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

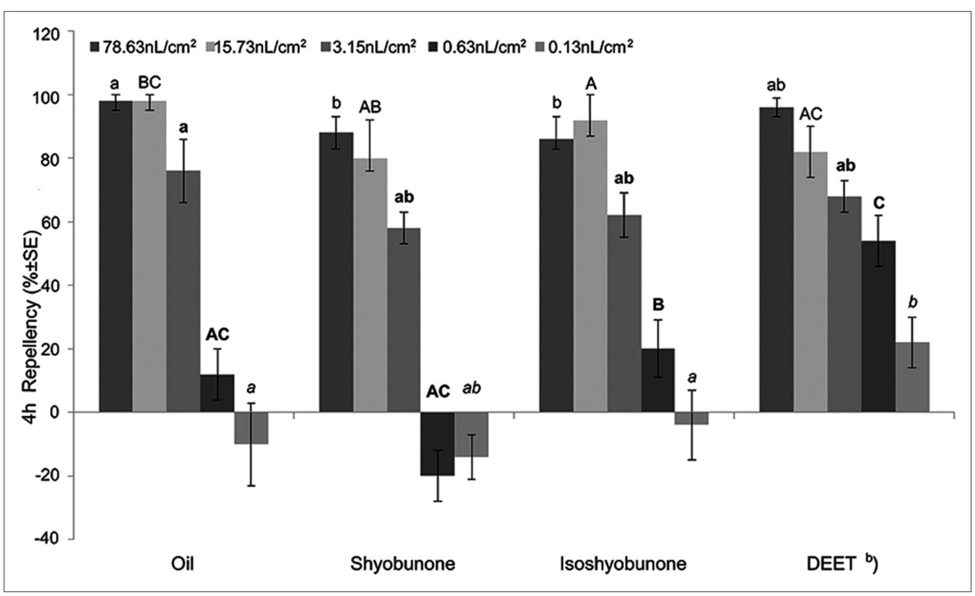

Figure 1: Percentage repellency (PR) of the essential oil from *Acorus calamus* rhizomes and its constituents against *Tribolium castaneum* at 4 h after exposure[a]. [a]Means in the same column followed by the same letters do not differ significantly (P > 0.05) in ANOVA and Tukey's tests. PR was subjected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

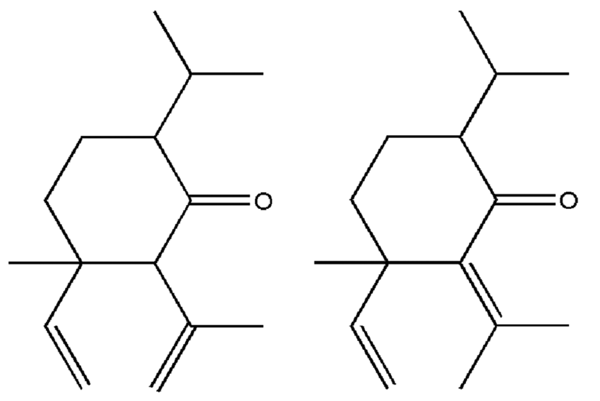

Figure 2: Constituent compounds isolated from the essential oil of *Acorus calamus* rhizomes

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

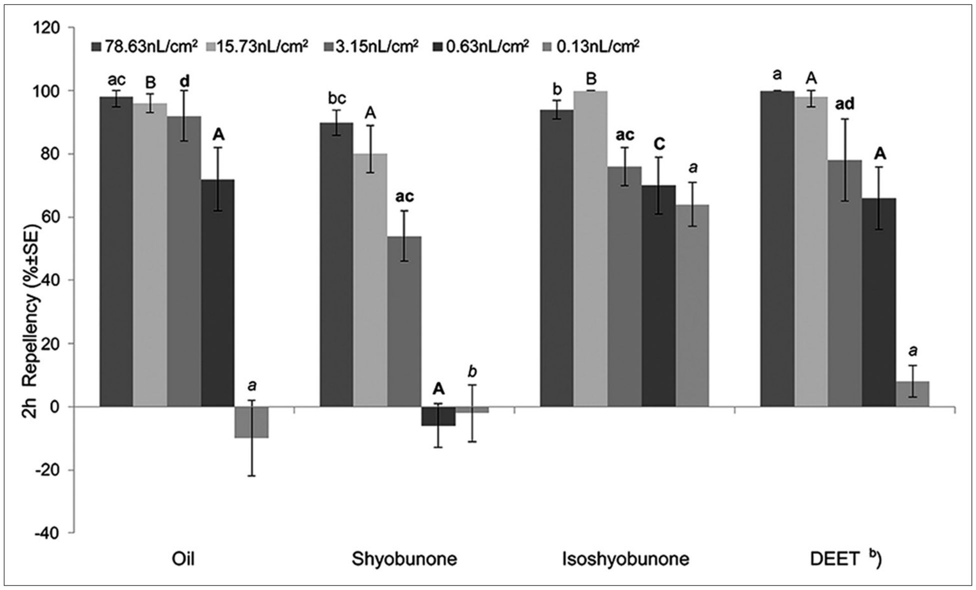

Figure 3: Percentage repellency (PR) of the essential oil from *Acorus calamus* rhizomes and its constituents against *Tribolium castaneum* at 2 h after exposure[a]. [a]Means in the same column followed by the same letters do not differ significantly ($P > 0.05$) in ANOVA and Tukey's tests. PR was subjected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

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