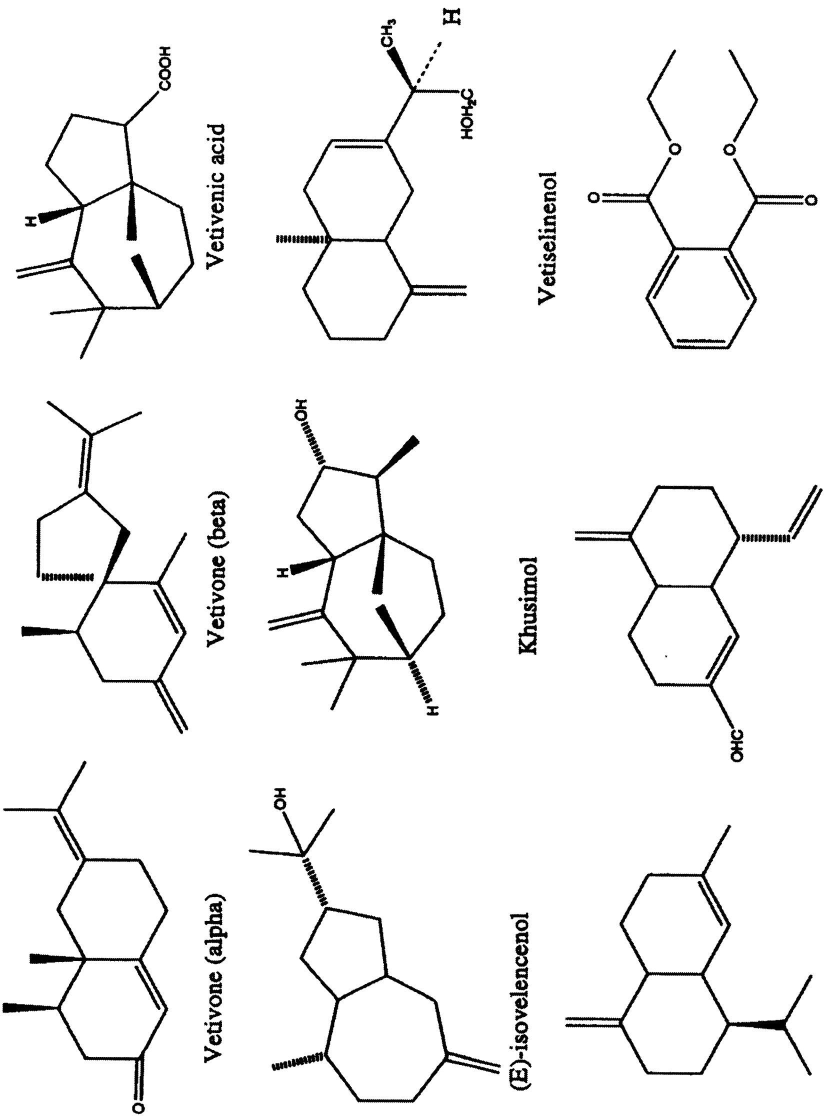

Fig. 2. Chemical structures of the main components of vetiver oil from different origins.

Repellency of vetiver oils against the Formosan subterranean termites

Six concentrations of vetiver oil were tested for determination of the lowest effective concentration, the threshold value, against Formosan subterranean termites at various observation times. The threshold values of "Haitian type" vetiver oil and vetivones against Formosan termites are shown in Table 3. Almost all of "Haitian type" vetiver oils exhibited repellency to Formosan termites with Haiti, Texarome (6477) having the greatest repellency, with a threshold value of 2.5 µg/dish from 1 h up to 24 h. Aged Haitian oil "Vetivert" from St. John's herbs (9584) had the lowest repellency. Its threshold value was 50-75 µg/dish and the repelling activity lasted for a few hours. Vetiver oils of "North Indian type" (9595, 9667) exhibited potential repelling activity against Formosan subterranean termites (Table 4) and the threshold value was similar. "Wild type" oils (9590, 9690) presented similar repelling activity to "North Indian type" as shown in Table 4.

Effects of known components on repellency of oils

All "Haitian type" vetiver oils besides oil 9584 and 9592 contain the same 5 identified components in different proportions (Table 1). In the multiple logistic regression models, except for β-vetivone, all the linear terms were not significant and all quadratic terms were significant according to the likelihood ratio statistics of the model (Table 5). Among the significant parameters, the estimates were positive for khusimol and β-vetivone.

According to the repellency threshold value measured in this study, Haiti origin vetiver oil is a strong acting and long-lasting repellent to Formosan subterranean termites. This agrees with our previous study (Zhu *et al.* 2001b). Although, it is not known how many compounds in the oil are repellents to termites, our results demonstrated that α-and β-vetivone, two well known components in Haitian vetiver oil were at least partially responsible for the repelling activity (Jain *et al.* 1982). All "Haitian type oil" exhibited strong repellency except the oil from an adulterated sample (9584) in which the major components were unknown and only a small amount of α-and β–vetivones were presented. The results of bioassay showed that the additional termite-active chemicals, besides α-and β-vetivones, also existed in "Haitian type" oils because the repellant activity of oils was stronger than the pure vetivones as shown in Table 3. It is possible that khusimol, a major constituent in Haitian oil, is one of the additional active chemicals against termites since its chemical structure is similar to khusimone and zizanal which are known as insect repellents (Jain *et al.* 1982). The

Table 3. Repellency of "Haitian type" Vetiver Oil against Formosan Subterranean Termites.

Vetiver oil #	Threshold value of repellency (μg/dish) 1 h	3 h	5 h	24 h
6477	2.5	2.5	2.5	2.5
9592	5	2.5	5	2.5
9591	12.5	2.5	5	2.5
9281	5	12.5	5	5
8059	5	5	5	12.5
9691	12.5	12.5	12.5	50
8060	25	25	12.5	25
9584	75	50	50	>100
α-, β-vetivone	5	5	25	25

Threshold value is the lowest effective concentration (μg/dish) for repellency.

Table 4. Repellency of Vetiver Oils from North Indian and Wild Varieties against Formosan Subterranean Termites.

Vetiver oil #	Threshold value for repellency (μg/dish) 1 h	3 h	5 h	24 h
9595	12.5	12.5	25	12.5
9690	12.5	25	25	25
9590	25	25	25	12.5
9667	12.5	5	25	25

Threshold value is the lowest effective concentration (μg/dish) for repellency.

Table 5. Parameter Estimates and the Test Statistics of the Multiple Logistic Regression Models for the Effect of 5 Components on Proportion of Termites Stayed on the Treated Side of a Dish.

Parameter	Estimate	ASE	X^2	P-value
Intercept	-0.9333	0.2316	16.24	<0.0001
Vetiselinenol linear term	0.2943	0.2021	2.12	0.1452
Khusimol linear term	-0.0818	0.2460	0.11	0.7395
Isovelencenol linear term	-0.0184	0.1420	0.02	0.8968
α-vetivone linear term	0.3801	0.2850	1.78	0.1823
β- vetivone linear term	-1.6302	0.4140	15.51	<0.0001
Vetiselinenol quadratic term	-0.2918	0.1151	6.43	0.0112
Khusimol quadratic term	0.3239	0.0932	12.08	0.0005
Isovelencenol quadratic term	-0.4923	0.0674	53.34	<0.0001
α-vetivone quadratic term	-0.8164	0.1404	33.80	<0.0001
β-vetivone quadratic term	1.1708	0.1972	35.24	<0.0001

logistic regression suggested that the relationship between the concentrations of vetiselinenol, khusimol, isovelencenol, α-vetivone and β-vetivone and the tendency of termites staying on the treated side is not linear, but curvilinear and all 5 components contribute to the observed repellency to termites. The model also indicates a negative relation between the concentrations of khusimol and β-vetivone and the repellent effect on termites.

"North India type" (Khus) oil from Nepal (9595) and India (9667) exhibited strong repellency to termites, and the distinguishing components in the oil were khusinol and khusilal. These two compounds might play important roles against termites.

We found that the oils from "wild type" of vetiver plant possessed unique components such as oil from Guang Dong, China (9690) possessed 73.2 % of vetivenic acid and exhibited repelling activity. We believe that vetivenic acid may responsible for the repellency against Formosan subterranean termites because the chemical structure is similar to khusimol. The adulterated "vetiver, wild, super India" vetiver oil from India (9590) contained 83.6% diethyl phthalate which is a very common industrial plasticizer and toxic to humans (Kamrin & Mayor 1991). This compound appears to be an industrial contaminant or deliberate dilutent.

Our results showed that vetiver oils from different origins possess different compositions (Table 1 and 2). Despite the differences between origins, the most of vetiver oils, except the adulterated product oil from aged Haitian vetiver plant, showed repelling activity against termites. The repelling activity of vetiver oils from different origins is due to one or more components or combinations thereof. The abundance of active components in vetiver oils, except the oil from aged vetiver plant, makes vetiver oil a good candidate for finding repellents against Formosan subterranean termites.

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