Contact toxicity of sweet flag rhizome (*Acorus calamus* L.) crude extracts on maize weevil, *Sitophilus zeamais* Motschulsky

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Abstract

Rhizome crude extracts of *Acorus calamus* L. were tested against maize grain weevil, *Sitophilus zeamais* adults for their contact toxicities under laboratory conditions. Dried rhizomes were grounded into powder before extracting with hexane, dichloromethane and methanol, respectively. A drop of 0.5 µL of each crude extract dilution (1, 3, 5, 7 and 9%) was dropped directly onto the thorax of 7 days old *S. zeamais* adults using a Burkard Arnold microapplicator (Burkard Manufacturing Company Ltd. England®). The crude hexane extract was found to possess moderate contact toxicity against *S. zeamais* with maximum mortality of 68% at 9% (v/v) 7 days after exposure. Conversely, the crude dichloromethane and methanol extracts showed low contact toxicity on *S. zeamais*. The mortality rate of *S. zeamais* were 0 to 38% and 0 to 10% during the experiment after the applications of crude dichloromethane and methanol extracts, respectively.

Keywords: *Sitophilus zeamais*, *Acorus calamus*, crude extract

1. Introduction

Thailand is one of the most botanically diverse countries in the world. Thai farmers cultivate rice, the main food of Thailand, for consumption in r households and also export. Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is an important pest of stored products such as maize, sorghum, wheat, barley, rice and paddy (Chankaewmanee, 1997). Larvae and adults of *S. zeamais* damage seed products which are stored in the barnr by feeding inside the seeds. Feeding by this insect can reduce both quality and quantity of stored products.

Phosphine, methyl bromide, sulphuryl fluoride and ethyl formate are commonly used to control stored-grain insects (Yao et al., 2012). Recent reports indicate moderate to high levels of phosphine resistance in field collected Brazillian populations of *Oryzaephius surinamensis* (L.) (Coleoptera: Silvanidae), *Rhizopertha dominica* (F.) (Coleoptera: Bostrichidae), *Triborium castaneum* (Herbst) (Coleoptera : Tenebrionidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Lorini et al., 2007; Pimentel et al., 2007, 2009). Consequently, the development of control techniques that provide efficient insect control without serious effects on product, environment and public health are required.

Botanical pesticides are one alternative for controlling stored product insects. Many researchers have investigated efficacy of plant crude extracts for controlling insects, with the aim to develop potential of crude extracts as new botanical insecticides. For example, the crude extract of *Millettia ferruginea* (Hochest) seeds showed contact toxicity to *S. zeamais* adults (Bekele, 2002). *Hyptis spicigera* Lam. fresh leaves, powder and crude oil extracts revealed insecticidal activities against *S. zeamais* (Othira et al., 2009). One of the most popular plant species that was used to control stored product insects is *Acorus calamus* L.

(sweet flag) which belongs to family Araceae. This plant has been known to have antibiotic effects against bacteria and insect pests (Yao et al., 2012). Previous research results demonstrated that ß-asarone was the main bioactive insecticidal compound of *A. calamus* (Schmidt and Streloke, 1994; Yao et al, 2008). Therefore, this experiment aims to determine the contact toxicity of *A. calamus* crude extracts against *S. zeamais* adults.

2. Materials and Methods

2.1. Test insects

The stock colonies of *S. zeamai*s used in this study were obtained from the Department of Agriculture (DOA), Ministry of Agriculture and Co-operatives (MOAC), Thailand. Three hundred *S. zeamai*s adults were cultured on 250 g of brown rice. The cultures were maintained in the labolatory at 28±2°C, 70±5% r.h. Seven-day-old adults were used in this experiment. All bioassays were conducted and carried out under the same environmental conditions as the cultures.

2.2. Crude extracts

Rhizomes of *A. calamus* were dried and grounded into powder by using a motor grinder. Dried powder (1 kg) was extracted 3 times with hexane at room temperature. The hexane solutions were then combined, filtered through a Whatman # 1 filter paper and evaporated under reduced pressure to give a crude hexane extract. The residue was consequently extracted with dichloromethane and methanol, respectively at the same condition to obtain the dichloromethane and methanol crude extracts, respectively. All crude extracts were kept in the refrigerator at 10-12°C for further study.

2.3. Contact toxicity

A drop of 0.5 µL of each concentration (1, 3, 5, 7, and 9% or 5, 15, 25, 35 and 45 µg/insect) was applied topically on the thorax of *S. zeamai*s adults by using a Burkard Arnold microapplicator (Burkard Manufacturing Company Ltd. UK). The control received the same volume of 95% ethanol or distilled water. The solvent was allowed to evaporate completely at room temperature. Ten test insects were introduced to the plastic cup (4.5 cm diameter and 2.7 cm height) and the plastic lid with net cover was placed on the plastic cup. Culture media was added to each treatment after 24 hours. There were 5 replications for each treatment (10 insects/replicate). Mortality of the adult insects was recorded daily for 1week.

2.4. Statistical analysis

The percentage of mortality of the adult was subjected to analysis of variance (ANOVA) using SPSS software. Means of treatments were separated with Duncan's multiple range test at $P = 0.05$.

3. Results and Discussion

The contact toxicities of *A. calamus* rhizome crude extracts using topical application against *S. zeamais* adults were shown in Table 1. At the highest concentration (9% v/v or 45 µg/insect), the crude hexane extract induced significantly higher mortalities of 40, 54 and 68% at 5, 6 and 7 days after treatment, respectively. The contact toxicity of hexane crude extract tended to increase with dosage from 3 days after treatment to the end of experiment. The lethal dose for 50 and 95% (LD_{50} and LD_{95}) contact toxicities of hexane crude extract was 40.28 and 56.01 µg/insect, respectively. In contrast, dichloromethane and methanol crude extracts at all concentrations demonstrated low contact toxicities which were ranging from 0-38 % and 0-10 % at the end of the experiment, respectively.

The previous studies documented that the crude extracts of *A. calamus* could be used to control some stored-grain insects (Paneru et al., 1997; Rahman and Schmidt, 1999; Kim et al., 2003a; Kim et al., 2003b; Hossain et al., 2008). Reports published involve with *A. calamus* rhizomes dominate contact toxicity against *S. zeamais* such as the ethanol extract of *A. calamus* had strong contact effect against adults of *S. zeamais* (Yao et al., 2008); supercritical fluid Co_2 extract of *A. calamus* exhibited contact toxicity agianst *S. zeamais* (Yao et al., 2012).

In this study, the hexane crude extract of *A. calamus* showed stronger adulticidal effect against *S. zeamais* than dichloromethane and methanol crude extracts. However, the maximum concentration of the hexane crude extract applied of *A. calamus* demonstrated moderate toxicity against *S. zeamais* at the end of experiment (68%). The hexane fraction obtained from the methanol extract of *A. gramineus* rhizome showed high mortality against adults of *S. oryzae* and *Callosobruchus chinensis* (L.) and exhibited moderate mortality on *Lasioderma serricorne* (F.) adults by direct contact application (Park et al., 2003). Yao et al. (2008) reported the contact toxicity of the ethanol crude extract of *A. calamus* on *S. zeamais* adults depended on both dose and exposure time. Their study was similar to our study since the contact toxicity of hexane crude extract of *A. calamus* varied with doses and exposure times. The hexane crude extract was of median value to control *S. zeamais* adults via contact action, indicating its activity might be due to an active chemical compounds in the extract. The major active constituents that were generally found in *Acorus* species are α-and β-asarones (Du et al., 2008; Ganjewala and Srivastava, 2011). However, a number of compounds were found and identified in *A. calamus* rhizome (Dong et al., 2010a, Dong et al., 2010b; Zhu et al., 2010)

As the result, the hexane crude extract of *A. calamus* from this study is possible to use for controlling *S. zeamais* adults via topical application. It is essential to continue doing research in field of analysis of active compounds in the hexane crude extract of *A. calamus*, to help preserve stored products. *Acorus calamus* hexane crude extract derived insect-control agent can be used for intergated pest management due to its rapid degradation and low environmental risks.

4. Conclusions

This experiment was carried out to investigate the contact toxicities of the crude extracts of *A. calamus* rhizomes on *S. zeamais* adults. The results showed that crude hexane extract of *A. calamus* rhizome had moderate contact toxicity against *S. zeamais*. The 54 and 68% mortalities were occurred at 9% v/v concentration at 6 and 7 days after treatment. In contrast, crude dichloromethane and methanol extracts of *A. calamus* indicated less contact toxicities on *S. zeamais* since the maximum mortalities of 38 and 10% were shown at 7 and 1% concentrations, respectively. The crude hexane extract of *A. calamus* rhizome could be used as an alternative seed protectant from *S. zeamais* damaging. This botanical extract needs to be investigated in more detail for use in controlling other stored-product insects.

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Table 1 Mortality (%) of *Sitophilus zeamais* adults caused by *Acorus calamus* rhizome crude extracts.

Solvent	Doses (µg/insect)	Day after treatment [a] 1	2	3	4	5	6	7
n-Hexane	control	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c
	5	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c
	15	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c
	25	0.0±0.0b	0.0±0.0b	2.0±2.1ab	2.0±2.1b	2.0±2.1c	6.0±3.0c	6.0±3.0c
	35	8.0±2.1a	8.0±2.1a	8.0±2.1ab	16.0±3.0ab	20.0±2.7b	28.0±2.9b	30.0±3.5b
	45	4.0±2.3b	6.0±3.0b	14.0±4.4a	30.0±5.0a	40.0±5.0a	54.0±5.0a	68.0±3.9a
	P	0.002	0.019	0.104	0.003	0.000	0.000	0.000
	LD_{50}	-	-	-	-	-	-	40.28
	LD_{95}	-	-	-	-	-	-	56.01
Dichloromethane	control	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0b
	5	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0b
	15	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c	2.0±2.1b
	25	0.0±0.0a	0.0±0.0a	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0c	2.0±2.1b
	35	2.0±2.1a	2.0±2.1a	14.0±3.0a	16.0±3.4a	24.0±3.7a	36.0±6.0a	38.0±4.4a
	45	2.0±2.1a	2.0±2.1a	4.0±2.3b	8.0±2.1b	14.0±3.0b	14.0±3.4b	28.0±2.9a
	P	0.623	0.623	0.000	0.001	0.000	0.000	0.000
Methanol	control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	10.0±4.5a
	15	0.0±0.0	0.0±0.0	0.0±0.0	2.0±2.1a	2.0±2.1a	2.0±2.1a	4.0±2.3a
	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	35	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	45	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	P	-	-	-	0.431	0.431	0.431	0.479

[a]Values were based on 5 levels of content (1, 3, 5, 7 and 9%), five replicates of 10 insects in each replication. For each solvent, means in the same column followed by the same letters do not differ significantly ($P > 0.05$) as determined by Duncan's Multiple Range Test.

References

Bekele, J., 2002. Evaluation of the toxicity potential of *Milletia ferruginea* (Hochest) bager against *Sitophilus zeamais* (Motsch). International Journal of Pest Management 48, 29–32.

Chankaewmanee, B., 1997. Training course of insect and animal pests and their control 9th: Stored product insect pests. Department of Agriculture, Bangkok. 34 p. (in Thai)

Dong, W., Li, M., Yang, D., Lu, R., 2010a. Two new sesquiterpenes from *Acorus calamus*. Planta Medica 76, 1742–1745.

Dong, W., Yang, D., Lu, R., 2010b. Chemical constituents from the rhizome of *Acorus calamus* L. Planta Medica 46, 454–457.

Du, Z., Clery, R.A., Hammond, C.J., 2008. Volatiles from leaves and rhizomes of fragrant *Acorus* spp. (Acoraceae). Chemistry and Biodiversity 5, 887–895.

Ganjewala, D., Srivastava, A.K., 2011. An update on chemical composition and bioactivities of *Acorus* species. Asian Journal of Plant Sciences, 1–8.

Hossain, M.S., Zaman, S., Haque, A.B.M.H., Bhuiyan, M.P.I., Khondkar, P., Islam, M.R., 2008. Chemical and pesticidal studies on *Acorus calamus* rhizomes. Journal of Appllied Sciences Research 4, 1261–1266.

Kim, S.-Il, Park, C., Ohh, M.-H., Cho, H.-C., Ahn, Y.-J. 2003a. Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). Journal of Stored Products Research 39, 11–19.

Kim, S.-Il, Roh, J.-Y., Kim, D.-H., Lee, H.-S., Ahn, Y.-J. 2003b. Insecticidal acticities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. Journal of Stored Products Research 39, 293–303.

Lorini, I., Collins, P.J., Daglish, G.J., Nayak, M.K., Pavic, H. 2007. Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science 63, 358–364.

Othira, J.O., Onek, L.A., Deng, L.A., Omolo, E.O. 2009. Insecticidal potency of *Hyptis spicigera* preparations against *Sitophilus zeamais* (Motschulsky) and *Tribolium castaneum* (herbst) on stored maize grains. African Journal of Agricultural Research 4, 187–192.

Paneru, R.B., le Patourel, G.N.J., Kennedy, S.H., 1997. Toxicity of *Acorus calamus* rhizome powder from eastern Nepal to *Sitophilus granaries* (L.) and *Sitophilus oryzae* (L.) (Coleoptera, Curculionidae). Crop Protection 16, 759–763.

Park, C., Kim, S.-Il, Ahn, Y.-J., 2003. Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored-product insects. Journal of Stored Products Research 39, 333–342.

Pimentel, M.A.G., Faroni, L.R.D'A., Guedes, R.N.C., Sousa, A.H., Totola, M.R., 2009. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Stored Products Research 45, 71–74.

Pimentel, M.A.G., Faroni, L.R.D'A., Totola, M.R., Guedes, R.N.C., 2007. Phosphine resistance, respiration rate and fitness consequences in stored-product insects. Pest Management Science 63, 876–881.

Rahman, M.M., Schmidt, G.H., 1999. Effect of *Acorus calamus* (L.) (Araceae) essential oil vapous from various origins on *Callosobruchus phaseoli* (Gyllenhal) (Coleoptera: Bruchidae). Journal of Stored Products Research 35, 285–295.

Schmidt, G.H., Streloke, M., 1994. Effect of *Acorus calamus* L. (Araceae) oil and its main compound ß-asarone on *Prostephanus truncates* Horn. (Coleoptera: Bostrichidae). Journal of Stored Products Research 30, 227–235.

Yao, Y., Cai, W., Yang, C., Hua, H., 2012. Supercritical fluid CO_2 extraction of *Acorus calamus* L. (Arales: Araceae) and its contact toxicity to *Sitophilus zeamais* Motschusky (Coleoptera: Curculionidae). Natural Product Research 26, 1498–1503.

Yao, Y.J., Cai, W.L., Yang, C.J., Xue, D., Huang, Y.Z., 2008. Isolation and characterization of insecticidal activity of (*Z*)-asarone from *Acorus calamus* L. Insect Science 15, 229–236.

Zhu, M., Tan, N., Ji., C., Xu, J., He, W., Zhang, Y., 2010. Chemical constituents from petroleum ether fraction of ethanol extract of *Acorus tatarinowii*. Zhongguo Zhong Yao Za Zhi 35, 173–176.