Repellant and insecticidal activities of shyobunone and isoshyobunone derived from the essential oil of *Acorus calamus* rhizomes

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ABSTRACT

Context: It was found that the essential oil of *Acorus calamus* rhizomes showed insecticidal activity. **Aim:** The aim of this study was to determine the chemical composition of the essential oil from *A. calamus* rhizomes, evaluate insecticidal and repellant activity against *Lasioderma serricorne* (LS) and *Tribolium castaneum* (TC), and to isolate any insecticidal constituents from the essential oil. **Materials and Methods:** Essential oil from *A. calamus* was obtained by hydrodistillation and analyzed by gas chromatography (GC) flame ionization detector and GC-mass spectrometry. The insecticidal and repellant activity of the essential oil and isolated compounds was tested using a variety of methods. **Results:** The main components of the essential oil were identified to be isoshyobunone (15.56%), *β*-asarone (10.03%), bicyclo[6.1.0]non-1-ene (9.67%), shyobunone (9.60%) and methylisoeugenol (6.69%). Among them, the two active constituents were isolated and identified as shyobunone and isoshyobunone. The essential oil showed contact toxicity against LS and TC with LD_{50} values of 14.40 and 32.55 μg/adult, respectively. The isolated compounds, shyobunone and isoshyobunone also exhibited strong contact toxicity against LS adults with LD_{50} values of 20.24 and 24.19 μg/adult, respectively, while the LD_{50} value of isoshyobunone was 61.90 μg/adult for TC adults. The essential oil, shyobunone and isoshyobunone were strongly repellent (98%, 90% and 94%, respectively, at 78.63 nL/cm^2, after 2 h treatment) against TC. **Conclusion:** The essential oil, shyobunone and isoshyobunone possessed insecticidal and repellant activity against LS and TC.

Key words: *Acorus calamus,* contact toxicity, *Lasioderma serricorne*, repellency, *Tribolium castaneum*

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INTRODUCTION

The red flour beetle, *Tribolium castaneum* (TC) Herbst and the cigarette beetle, *Lasioderma serricorne* (LS) Fabricius are the most widespread and destructive primary insect pests of stored cereals.[1] The infestations of stored product insects currently not only cause significant losses due to the consumption of grains but also result in the rise of temperature and moisture which lead to an accelerated growth of molds, including toxigenic species.[2] Control of stored product insects relies heavily on the use of synthetic insecticides and fumigants, that has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on nontarget organisms in addition to direct toxicity to users.[3] These problems have necessitated a search for alternative eco-friendly insect pest control methods.[4] Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecules.[5,6] The use of essential oils or their constituents with low mammalian toxicity can effectively prevent and/or suppress insect pest especially in storage.[5] Essential oils from many plants have been evaluated with success for insecticidal/repellency activity

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against stored-product insects/mites, in some cases, have been proven more effective than traditionally used organophosphorus pesticides.[7-9] During our screening program for new agrochemicals from local wild plants and Chinese medicinal herbs, the essential oil from *Acorus calamus* rhizomes has been found to possess contact and repellent activities towards LS and TC.

Acorus calamus (Linn.), a member of the family Araceae, is a perennial and semiaquatic plant with creeping rhizomes. Commonly known as sweet flag, *A. calamus* wildly grows along swampy and marshy areas in the northern temperate and subtropical regions of Asia, North America and Europe.[10] *A. calamus* is well known for its beneficial and medicinal properties in Indian medical system. Pharmacological studies have revealed that the plant possesses a wide range of therapeutic activities, including behavior-modifying, anticonvulsant, acetyl cholinesterase inhibitory,[11,12] antispasmodic, antidepressant, anxiolytic,[13-15] anti-diabetic,[16] hypolipidemic,[17] antidiarrheal,[18] bronchodilatory,[19] anti-inflammatory,[20] cytoprotective[21] and analgesic properties.[22] In addition, the essential oil of *A. calamus* has been demonstrated to possess repellency activity against the maize weevil, *S. zeamais*[23] and insecticidal activity against many species of insects, e.g., the larger grain borer, *Prostephanus truncates*,[24] the tobacco armyworm, *Spodoptera litura*[25] and the booklouse, *Liposcelis bostrychophila*.[26] However, a literature survey has shown that there is no report on contact/repellency of *A. calamus* essential oil against the red flour beetle and the cigarette beetle, thus we decided to investigate the chemical constituents and contact/repellency activity of the essential oil of *A. calamus* against TC and LS for the first time and to isolate any biologically active compounds from its essential oil.

MATERIALS AND METHODS

Plants material

Rhizomes (3.5 kg) of *A. calamus* were collected in September 2012 in Dali City (35.23°N and 116.33°E), Yunnan province of China. The rhizomes were air-dried for one week and ground to a powder. The plant was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing, China) and a voucher specimen (BNU-CMH-Dushuahan-2012-11-25-006) was deposited at the Herbarium (BNU) of College of Life Sciences, Beijing Normal University.

Insects

Cigarette beetles (LS) and red flour beetles (TC) were obtained from laboratory cultures maintained for the last 2 years in dark in incubators at 29°C ± 1°C and 70–80% relative humidity. The insects were reared in glass containers (0.5 L) containing wheat flour at 12–13% moisture content mixed with yeast (10:1, w/w). Adults used in all the experiments were about 7 ± 2 days old regardless of gender.

Extraction and composition of essential oil

The ground powder of *A. calamus* rhizomes was subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h and extracted with n-hexane. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in airtight container in a refrigerator at 4°C.

Gas chromatography-mass spectrometry (GC-MS) analysis was performed on a Thermo Finnigan Trace DSQ instrument equipped with a flame ionization detector and an HP-5 MS (30 m × 0.25 mm × 0.25 μm) capillary column. The column temperature was programmed at 50°C for 2 min, then increased at 2°C/min to the temperature of 150°C and held for 2 min, and then increased at 10°C/min until the final temperature of 250°C was reached, where it was held for 5 min. The injector temperature was maintained at 250°C and the volume injected was 0.1 mL of 1% solution (diluted in *n*-hexane). The carrier gas was helium at flow rate of 1.0 mL/min. Spectra were scanned from 50 to 550 m/z. Most constituents were identified by comparison of their retention indices with those reported in the literatures. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_5–C_{36}) under the same operating conditions. GC retention time and their mass spectra that stored in NIST 05 and Wiley 275 libraries or from literature were used for identify the essential oil components.[27] Relative percentages of the individual components of the essential oil were obtained by averaging the GC peak area% reports.

Purification and characterization of two constitunent compounds

The crude essential oil (5 ml) was chromatographed on a silica gel (Qingdao Marine Chemical Plant, Shandong province, China) column (30 mm i.d., 500 mm length) by gradient elution with *n*-hexane first, then with *n*-hexane-ethyl acetate, and last with ethyl acetate to obtain 22 fractions. Based on contact toxicity/repellent test, fraction 3 and 15 were chosen for further fractionation. With PTLC, two purified compounds were obtained and they were analysised by various NMR techniques including ^{1}H NMR and ^{13}C NMR. Combining all the NMR spetra data, the two isolated compounds were finally recognized as shyobunone (**1**, 0.24 g)[28,29] and isoshyobunone (**2**, 0.35 g).[28,30] NMR experiments were

performed on Bruker Avance DRX 500 instrument using $CDCl_3$ as solvent with TMS as internal standard.

Contact toxic activity test

The contact toxicity of the essential oil/pure compounds against LS and TC adults was measured as described by Liu and Ho.[1] Range-finding studies were run to determine the appropriate testing concentrations. A serial dilution of the essential oil/compounds (five concentrations) was prepared in *n*-hexane. Aliquots of 0.5 μL of the dilutions were applied topically to the dorsal thorax of the insects. Controls were determined using *n*-hexane. Five replicates were carried out for all treatments and controls. Both treated and control insects were then transferred to glass vials (10 insects/vial) with culture media and kept in incubators. Mortality was recorded after 24 h and the LD_{50} values were calculated using Probit analysis.[31] Positive control, pyrethrins (pyrethrin I and II, 37%) were purchased from Dr Ehrenstorfer GmbH.

Repellency tests

The repellent activity of the essential oil/pure compounds to TC adults was tested using the area preference method.[32] The essential oil/compounds was diluted in *n*-hexane to different concentrations (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm^2), and *n*-hexane was used as the control. Filter paper (9 cm in diameter) was cut in half. 500 μL of treatment solution was placed on one half of the filter paper and allowed to dry for 30s. The other half was treated with 500 μL of *n*-hexane. The treated side was then joined to the control side by tape and placed in glass petri dishes (9 cm in diameter). Twenty insects were released in the center of each filter paper disk, and a cover was placed over the Petri dish. Five replicates were used. Counts of the insects present on each strip were made after 2 and 4 h. The percent repellency (PR) of each volatile oil/compound was then calculated using the formula:

$$PR\ (\%) = ([Nc - Nt]/[Nc + Nt]) \times 100$$

Where N_c is the number of insects present in the negative control half and N_t is the number of insects present in the treated half. Analysis of variance (One-Way ANOVA and GLM Univariate) and Tukey's test were conducted by using SPSS 20.0 (IBM, Armonk, NY) for Windows 2007. Percentage mortality data were subjected to arcsine square-root transformation before analysis of variance. A commercial repellent, N, N-diethyl-3-methylbenzamide (DEET), was purchased from the National Center of Pesticide Standards (8 Shenliao West Road, Tiexi District, Shenyang 110021, China) and used as a positive control.

RESULTS

Chemical compounds of the essential oil

The yield of *A. calamus* rhizomes essential oil was 1.00% (v/w) and the density of the essential oil was determined to be 0.93 g/ml. GC-MS analysis of the essential oil of *A. calamus* rhizomes led to the identification and quantification of a total of 56 major components, accounting for 89.21% of the total components present [Table 1].

Table 1: Chemical components of the essential oil of *A. calamus* rhizomes

RI*	Compounds	Content (%)
951	(1α,2α,4α,5α)-8-Methylenetricyclo [3.2.1.02,4]octane	0.18
957	Camphene	0.82
1011	3-Carene	0.08
1014	1,4-Cineole	2.60
1020	Limonene	0.49
1025	1,3,8-p-Menthatriene	0.19
1031	Eucalyptol	1.78
1043	Benzyl Alcohol	0.07
1050	2-Methylbutyl propionate	0.09
1061	2,2,4-trimethyl-3-Pentanone	0.09
1096	α-Linalool	1.58
1099	Myrcenol	2.31
1146	(-)-Alcanfor	0.18
1167	2,9-Bornanediol	0.07
1175	L-4-terpineol	0.31
1188	α-Terpineol	0.61
1202	Estragole	0.11
1295	Isobornyl acetate	0.38
1343	n-Butylbenzene	0.07
1422	5,7-diethyl-5,6-Decadien-3-yne	2.34
1436	(+)-Calarene	3.81
1453	Valencene	0.60
1461	Aromadendrene	0.07
1478	Germacrene D	0.87
1483	ë-Selinene	0.27
1492	2-Methylbenzoic acid phenacyl ester	0.07
1500	Methyl isoeugenol	6.69
1502	Shyobunone	9.60
1517	Calamenene	0.32
1518	Maltotriose	0.11
1520	(+)-delta-Cadinene	0.69
1522	Isoshyobunone	15.56
1531	4-Tertbutyloxytoluene	0.50
1537	Culmorin	2.47
1546	α-Calacorene	3.90
1559	Decahydro-1,6-methanonaphthalene	0.32
1580	(-)-Spathulenol	2.27
1584	2-Methyl-2-bornene	0.35
1602	1,3-Benzodioxole-5-(4-keto-butyric acid)	0.64
1606	Bicyclo[6.1.0]non-1-ene	9.67
1614	Dihydro-cis-α-copaene-8-ol	0.56

Contd...

Contact toxicity

The essential oil of *A. calamus* rhizomes showed strong contact toxicity against LS and TC adults with LD_{50} values of 14.40 and 32.55 μg/adult, respectively [Table 2]. Compared with the positive control pyrethrins (37% pyrethrin I and pyrethrin II), the crude essential oil demonstrated 60 and 125 times less toxicity against the two insect species because the pyrethrins had acute contact toxicity to LS and TC adult with LD_{50} values of 0.24 μg/adult and 0.26 μg/adult, respectively. The isolated compounds, shyobunone and isoshyobunone also exhibited strong contact toxicity against LS adults with LD_{50} values of 20.24 and 24.19 μg/adult, respectively [Table 2], while the LD_{50} value of isoshyobunone, was 61.90 μg/adult for TC adults.

Table 1: Contd...

RI*	Compounds	Content (%)
1616	α-Vatirenene	0.13
1621	2-Methylenebornane	0.24
1626	3-Cyclopentyl-6-methyl-3,4-heptadien-2-one	3.23
1631	β-Asarone	10.03
1667	4-(1-phenylethyl)-Phenol	0.65
1685	4-(Phenylethynyl) anisole	0.18
1704	2,2,5,7-Tetramethyltetralin	0.07
1710	Genistin	0.08
1717	DL^{-3}-Phenyllactic acid	0.41
1724	1,4-Cineole	2.60
1726	Tropic acid	0.10
1729	6,6-Dimethyl-10-methylene-1-oxaspiro [4.5]dec-3-ene	0.16
1746	2-Methoxy-3-benzofurancarbaldehyde	0.08
1749	Euparone methyl ether	0.09
1776	Scytalone	0.15
1789	3-Ethyl-3-methylheptane	0.10
	Total	89.21

*RI. RI as determined on a HP-5MS column using the homologous series of *n*-hydrocarbons. RI: Retention index

Repellent activity

The results of repellency assays for the essential oil and isolated compounds against TC adults are presented in Figures 1 and 2. However, the crude essential oil showed no obvious repellency against LS adults because the essential oil at dose of 78.63 and 15.73 nL/cm² has weak repellency (56% and 26%, respectively) to LS after 2 h treatment. *A. calamus* rhizomes oil at dose of 78.63 nL/cm² showed 98% and 98% repellency against TC adults at 2 and 4 h after exposure, respectively. The repellent responses of TC adults to the essential oil at dose of 15.73 nL/cm² ($P = 0.291$) and 3.15 nL/cm² ($P = 0.103$) were the same level compared to that at the highest concentration treatment. Shyobunone and isoshyobunone also showed obvious repellency (>80%) at dose of 78.63 and 15.73 nL/cm² after 4 h treatment. However, compared with shyobunone, isoshyobunone produced stronger repellency (100% and 92%, respectively, at 15.73 nL/cm², after 2 and 4 h treatment). At the lowest concentration (0.13 nL/cm²), isoshyobunone still showed repellency (64%) against TC adults at 2 h after exposure.

DISCUSSION

The main constituents of *A. calamus* rhizomes essential oil were isoshyobunone (15.56%), β-asarone (10.03%), bicyclo[6.1.0]non-1-ene (9.67%), shyobunone (9.60%) and methylisoeugenol (6.69%). The results were different from the previous reports. These differences might have been due to harvest time and local, climatic and seasonal

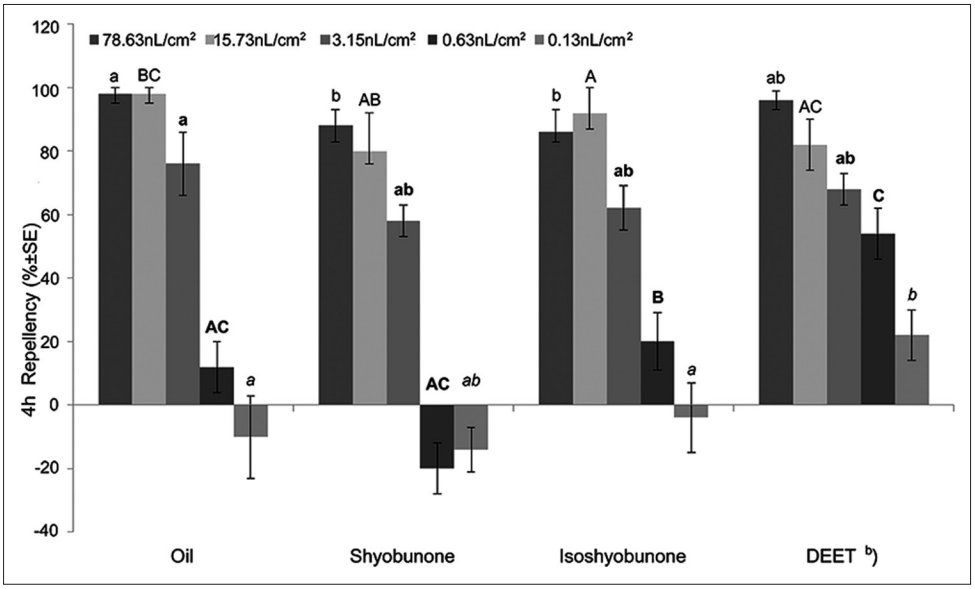

Figure 1: Percentage repellency (PR) of the essential oil from *Acorus calamus* rhizomes and its constituents against *Tribolium castaneum* at 4 h after exposure[a]. [a]Means in the same column followed by the same letters do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests. [b]Positive control

Table 2: Contact toxicity of essential oil of *Acorus calamus* rhizomes and its main components against LS and TC adults

Insects	Treatment	LD_{50} (μg/adult)[a]	Slope±SE	χ^2	*P*
LS	*A. calamus*	14.40 (10.97-17.41)	3.05±0.49	14.49	0.912
	Shyobunone	20.24 (17.14-23.08)	3.28±0.43	14.49	0.912
	Isoshyobunone	24.19 (20.47-27.73)	2.98±0.40	14.49	0.912
	Pyrethrins	0.24 (0.16-0.35)	2.98±0.40	17.36	0.791
TC	*A. calamus*	32.55 (27.69-40.62)	2.60±0.38	14.72	0.904
	Shyobunone	>189.92			
	Isoshyobunone	61.90 (56.18-68.37)	5.27±0.62	14.72	0.904
	Pyrethrins	0.26 (0.22-0.30)	3.34±0.32	13.11	0.950

[a]95% lower and upper fiducial limits are shown in parenthesis. *A. calamus: Acorus calamus*; LS: *Lasioderma serricorne*; TC: *Tribolium castaneum*

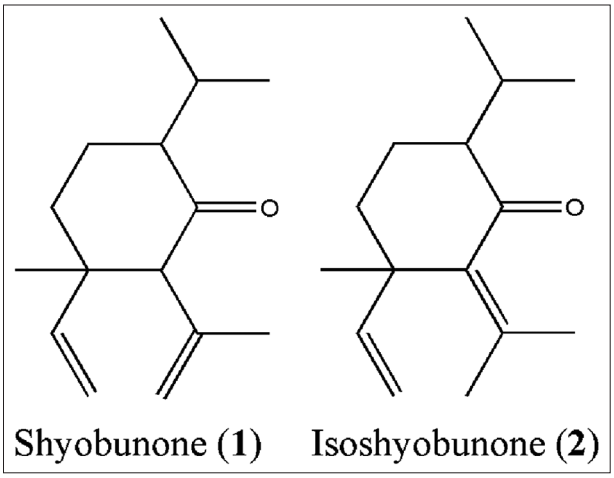

Figure 2: Constituent compounds isolated from the essential oil of *Acorus calamus* rhizomes

factors as well as storage duration of medicinal herbs.[33,34] For example, α-asarone (50.09%), (E)-methylisoeugenol (14.01%), methyleugenol (8.59%), β-asarone (3.51%), α-cedrene (3.09%) and camphor (2.42%) were the main components of the essential oil of *A. calamus* rhizomes obtained from China.[26] However, the essential oil of *A. calamus* rhizomes collected from Italy contained acorenone (21.6%), (Z)-sesquilavandulol (13.0%), shyobunone (7.0%), α-asarone (5.1%) and dehydroxyisocalamendiol (3.5%)[35] while the essential oil of *A. calamus* collected from Quebec, Canada contained preisocalamenediol (18.0%), acorenone (14.2%), shyobunone (13.3%) and cryptoacorone (7.5%).[36] The essential oil of *A. calamus* contained various chemical constituents, and the proportion of each chemical constituent of the oil particularly β-asarone varied in different genotypes and corresponds to the ploidy level.[13] It is reported that the tetraploids have higher (70–96%) β-asarone content, than the triploids (5–19%), and almost negligible in diploid genotypes.[37,38] The above discussions suggest that further studies on plant cultivation and essential oil standardization would be expected because chemical composition of the essential oil varies greatly among the plant population.

To our knowledge, this is the first report regarding to insecticidal action of shyobunone and isoshyobunone against stored-grain insects, as exemplified here with LS and TC. Shyobunone showed more toxicity against LS and much less toxicity against TC than isoshyobunone [Table 2]. However, all the two isolated constituent compounds possessed less activity against LS adult than the crude essential oil [Table 2], suggesting that there may be some other stronger active compounds in small amounts in the essential oil or may be some synergistic action between the various compounds. In addition, we have an interesting discovery in this work. Shyobunone (1) and isoshyobunone (2) have the same molecular formula ($C_{15}H_{24}O$). They are a pair of isomers with a double bond located at different positions along the isopropyl side chain [Figure 3], but their contact toxicity is very different. Differences in the biological activities of geometric isomers were reported in coleopteran pests of stored products and in a yellow fever vector mosquito. In previous research, similar phenomena were also observed. cis-Asarone is toxic in addition to having strong antifeedant activity, whereas the trans isomer acts only as an antifeedant with no appreciable toxicity.[39] Park *et al.*[40] reported that the insecticidal activity against *Sitophilus oryzae* (L.), *Callosobruchus chinensis* (L.), and LS (F.) was more evident in (*Z*)-asarone than that in (*E*)-asarone. In addition, (*Z*)-9-octadecenoic acid was a more potent repellent agent than (*E*)-9-octadecenoic acid against *Aedes aegypti* (L.) adult females.[41] The tiny structural difference of these compounds may account for the significant differences in their insecticidal action. This action includes insect mortality and sublethal effects on behavior, depending on insect and mode of application.

Many essential oils and their constituents have been evaluated for repellency against insects.[42] For example, Zhang *et al.* reported that geraniol and citronellol exhibited stronger repellency against TC adults than DEET, whereas limonene and citronella showed the same level of repellency against TC adults compared with DEET.[32] The origanum oil, linalool and p-cymene at dose of 0.03 mg/cm^2 showed

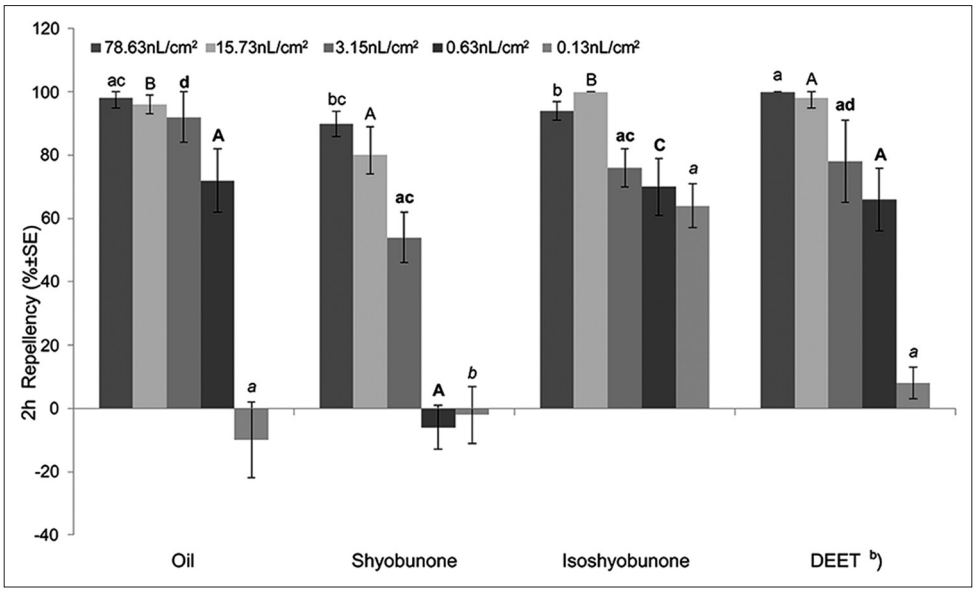

Figure 3: Percentage repellency (PR) of the essential oil from *Acorus calamus* rhizomes and its constituents against *Tribolium castaneum* at 2 h after exposure[a]. [a]Means in the same column followed by the same letters do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. PR was subjected to an arcsine square-root transformation before ANOVA and Tukey's tests. [b]Positive control

98%, 83% and 85% repellency (after 2 h treatment) against TC adults, respectively.[43] However, in this paper, we report the repellency action of shyobunone and isoshyobunone for the first time. In this study, compared with the positive control, DEET, essential oil ($P = 0.051$), isoshyobunone ($P = 0.721$) exhibited the same level of repellency against TC adults, while shyobunone demonstrated less repllency than isoshyobunone [Figure 1].

CONCLUSION

The above discussions suggest that the essential oil and its four compounds show the potential to be developed as natural insecticides and repellents against stored-products insects. However, for the practical application of the essential oil and the four compounds as novel insecticides/repellents, further studies on the safety of the essential oil and its four compounds toward human beings and on the development of formulations are necessary to improve the efficacy and stability, and to reduce cost.

REFERENCES

1. Liu ZL, Ho SH. Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook f. Thomas ET. Against the grain storage insects, *Sitophilus zeamais* Motsch and *Tribolium castaneum* (Herbst). J Stored Prod Res 1999;35:317-28.
2. Magan N, Hope R, Cairns V, Aldred D. Postharvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. Eur J Plant Pathol 2003;109:723-30.
3. Isman MB. Plant essential oils as green pesticides for pest and disease management. ACS Symp 2004;887:41-51.
4. Phillips TW, Throne JE. Biorational approaches to managing stored-product pests. Ann Rev Entomol 2010;55:375-97.
5. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 2006;51:45-66.
6. Isman MB. Botanical insecticides: For richer, for poorer. Pest Manag Sci 2008;64:8-11.
7. Chu SS, Liu QR, Liu ZL. Insecticidal activity and chemical composition of the essential oil of Artemisia vestita from China. Biochem Syst Ecol 2010;38:489-92.
8. Fang R, Jiang CH, Wang XY, Zhang HM, Liu ZL, Zhou L, *et al*. Insecticidal activity of essential oil of *Carum carvi* fruits from China and its main components against two grain storage insects. Molecules 2010;15:9391-402.
9. Rajendran S, Srianjini V. Plant products as fumigants for stored-product insects control. J Stored Prod Res 2008;44:126-35.
10. Mukherjee PK, Kumar V, Mal M, Houghton PJ. *Acorus calamus*: Scientific validation of ayurvedic tradition from natural resources. Pharm Biol 2007;45:651-66.
11. Oh MH, Houghton PJ, Whang WK, Cho JH. Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. Phytomedicine 2004;11:544-8.
12. Vohora SB, Shah SA, Dandiya PC. Central nervous system studies on an ethanol extract of Acorus calamus rhizomes. J Ethnopharmacol 1990;28:53-62.
13. McGaw LJ, Jager AK, van Staden J. Isolation of β-asarone, an antibacterial and anthelmintic compound, from *Acorus calamus* from South Africa. S Afr J Bot 2002;68:31-5.
14. Raina VK, Srivastava SK, Syamasunder KV. Essential oil composition of *Acorus calamus* L. from the lower region of the Himalayas. Flavour Fragr J 2003;18:18-20.
15. Bertea CM, Azzolin CM, Bossi S, Doglia G, Maffei ME. Identification of an EcoRI restriction site for a rapid and precise determination of beta-asarone-free *Acorus calamus* cytotypes. Phytochemistry 2005;66:507-14.

16. Wu HS, Zhu DF, Zhou CX, Feng CR, Lou YJ, Yang B, *et al*. Insulin sensitizing activity of ethyl acetate fraction of *Acorus calamus* L. *in vitro* and *in vivo*. J Ethnopharmacol 2009;123:288-92.
17. Parab RS, Mengi SA. Hypolipidemic activity of *Acorus calamus* L. in rats. Fitoterapia 2002;73:451-5.
18. Shoba FG, Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. J Ethnopharmacol 2001;76:73-6.
19. Shah AJ, Gilani A. Bronchodilatory effect of *Acorus calamus* (Linn.) is mediated through multiple pathways. J Ethnopharmacol 2010;131:471-7.
20. Kim H, Han TH, Lee SG. Anti-inflammatory activity of a water extract of *Acorus calamus* L. leaves on keratinocyte HaCaT cells. J Ethnopharmacol 2009;122:149-56.
21. Smit HF, Woerdenbag HJ, Singh RH, Meulenbeld GJ, Labadie RP, Zwaving JH. Ayurvedic herbal drugs with possible cytostatic activity. J Ethnopharmacol 1995;47:75-84.
22. Almeida RN, Navarro DS, Barbosa-Filho JM. Plants with central analgesic activity. Phytomedicine 2001;8:310-22.
23. Yao YJ, Cai WL, Yang CJ, Xue D, Huang YZ. Isolation and characterization of insecticidal activity of (Z)-asarone from *Acorus calamus* L. Insect Sci 2008;15:229-36.
24. Schmidt GH, Streloke M. Effect of *Acorus calamus* (L.) (*Araceae*) oil and its main compound β-asarone on *Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*). J. Stored Prod Res 1994;30:227-35.
25. Sharma PR, Sharma OP, Saxena BP. Effect of sweet flag rhizome oil (*Acorus calamus*) on hemogram and ultrastructure of hemocytes of the tobacco armyworm, *Spodoptera litura* (*Lepidoptera*: *Noctuidae*). Micron 2008;39:544-51.
26. Liu XC, Zhou LG, Liu ZL, Du SS. Identification of insecticidal constituents of the essential oil of *Acorus calamus* rhizomes against *Liposcelis bostrychophila* Badonnel. Molecules 2013;18:5684-96.
27. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Carol Stream, IL: Allured Publishing; 2001.
28. Iguchi M, Nishiyama A, Koyama H, Yamamura S, Hirata Y. Isolation and structures of three new sesquiterpenes. Tetrahedron Lett 1968;9:5315-8.
29. Niwa M, Terada Y, Iguchi M, Yamamura S. Stereochemical studies on the elemente-types sesquiterpenes from *Acorus calamus* L. Chem Lett 1977;72:1415-8.
30. John RW, James FC. Photocycloaddition of methy-lcy-clobutene and (-)-piperitone: Synthesis of (-)-shyobunone and related sesquiterpenes. J Org Chem 1980;45:4475-8.
31. Sakuma M. Probit analysis of preference data. Appl Entomol Zool 1998;33:339-47.
32. Zhang JS, Zhao NN, Liu QZ, Liu ZL, Du SS, Zhou L, *et al*. Repellent constituents of essential oil of *Cymbopogon* distans aerial parts against two stored-product insects. J Agric Food Chem 2011;59:9910-5.
33. Galambosi B, Peura P. Agrobotanical features and oil content of wild and cultivated forms of caraway (*Carum carvi* L.). J Essent Oil Res 1996;8:389-97.
34. Laribi B, Bettaieb I, Kouki K, Sahli A, Mougou A, Marzouk B. Water deficit effects on caraway (*Carum carvi* L.) growth, essential oil and fatty acid composition. Ind Crop Prod 2009;30:372-9.
35. Marongiu B, Piras A, Porcedda S, Scorciapino A. Chemical composition of the essential oil and supercritical CO2 extract of *Commiphora myrrha* (Nees) Engl. and of *Acorus calamus* L. J Agric Food Chem 2005;53:7939-43.
36. Garneau FX, Collin G, Gagnon H, Belanger A, Lavoie S, Savard N, *et al*. Aromas from Quebec. I. Composition of the essential oil of the rhizomes of *Acorus calamus* L. J Essent Oil Res 2008;20:250-4.
37. Rost LC, Bos R. Biosystematic investigation with *Acorus* L. 3 communication. Constituents of essential oils. Planta Med 1979;36:350-61.
38. Todorova MN, Ognyanov IV, Shatar S. Chemical composition of essential oil from Mongolian *Acorus calamus* L. rhizomes. J Essent Oil Res 1995;7:191-3.
39. Koul O, Smirle MJ, Isman MB. Asarones from *Acorus calamus* L. Oil: Their effect on feeding behavior and dietary utilization in *Peridroma saucia*. J Chem Ecol 1990;16:1911-20.
40. Park C, Kim SI, Ahn YJ. Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored-product insects. J Stored Prod Res 2003;39:333-42.
41. Kim DH, Kim SI, Chang KS, Ahn YJ. Repellent activity of constituents identified in *Foeniculum vulgare* fruit against *Aedes aegypti* (*Diptera*: *Culicidae*). J Agric Food Chem 2002;50:6993-6.
42. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: A review. Bioresour Technol 2010;101:372-8.
43. Kim SI, Yoon JS, Jung JW, Hong KB, Ahn YJ, Kwon HW. Toxicity and repellency of oregano essential oil and its components against *Tribolium castaneum* (*Coleoptera*: *Tenebrionidae*) adults. J Asia Pac Entomol 2010;13:369-73.

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