SYNERGISTIC CONTACT ACTIVITY CONSTITUENTS OF *Acorus gramineus* AND *Rosmarinus officinalis* ESSENTIAL OILS AGAINST *Sitophilus oryzae*

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ABSTRACT

Plant essential oils exhibit multiple insecticidal activities and serve as an important source of botanical insecticides. This study evaluated the contact toxicity of 26 plant essential oils, alone and in binary mixtures, against *Sitophilus oryzae* (Coleoptera: Curculionidae; Linnaeus, 1763). Essential oils were extracted by steam distillation. The active constituents of the two oils with the strongest individual and combined activities were identified by gas chromatography-mass spectrometery and a compound-elimination assay. The essential oil of *Acorus gramineus* (Acorales: Acoraceae; Aiton, 1789) showed the strongest contact toxicity against *Sitophilus oryzae*, with an LD_{50} value of 4.01 μg/adult. When *Acorus gramineus* oil was combined with the other 25 oils, *Rosmarinus officinalis* (Lamiales: Lamiaceae; Linnaeus, 1753) oil produced the strongest synergistic effect, with a co-toxicity factor of 60.87. The major constituents of *Acorus gramineus* oil were β-asarone (69.94%), isoeugenol methyl ether (14.04%) and aristolone (5.14%), whereas those of *Rosmarinus officinalis* oil were patchouli alcohol (64.33%), eucalyptol (8.63%), (+)-2-bornanone (5.56%) and L(-)-borneol (4.49%). The compound-elimination assay identified β-asarone and patchouli alcohol as the principal active constituents of *Acorus gramineus* and *Rosmarinus officinalis* oils, with LD_{50} values of 3.82 and 39.93 μg/adult, respectively. β-Asarone showed stronger contact toxicity than *Acorus gramineus* essential oil. A mixture of β-asarone and patchouli alcohol at a volume ratio of 1:9 had an LD_{50} value of 35.42 μg/adult and a synergistic ratio of 0.58, indicating an additive effect. *Acorus gramineus* essential oil showed strong contact toxicity against *Sitophilus oryzae*. Its combination with *Rosmarinus officinalis* oil produced the strongest synergistic effect among the tested oil mixtures. The main active constituents were β-asarone and patchouli alcohol, and their binary mixture showed additive contact toxicity against *Sitophilus oryzae*.

Key words: β-asarone; patchouli alcohol; *Sitophilus oryzae*; compound-elimination assay; binary mixture

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INTRODUCTION

Rice weevil *Sitophilus oryzae* is a major stored-grain pest worldwide and causes serious damage to stored products, including wheat, rice, sorghum, beans, dried fruits and medicinal herbs (Gyawali *et al.*, 2026). Infestation by *S. oryzae* increases grain moisture, the proportion of insect-damaged kernels and powder accumulation, while reducing thousand-grain weight and overall grain quality; heavily infested wheat can become mouldy, clumped and unsuitable for consumption (Lougraimzi *et al.*, 2018; Ahmad *et al.*, 2017). Insecticides are vital to modern agriculture because they effectively suppress pest populations, reduce insect-induced crop losses, and ensure stable crop yields and food supply. Nevertheless, their drawbacks including insect resistance, non-target toxicity and environmental contamination have led to the replacement of traditional insecticides with safer and high-efficiency substitutes (Zakaria and Zuharah, 2025; Nguyen *et al.*, 2015). Therefore, efficient, safe and environmentally benign grain fumigants and protectants are urgently needed. Plant essential oils act as semiochemicals and can exert multiple effects on insects, including fumigant and contact toxicity (Sheikh *et al.*, 2024). They rapidly penetrate the insect cuticle and act on physiological targets, altering the activities of ALP and GOT and ultimately causing mortality in pests.(Gaire *et al.*, 2019; Omar *et al.*, 2024). Given their degradable nature, limited environmental persistence and low resistance-inducing risk, plant essential oils possess great practical potential for controlling stored grain insects (Yallappa *et al.*, 2025; Vendan *et al.*, 2017).

Acorus gramineus is a perennial herb in the genus Acorus. It grows in wet habitats, including stream banks and rocky areas at elevations below 1800 m a.s.l. and is cultivated widely for medicinal use (Ryuk *et al.*, 2014). Extracts of *A. gramineus* have shown insecticidal activity. For example, the methanol extract of *A. gramineus* rhizomes caused an

83.00% corrected mortality rate in *S. oryzae* and *Callosobruchus chinensis* (Coleoptera: Chrysomelidae; Linnaeus, 1758) after 48 h at 3.5 mg/cm2 in a filter-paper diffusion assay (Kim *et al.*, 2003). In another study, n-hexane, chloroform, ethyl acetate and water extracts of *A. gramineus* rhizomes caused corrected mortality rates of 83.00% in *S. oryzae*, 100% in *C. chinensis* and 57% in *Lasioderma serricorne* (Coleoptera: Ptinidae; Fabricius, 1792) after 48 h at 0.51 mg/cm2 (Kim *et al.*, 2003). The n-hexane extract of *A. gramineus* was active against *S. oryzae*, *C. chinensis* and *L. serricorne*, with corrected mortality rates of 100%, 100% and 57%, respectively, after 48 h at 0.51 mg/cm^2 (Kim *et al.*, 2003). *Acorus gramineus* n-hexane extract has also shown activity against larvae of *Lucilia sericata* (Diptera: Calliphoridae; Meigen, 1826), larvae of *Boophilus microplus* (Ixodida: Ixodidae; Canestrini, 1887) and adults of *Ornithodoros moubata* (Ixodida: Argasidae; Murray, 1877), whereas an aqueous decoction of *A. gramineus* was active against *Ascaris suum* (Ascaridida: Ascarididae; Goeze, 1782) (Perrett and Whitfield, 1995).

Rosmarinus officinalis (Lamiales: Lamiaceae; Linnaeus, 1753) is an aromatic herb in the genus Rosmarinus. It is native to Europe and the Mediterranean coast of North Africa and has been introduced into China. The plant is used as a source of aromatic oil for soap and cosmetic fragrances and is also grown as an ornamental species (Peng *et al.*, 2005). *Rosmarinus officinalis* essential oil has shown fumigant activity, with LD_{50} values of 43.00 and 19.28 μL/L after 12 and 24 h, respectively. At 10 μL/mL, the oil showed repellent activity against adults of *Aphis gossypii* (Hemiptera: Aphididae; Glover, 1877) (Koorki, 2018). In addition, *R. officinalis* oil showed 24- and 48-h fumigant LD_{50} values of 222.82 and 55.79 μL/L, respectively, against *Culex pipiens* (Diptera: Culicidae; Linnaeus, 1758) (Ramzi *et al.*, 2022). These findings indicate that *A. gramineus* and *R. officinalis* oils contain insecticidal constituents. In preliminary screening of 26 plant essential oils, we found that *A. gramineus* oil had strong contact toxicity against *S. oryzae*. When the LD_{30} dose of *A. gramineus* oil was mixed with the LD_{30} doses of other oils, the combination with *R. officinalis* oil showed a strong synergistic effect. Therefore, we analysed the chemical composition of *A. gramineus* and *R. officinalis* oils by gas chromatography-mass spectrometry and used a compound-elimination assay to identify their active constituents. We evaluated the activity of the active-constituent mixture. These results provide a basis for developing *A. gramineus* and *R. officinalis* oil resources for the control of *S. oryzae*.

MATERIALS AND METHODS

Test materials: Twenty-six plant samples were identified by Professor Du Fan from the Botany Teaching and Research Office, College of Forestry, Southwest Forestry University. The scientific names, family names, collection locations and plant parts used are listed in Table 1. Samples were collected from July to October 2021.

Chemical reagents: β-asarone (≥98%), isoeugenol methyl ether (≥98%), aristolone (≥98%), eucalyptol (≥99%), eucalyptol (≥96%) and L(-)-borneol (≥98%) were purchased from Shanghai Yuan Ye Biotechnology Co., Ltd. Patchouli alcohol (≥98%) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. n-Hexane of analytical grade was purchased from Tianjin Damao Chemical Reagent Factory.

Instruments and equipment: The instruments used in this study included an HP7890A-5975C gas chromatography-mass spectrometry system (Agilent Technologies, USA), a constant-temperature incubator (Shanghai Kuntian Experimental Instrument Co., Ltd.), an electric heating mantle (Shanghai Lichen Co., Ltd.) and a micropipette (Shanghai Dalong Medical Equipment Co., Ltd.).

Extraction of plant essential oils: Essential oils from the 26 plant samples were extracted according to Method A in Appendix XD of the Chinese Pharmacopoeia. Each sample was placed in a 1 L round-bottom flask, and 300-500 mL of water was added. The flask was fitted with a volatile-oil extractor and a reflux condenser and heated with an electric heating mantle for 5-6 h until no further increase in oil volume was observed. The oil phase was collected, freeze-dried and stored under refrigeration (4℃) until use.

Determination of essential-oil components: Chemical components were analysed using an HP7890A-5975C gas chromatography-mass spectrometry system equipped with a quartz capillary column (30 m × 0.25 mm × 0.25 μm). The oven temperature was held at 100 °C for 2 min, increased to 250 °C at 4 °C/min and then held at 250 °C for 10 min. The injector temperature was 250 °C, the column pressure was 100 kPa, the injection volume was 0.2 L, the split ratio was 100:1 and helium was used as the carrier gas at 1.2 mL/min.

Mass spectrometry conditions: The ion source was operated in electron-impact mode at 70 eV. The gas chromatography-mass spectrometry interface, ion source and quadrupole temperatures were 280, 230 and 150 °C, respectively. The electron multiplier voltage was 1894 V. Compounds were identified by comparison with the Wiley 7n.l standard mass spectral library, and relative contents were calculated by peak-area normalization.

Table 1 Scientific names, collection sites and plant parts of the tested plant samples.

No.	Scientific name	Family	Collection location	Parts
1	*Acorus gramineus* (Acorales: Acoraceae; Aiton, 1789)	Acoraceae	33°30′N, 116°53′E	root
2	*Angelica sinensis* (Apiales: Apiaceae; Diels, 1900)	Apiaceae	34°26′N, 104°02′E	root
3	*Foeniculum vulgare* (Apiales: Apiaceae; Miller, 1768)	Apiaceae	26°86′N, 100°25′E	fruit
4	*Notopterygium incisum* (Apiales: Apiaceae; H.T.Chang, 1975)	Apiaceae	30°54′N, 02°32′E	root and stock
5	*Asarum heterotropoides* (Piperales: Aristolochiaceae; F.Schmidt, 1868)	Aristolochiaceae	40°44′N, 124°47′E	root
6	*Artemisia argyi* (Asterales: Asteraceae; H.Leveille & Vaniot, 1910)	Asteraceae	25°23′N, 102°20′E	leaf
7	*Nardostachys jatamansi* (Dipsacales: Caprifoliaceae; de Candolle, 1830)	Caprifoliaceae	22°50′N, 110°10′E	herb
8	*Caryopteris forrestii* (Lamiales: Lamiaceae; Diels, 1912)	Lamiaceae	28°17′N, 98°52′E	aerial part
9	*Mosla chinensis* (Lamiales: Lamiaceae; Maximowicz, 1883)	Lamiaceae	25°20′N, 102°10′E	leaf
10	*Mentha canadensis* (Lamiales: Lamiaceae; Linnaeus, 1753)	Lamiaceae	25°23′N, 102°20′E	stem and leaf
11	*Nepeta cataria* (Lamiales: Lamiaceae; Linnaeus, 1753)	Lamiaceae	35°26′N 118°22′E	ear
12	*Pogostemon cablin* (Lamiales: Lamiaceae; Bentham, 1848)	Lamiaceae	18°50′N, 110°20′E	stem and leaf
13	*Rosmarinus officinalis* (Lamiales: Lamiaceae; Linnaeus, 1753)	Lamiaceae	25°4′N, 102°27′E	aerial part
14	*Cinnamomum cassia* (Laurales: Lauraceae; J.Presl, 1825)	Lauraceae	25°5′N, 99°6′E	bark
15	*Yulania biondii* (Magnoliales: Magnoliaceae; D.L.Fu, 2001)	Magnoliaceae	33°29′N, 112°26′E	bud
16	*Myristica fragrans* (Magnoliales: Myristicaceae; Houttuyn, 1774)	Myristicaceae	23°25′N, 115°50′E	kernel
17	*Syzygium aromaticum* (Myrtales: Myrtaceae; Merrill & L.M.Perry, 1939)	Myrtaceae	23°15′N, 109°11′E	bud
18	*Cymbopogon citratus* (Poales: Poaceae; Stapf, 1906)	Poaceae	21°54′N, 101°32′E	aerial part
19	*Citrus aurantium* (Sapindales: Rutaceae; Linnaeus, 1753)	Rutaceae	33°9′N, 107°20′E	fruit
20	*Zanthoxylum bungeanum* (Sapindales: Rutaceae; Maximowicz, 1871)	Rutaceae	29°23′N, 102°42′E	fruit
21	*Illicium verum* (Austrobaileyales: Schisandraceae; Hooker, 1888)	Schisandraceae	23°53′N, 105°40′E	fruit
22	*Amomum villosum* (Zingiberales: Zingiberaceae; Loureiro, 1790)	Zingiberaceae	22°5′N, 107°2′E	fruit
23	*Amomum kravanh* (Zingiberales: Zingiberaceae; Gagnepain, 1906)	Zingiberaceae	30°43′N, 103°32′E	fruit
24	*Curcuma longa* (Zingiberales: Zingiberaceae; Linnaeus, 1753)	Zingiberaceae	24°45′N, 104°11′E	root
25	*Curcuma phaeocaulis* (Zingiberales: Zingiberaceae; Valeton, 1918)	Zingiberaceae	22°24′N, 109°17′E	root
26	*Zingiber officinale* (Zingiberales: Zingiberaceae; Roscoe, 1807)	Zingiberaceae	24°50′N, 104°23′E	root

Rearing of *S. oryzae*: The test insects were maintained in an incubator at 26-28 °C, 65-75% relative humidity and a 16 h light/8 h dark photoperiod. The insects were reared on rice. Adults were removed after 7 days, and newly emerged adults were collected 1-2 weeks later for bioassays.

Contact-toxicity assay: Contact toxicity against *S. oryzae* was determined using the method described by Sang *et al.* (2023) and Fang *et al.* (2010), with n-hexane as the solvent. Test concendtrations were selected on the basis of preliminary assays. Using a microsyringe, 0.50 μL of eacdh test solution was applied to the dorsal thoracic plate of each adult insect. Ten insects were used poer replicate, and each treatment was replicated five times. Treated insects were maintained in an iprncubator, with n-hexane as the negative control and cypermethrin as the positive control. Survival was recorded after 24 h. Mortality and corrected mortality were calculated using formulas (1) and (2). LD_{50} values were estimated by probit regression in IBM SPSS 25.0 (LD_{50} values, along with their 95% confidence intervals and standard errors, were estimated by probit regression using IBM SPSS 25.0. Concentration data were $\log_{10}$-transformed prior to modelling. Pearson's chi-square test was applied to evaluate the goodness-of-fit of the probit model at a significance level of $\alpha = 0.05$).

$$\text{mortality rate} = \frac{N_l}{N_m} \times 100\% \quad (1)$$

$$\text{Correction of mortality rate} = \frac{N_o - N_p}{1 - N_p} \times 100\% \quad (2)$$

In these formulas, N_l is the number of dead insects, Nm is the total number of insects in the treatment group, No is the mortality rate of the treatment group and Np is the mortality rate of the control group.

Binary mixtures of *A. gramineus* oil with the other 25 essential oils: The synergistic effects of *A. gramineus* oil mixed with each of the other 25 essential oils were evaluated according to Abbassy *et al.* (2009) and Dhinakaran *et al.* (2019). LD_{30} solutions were prepared for each essential oil. The corrected contact mortality of each individual oil against *S. oryzae* and that of each binary mixture were determined. The co-toxicity factor (CTF) was calculated using formula (3).

$$CTF=\frac{\text{percentage observed mortality-percentage expected mortality}}{\text{percentage expected mortality}}\times 100 \quad (3)$$

In formula (3), the expected corrected mortality is the sum of the corrected mortality rates of the two individual oils. A CTF value of 20 or greater indicates synergism, a value between -20 and 20 indicates an additive effect, and a value of -20 or lower indicates antagonism.

Compound-elimination assay for the main constituents of *A. gramineus* and *R. officinalis* oils: The contribution of major constituents to contact toxicity against *S. oryzae* was evaluated according to Kim *et al.* (2021) and Yeom *et al.* (2012). Compounds present at more than 3% in *A. gramineus* or *R. officinalis* oils were mixed according to their natural proportions. For *A. gramineus* oil, the following treatments were prepared: the LD_{90} concentration of the whole oil (15.89 μg/adult); an LD_{90} mixed-constituent solution containing isoeugenol methyl ether, β-asarone and aristolone at a natural ratio of 14.04:69.94:5.14; and mixed-constituent solutions in which isoeugenol methyl ether, β-asarone or aristolone was omitted. For *R.officinalis* oil, the following treatments were prepared: the LD_{90} concentration of the whole oil (454.23 μg/adult); an LD_{90} mixed-constituent solution containing eucalyptol, (+)-2-bornanone, L(-)-borneol and patchouli alcohol at a natural ratio of 8.63:5.56:4.49:64.33; and mixed-constituent solutions in which eucalyptol, (+)-2-bornanone, L(-)-borneol or patchouli alcohol was omitted. Each treatment was tested as described in the contact-toxicity assay.

Binary mixture of β-asarone and patchouli alcohol: The combined lethal effect of β-asarone and patchouli alcohol against *S. oryzae* was evaluated according to Chen *et al.* (2021) and Jiang *et al.* (2021). The two compounds were mixed at different volume ratios, and the expected LD_{50} value and synergistic ratio were calculated using formulas (4) and (5).

$$\text{Expected } LD_{50}=\frac{a+b}{\frac{a}{LD_{50}(A)}+\frac{b}{LD_{50}(B)}} \quad (4)$$

$$\text{synergistic ratio (R)}=\frac{\text{Expected } LD_{50}}{\text{Observed } LD_{50}} \quad (5)$$

RESULTS

The LD_{30} and LD_{50} values for the contact toxicity of the 26 plant essential oils against *S. oryzae* are shown in Table 2.

Table 2 Contact toxicity of 26 essential oils against *S. oryzae*.

Treatment	LD_{30} (95% CI)/μg·adult-1	LD_{50} (95% CI)/μg·adult-1	Slope ± SE	χ2	P value
A. gramineus	2.28（0.86~3.56）	4.01（2.21-5.76）	2.14±0.20	6.98	0.073
A. sinensis	5.54（4.93~6.12）	7.59（6.89 ~ 8.35）	3.83±0.29	1.70	0.638
P. cablin	8.41（6.88~10.00）	12.93（10.92~15.35）	2.81±0.27	2.69	0.442
C. cassia	14.57（13.06~15.67）	16.13（14.86 ~17.13）	11.88±1.61	0.95	0.813
M. chinensis	11.85（3.62~23.41）	20.56（10.63-72.73）	2.19±0.18	22.33	0.000
M. canadensis	12.94（4.06~34.70）	22.32（8.62~65.93）	2.22±0.18	18.65	0.000
S. aromaticum	18.08（15.43~20.82）	24.48（21.27~28.57）	3.98±0.45	0.04	0.998
A. heteropoides	21.76（18.85~24.37）	29.51（26.53~32.53）	3.96±0.37	0.33	0.954
M. fragrans	18.66（1.37~53.93）	34.67（7.53~154.37）	1.95±0.15	28.13	0.000
N. cataria	32.17（7.27~69.04）	54.20（21.22~152.96）	2.32±0.20	17.90	0.000
C. forrestii	30.29（0.60~24.55）	59.94 (10.00~998.78)	1.77±0.14	33.94	0.000
C. longa	30.67（0.00~332.96）	66.09（2.97~8.466539729）	1.57±0.13	45.33	0.000
N. incisum	24.56（0.05~296.16）	72.88（6.74~49436.08）	1.11±0.10	31.00	0.000
Z. bungeanum	63.11（36.85~89.42）	90.68（62.00~138.55）	3.33±0.37	5.80	0.122

Treatment	LD_{30} (95% CI)/μg·adult-1	LD_{50} (95% CI)/μg·adult-1	Slope ± SE	χ2	P value
A. kravanh	53.23（12.84~142.42）	121.98（47.15~567.68）	1.46±0.13	14.91	0.002
C. citratus	56.45（4.71~219.59）	123.43 (33.48~1750.20)	1.54±0.13	24.04	0.000
A. villosum	66.07（0.01~493.82）	130.94（17.79~803890.24）	1.77±0.16	34.59	0.000
F. vulgare	100.18（72.56~127.32）	145.39（113.58~189.02）	3.24±0.25	5.98	0.113
Z. officinale	59.36（0.08~1763.73）	153.63 (26.20~9.50E+8)	1.27±0.12	32.70	0.000
I. verum	58.76（0.40~602.31）	161.27（31.60~704380.61）	1.20±0.11	27.22	0.000
R. officinalis	107.35（12.35~247.58）	163.20 (52.38~657.10)	2.88±0.27	21.89	0.000
Y. biondii	127.78（115.17~140.27）	166.18（151.18~185.89）	4.60±0.53	1.39	0.707
C. phaeocaulis	26.36（0.00~95373.88）	193.29（33.68~9.73E+43）	1.30±0.12	32.32	0.000
N. jatamansi	138.40（0.00~333.43）	195.01（39.29~2.70E+12）	4.42±0.38	40.03	0.000
C. aurantium	120.66（57.58~198.70）	200.20（118.53~382.26）	2.39±0.24	7.72	0.052
A. argyi	31.86（0.071~4391248562）	238.16（16.61~1.01E+35）	0.60±0.06	27.13	0.000
Deltamethrin	0.006（0.005~0.007）	0.012（0.010~0.014）	1.75±0.16	3.77	0.290

As shown in Table 2, *A. gramineus* essential oil showed the strongest contact toxicity against *S. oryzae*. The oils of *A. sinensis*, *P. cablin* and *C. cassia* also showed strong contact toxicity, with LD_{50} values below 20.00 μg/adult. The oils of *Mosla chinensis*, *Mentha canadensis*, *Syzygium aromaticum*, *Asarum heteropoides* and *Myristica fragrans* showed moderate activity, with LD_{50} values below 50.00 μg/adult. In contrast, *Zingiber officinale*, *Illicium verum*, *Rosmarinus officinalis*, *Yulania biondii*, *Curcuma phaeocaulis*, *Nardostachys jatamansi*, *Citrus aurantium* and *Artemisia argyi* showed relatively weak contact toxicity. The effects of binary mixtures of *A. gramineus* oil with the other 25 essential oils against *S. oryzae* are shown in Table 3.

Table 3 Effects of binary mixtures of 26 essential oils on contact toxicity against *S.oryzae*.

Essential oil A	Essential oil B	Observed mortality/%		Binary mixture/%		Co-toxicity factor	Effect
		Observed A	Observed B	Expected	Observed		
A. gramineus	*A. sinensis*	22.00±3.74	6.00±2.45	28.00	32.00±6.63	14.29	additive
A. gramineus	*P. cablin*	22.00±3.74	74.00±8.12	96.00	94.00±2.45	-2.08	additive
A. gramineus	*C. cassia*	22.00±3.74	22.00±15.62	44.00	30.00±12.65	-31.82	antagonistic
A. gramineus	*M. chinensis*	22.00±3.74	2.00±2.00	24.00	18.00±4.90	-25.00	antagonistic
A. gramineus	*M. canadensis*	22.00±3.74	6.00±2.45	28.00	42.00±10.68	50.00	synergistic
A. gramineus	*S. aromaticum*	22.00±3.74	44.00±15.68	66.00	16.00±6.78	-75.76	antagonistic
A. gramineus	*A. heteropoides*	22.00±3.74	36.00±9.27	58.00	70.00±5.48	20.69	synergistic
A. gramineus	*M. fragrans*	22.00±3.74	20.00±6.32	42.00	18.00±7.35	-57.14	antagonistic
A. gramineus	*N. cataria*	22.00±3.74	20.00±17.61	42.00	34.00±12.49	-19.05	additive
A. gramineus	*C. forrestii*	22.00±3.74	4.00±2.45	26.00	8.00±3.74	-69.23	antagonistic
A. gramineus	*C. longa*	22.00±3.74	28.00±9.70	50.00	12.00±3.74	-76.00	antagonistic
A. gramineus	*N. incisum*	22.00±3.74	0.00±0.00	22.00	22.00±19.60	0.00	additive
A. gramineus	*Z. bungeanum*	22.00±3.74	62.00±10.68	84.00	26.00±5.10	-69.05	antagonistic
A. gramineus	*A. kravanh*	22.00±3.74	6.00±4.00	28.00	2.00±2.00	-92.86	antagonistic
A. gramineus	*C. citratus*	22.00±3.74	32.00±7.35	54.00	46.00±20.64	-14.81	additive
A. gramineus	*A. villosum*	22.00±3.74	4.00±2.45	26.00	2.00±2.00	-92.31	antagonistic
A. gramineus	*F. vulgare*	22.00±3.74	34.00±11.66	56.00	68.00±3.74	21.43	synergistic
A. gramineus	*Z. officinale*	22.00±3.74	38.00±10.68	60.00	26.00±7.48	-56.67	antagonistic
A. gramineus	*I. verum*	22.00±3.74	34.00±9.27	56.00	8.00±8.00	-85.71	antagonistic
A. gramineus	*R. officinalis*	22.00±3.74	24.00±8.72	46.00	74.00±12.08	60.87	synergistic
A. gramineus	*Y. biondii*	22.00±3.74	58.00±13.19	80.00	82.00±9.17	2.50	additive
A. gramineus	*C. phaeocaulis*	22.00±3.74	58.00±12.41	80.00	36.00±4.00	-55.00	antagonistic
A. gramineus	*N. jatamansi*	22.00±3.74	8.00±5.83	30.00	12.00±4.90	-60.00	antagonistic
A. gramineus	*C. aurantium*	22.00±3.74	24.00±5.10	46.00	24.00±7.48	-47.83	antagonistic
A. gramineus	*A. argyi*	22.00±3.74	4.00±2.45	26.00	16.00±2.45	-38.46	antagonistic

As shown in Table 3, mixtures of *A. gramineus* oil with *M. canadensis*, *A. heteropoides*, *F vulgare* or *R. officinalis* showed synergistic effects, with the *A. gramineus* + *R. officinalis* mixture producing the strongest effect. Mixtures of *A. gramineus* oil with *A. sinensis*, *P. cablin*, *N. cataria*, *N. incisum*, *C. citratus* or *Y. biondii* showed additive effects. Mixtures with *C. cassia*, *M. chinensis*, *S. aromaticum*, *M. fragrans*, *C. forrestii*, *C. longa*, *Z. bungeanum*, *A. kravanh*, *A. villosum*, *Z. officinale*, *I. verum*, *C. phaeocaulis*, *N. jatamansi*, *C. aurantium* or *A. argyi* showed antagonistic effects.

The chemical compositions of *A. gramineus* and *R. officinalis* essential oils are shown in Tables 4 and 5.

Table 4 Chemical constituents of *A. gramineus* essential oil.

No.	Retention time/min	Compounds	Relative content/%
1	4.897	camphor	0.54
2	12.516	isoeugenol methyl ether	14.04
3	13.439	6-epishyobunone	0.58
4	14.034	shyobunone	2.08
5	16.559	patchoulane	0.86
6	17.395	β-asarone	69.94
7	17.732	asarone	0.74
8	18.707	*aristolone*	5.14
9	18.879	1,7-dimethyl-4-(1-methylethyl)-spiro[4.5]dec-6-en-8-one	0.78
10	20.308	(Z)-dodec-5-en-4-olide	0.95

As shown in Table 4, the main constituents of *A. gramineus* essential oil were β-asarone, isoeugenol methyl ether and aristolone, with relative contents of 69.94%, 14.04% and 5.14%, respectively. Together, these three constituents accounted for 89.14% of the oil.

Table 5 Chemical constituents of *R. officinalis* essential oil.

No.	Retention time/min	Compounds	Relative content/%
1	2.62	α-pinene	2.33
2	3.58	eucalyptol	8.63
3	5.39	(+)-2-bornanone	5.56
4	5.77	L(-)-borneol	4.49
5	6.23	α-terpineol	2.95
6	6.68	(-)-verbenone	2.79
7	8.66	tridecane	1.59
8	18.46	β-selinene	2.62
9	18.61	patchouli alcohol	64.33
10	26.99	hexadecanoic acid, ethyl ester	2.84

As shown in Table 5, the main compounds in *R. officinalis* oil with relative contents greater than 3% were eucalyptol, (+)-2-bornanone, L(-)-borneol and patchouli alcohol, with contents of 8.63%, 5.56%, 4.49% and 64.33%, respectively. Together, these compounds accounted for 83.01% of the oil.

The contributions of the main constituents of *A. gramineus* and *R. officinalis* oils to contact toxicity against *S. oryzae* are shown in Figures 1 and 2, respectively.

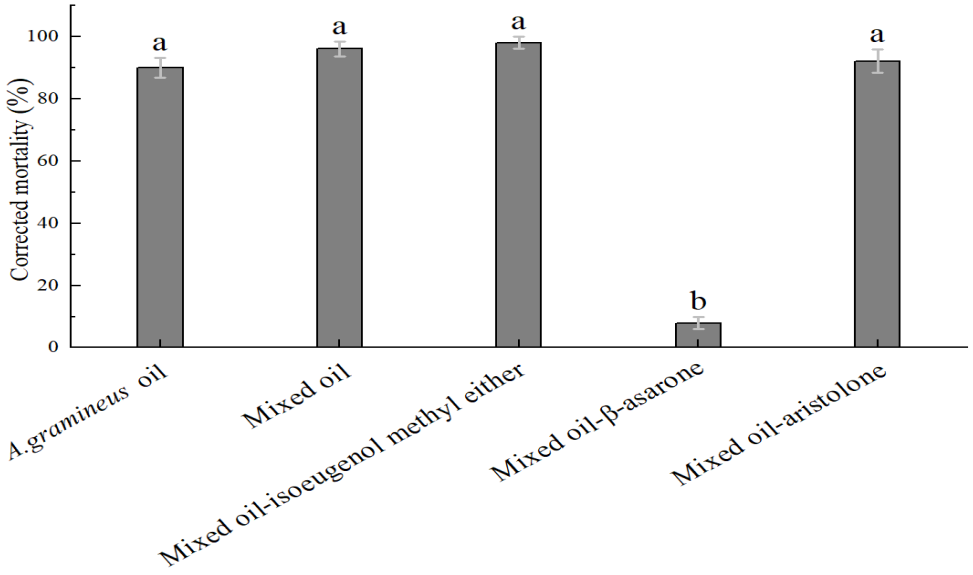


Figure 1 Compound-elimination assay of *A. gramineus* essential oil.: As shown in Figure 1, the corrected mortality rates caused by *A. gramineus* essential oil, the complete mixed-constituent solution, the isoeugenol methyl ether-eliminated mixture, the β-asarone-eliminated mixture and the aristolone-eliminated mixture were 90.0%, 96.0%, 98.0%, 8.0% and 92.0%, respectively. The β-asarone-eliminated mixture produced a significantly lower corrected mortality rate than the whole oil, the complete mixture and the other two elimination treatments. These results indicate that β-asarone is the principal active constituent of *A. gramineus* essential oil.

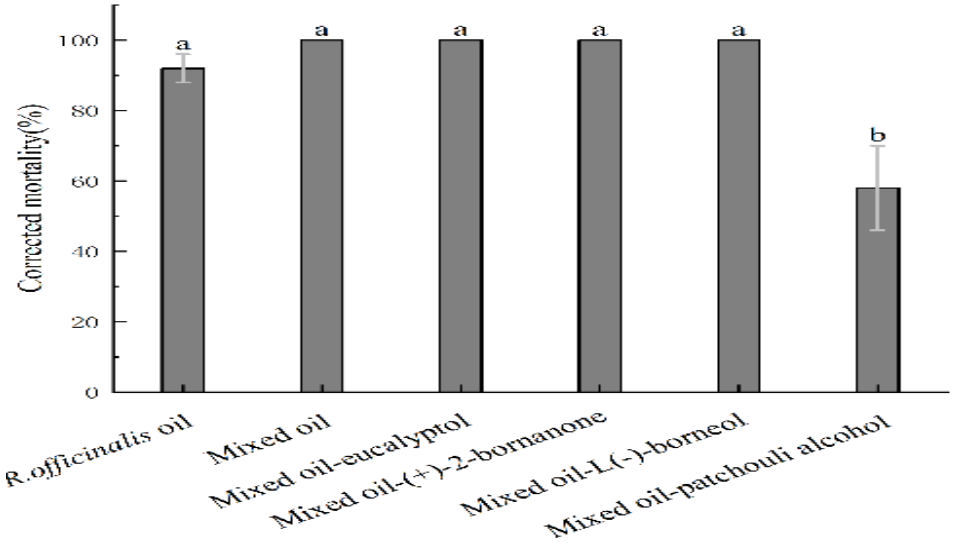


Figure 2 Compound-elimination assay of *R. officinalis* essential oil.: As shown in Figure 2, the corrected mortality rates caused by *R. officinalis* essential oil, the complete mixed-constituent solution, the eucalyptol-eliminated mixture, the (+)-2-bornanone-eliminated mixture, the L(-)-borneol-eliminated mixture and the patchouli alcohol-eliminated mixture were 92.0%, 100.0%, 100.0%, 100.0%, 100.0% and 58.0%, respectively. The patchouli alcohol-eliminated mixture produced a significantly lower corrected mortality rate than the whole oil, the complete mixture and the other elimination treatments. These results indicate that patchouli alcohol is an important active constituent of *R. officinalis* essential oil.

The contact toxicities of β-asarone and patchouli alcohol against *S. oryzae* are shown in Table 6.

Table 6 Contact toxicity of β-asarone and patchouli alcohol against *S. oryzae*.

No	Treatment	LD_{50}/μg·adult-1	95% CI	Slope ± SE	$\chi 2$	P-value
1	*A. gramineus* essential oil	4.01	2.21~5.76	2.14±0.20	6.98	0.073
2	*R. officinalis* essential oil	163.20	52.38~657.10	2.88±0.27	21.89	0.000
3	β-asarone	3.82	2.74～5.28	4.11±0.30	11.51	0.009
4	patchouli alcohol	39.93	24.96～59.19	3.18±0.26	12.81	0.005

As shown in Table 6, β-asarone and patchouli alcohol, which were identified by the compound-elimination assay as active constituents, showed stronger contact toxicity against *S. oryzae* than their corresponding whole essential oils.

The contact toxicity of β-asarone and patchouli alcohol mixtures against *S. oryzae* is shown in Table 7.

Table 7 Effects of binary mixtures of β-asarone and patchouli alcohol against *S. oryzae*.

β-asarone: patchouli alcohol (V/V)	Expected mortality rate/%	Observed corrected mortality rate/%	Co-toxicity factor	Effect
0:10	62	62	0	additive
1:9	32	40	25	synergistic
2:8	30	20	-33	antagonistic
3:7	25	20	-20	antagonistic
4:6	16	14	-13	additive
5:5	24	10	-58	antagonistic
6:4	22	4	-82	antagonistic
7:3	48	20	-58	antagonistic
8:2	52	22	-58	antagonistic
9:1	58	46	-21	antagonistic
10:0	68	68	0	additive

As shown in Table 7, the 1:9 mixture of β-asarone and patchouli alcohol showed a synergistic effect against *S. oryzae* based on the co-toxicity factor. Mixtures at ratios of 4:6, 0:10 and 10:0 showed additive effects, whereas the other ratios showed antagonistic effects.

The contact toxicity of the 1:9 β-asarone + patchouli alcohol mixture is shown in Table 8.

Table 8 Contact toxicity of the 1:9 β-asarone + patchouli alcohol mixture against *S. oryzae*.

β-asarone:patchouli alcohol (V/V)	Expected LD_{50}/μg·adult-1	Observed LD_{50}/μg·adult-1	Synergistic ratio	Effect
1:9	20.41	35.42	0.58	additive

As shown in Table 8, the 1:9 mixture of β-asarone and patchouli alcohol was less toxic than β-asarone alone but more toxic than patchouli alcohol alone or *R. officinalis* essential oil.

DISCUSSION

Food security is closely linked to economic development and social stability, and stored-grain pests cause substantial losses during storage. In this study, we screened 26 plant essential oils against *S. oryzae* and found that *A. gramineus* oil had the strongest contact toxicity, with an LD_{50} value of 4.01 μg/adult. β-asarone, the principal active constituent of *A. gramineus* oil, had an LD_{50} value of 3.82 μg/adult. Reported LD_{50} values for essential oils from *Elsholtzia kachinensis* (Lamiales: Lamiaceae; Prain, 1904), *Melaleuca bracteata* (Myrtales: Myrtaceae; F.Muell., 1858), *Rhododendron heliolepis* (Ericales: Ericaceae; Franch., 1887), *Rhododendron davidsonianum* (Ericales: Ericaceae; Rehd. et Wils., 1913), *Artemisia judaica* (Asterales: Asteraceae; Linnaeus, 1753), *Rhododendron strigillosum* (Ericales: Ericaceae; Franch., 1886) and *Lobularia maritima* (Brassicales: Brassicaceae; Desv., 1814) against *S. oryzae* are 29.62, 20.40, 15.02, 10.20, 9.62, 9.18 and 6.23 μg/adult, respectively (Zhang *et al.*, 2022; Zhang *et al.*, 2021; Sang *et al.*, 2022;

Abdelgaleil *et al.*, 2016; Wang *et al.*, 2020). Reported LD_{50} values for methyl eugenol, dehydrothymoquinone, carvone, methyl cinnamate, myristic acid and 3-pentenone against *S. oryzae* are 30.70, 28.82, 22.35, 10.70, 6.13 and 5.47 μg/adult, respectively (Zhang *et al.*, 2021; Zhang *et al.*, 2022; Sang *et al.*, 2023; Wang *et al.*, 2020). Compared with these reported values, *A. gramineus* oil and β-asarone showed strong contact toxicity against *S. oryzae* under similar bioassay conditions.

Insecticidal activity and possible mechanisms of β-asarone and patchouli alcohol: The compound-elimination assay was used to evaluate the contribution of three major constituents of *A. gramineus* oil (isoeugenol methyl ether, β-asarone and aristolone) and four major constituents of *R. officinalis* oil (eucalyptol, (+)-2-bornanone, L(-)-borneol and patchouli alcohol) to contact toxicity against *S. oryzae*. The results identified β-asarone and patchouli alcohol as the key constituents responsible for the contact toxicity of *A. gramineus* and *R. officinalis* oils, respectively.

Previous studies reported that the 48-h fumigant LC_{50} of β-asarone against *Rhyzopertha dominica* (Coleoptera: Bostrichidae; Fabricius, 1792) was 4.62 μL/L, with a contact LD_{50} of 42.56 μg/adult (Song, 2007). At 120 h, the fumigant LC_{50} values of β-asarone against *Sitophilus zeamais* (Coleoptera: Curculionidae; Motschulsky, 1855), *Rhyzopertha dominica*, *Tribolium castaneum* (Coleoptera: Tenebrionidae; Herbst, 1797) and *C. maculatus* (Coleoptera: Chrysomelidae; Fabricius, 1775) were 17.82, 4.42, 116.48 and 0.73 μL/L, respectively (Yao *et al.*, 2009). Against second-instar larvae of *Spodoptera litura* (Lepidoptera: Noctuidae; Fabricius, 1775) and *Mythimna separata* (Lepidoptera: Noctuidae; Walker, 1865), the 24-h contact LD_{50} values were 6.24 and 1.22 μg/adult, respectively (Yooboon *et al.*, 2023).

For patchouli alcohol, the 24-h fumigant LD_{50} against *Coptotermes formosanus* (Blattodea: Rhinotermitidae; Shiraki, 1909) was 5.33 μg/adult (Zhu *et al.*, 2003). The 24-h contact LD_{50} values against *T. castaneum* and *Lasioderma serricorne* were 96.60 and 13.08 μg/adult, respectively (Feng *et al.*, 2019).

Previous work showed that *S. oryzae* exposed to β-asarone in contact and fumigation assays passed through four behavioural stages: excitation, convulsion, paralysis and either death or recovery (Qiu *et al.*, 2014). Treatment with β-asarone inhibited acetylcholinesterase and glutathione S-transferase activities and induced carboxylesterase activity, suggesting that β-asarone may act as a neurotoxic compound. In Sf9 insect cells, β-asarone induced apoptosis, as indicated by cell shrinkage, rounding, membrane blebbing, chromatin condensation, apoptotic-body formation and nuclear DNA fragmentation (Yooboon *et al.*, 2019). Annexin V staining further confirmed β-asarone-induced apoptosis. Thus, β-asarone may exert insecticidal effects by disrupting neural function and inducing apoptosis. Patchouli alcohol also appears to have neurotoxic effects. In termites, patchouli alcohol caused convulsions and tremors within minutes, followed by paralysis, rigidity and death within 1 h (Zhu *et al.*, 2003). After 24 h, the termite exoskeleton remained intact, but internal tissues were dissolved, the body became semi-transparent and severe damage occurred at the application site. These observations suggest that patchouli alcohol may act through both neurotoxicity and disruption of the cuticular wax layer, thereby increasing body-wall permeability and causing internal tissue damage. In *S. oryzae*, β-asarone may therefore disrupt neural function and induce apoptosis, whereas patchouli alcohol may disrupt neural function and damage the cuticular barrier.

Mixtures of β-asarone and patchouli alcohol and their insecticidal implications: To evaluate the interaction between β-asarone and patchouli alcohol, the two compounds were mixed at a volume ratio of 1:9 and tested against *S. oryzae*. The mixture showed stronger contact toxicity than *R. officinalis* oil and patchouli alcohol alone, but weaker toxicity than *A. gramineus* oil and β-asarone alone. Previous work reported that capsaicin mixed with β-asarone at 3.00, 6.00, 12.00 and 18.00 μg/adult increased toxicity against second-instar larvae of *S. litura*, with enhancement factors of 1.87, 3.08, 8.47 and 11.08, respectively. Similar mixtures against *M. separata* larvae produced enhancement factors of 3.29, 8.45, 7.23 and 44.23, respectively (Yooboon *et al.*, 2023). In the present study, *A. gramineus* oil combined with *R. officinalis* oil showed synergism, whereas β-asarone combined with patchouli alcohol showed an additive effect. This difference indicates that the activity of plant essential oils may depend on interactions among multiple constituents, including minor or weakly active compounds, rather than only on the dominant active constituents. For example, thymol was identified as the active constituent of *Thymus mongolicus* (Lamiales: Lamiaceae; Ronniger, 1934) oil against *Tetranychus cinnabarinus* (Trombidiformes: Tetranychidae; Boisduval, 1867). The LC_{50} values of *T. mongolicus* oil and thymol against *T. cinnabarinus* were 762.1 and 1183.9 mg/L, respectively, indicating that the whole oil was more active than thymol alone. When thymol was mixed with less active compounds such as linalool, terpinene, umbelliferone and parthenolide, or with inactive compounds such as α-pinene, benzoic acid and gallic acid ethyl ester, corrected mortality increased significantly compared with thymol alone (Wu *et al.*, 2017).

Similarly, the active constituent of *Salvia hispanica* (Lamiales: Lamiaceae; Linnaeus, 1753) oil against *Spodoptera exigua* (Lepidoptera: Noctuidae; Hübner, 1808) was α-olivetone. When α-olivetone was mixed with less active compounds, including eucalyptol, camphene, α-caryophyllene, limonene, umbelliferone, citronellol and linalool, or with inactive compounds, including camphene, menthol, eucalyptol, β-pinene, 3-carene and β-caryophyllene, corrected mortality increased significantly relative to α-olivetone alone (Chen *et al.*, 2021). Miresmailli *et al.* (2006) also found

that eliminating minor constituents such as umbelliferone (0.9%), α-terpineol (1.1%) and citronellol (2.2%) from *R. officinalis* oil affected mortality in *Tetranychus urticae* (Trombidiformes: Tetranychidae; Koch, 1836) on *Phaseolus vulgaris* (Fabales: Fabaceae; Linnaeus, 1753), although eliminating the two most abundant constituents, eucalyptol (31.5%) and α-pinene (17.5%), had a stronger effect. These findings indicate that low-abundance constituents can still contribute to the overall insecticidal activity of essential oils. Therefore, the biological activity of plant essential oils is not always determined by a single active compound; it may instead arise from interactions among multiple constituents that jointly affect insect physiology and behaviour. Because essential oils are chemically complex, studying interactions among their constituents is important for developing combined botanical insecticides and for improving biological control strategies. Such interactions may also explain why essential oils differ from synthetic pesticides in their dose-response patterns. Synthetic pesticides often act on specific targets and produce relatively linear dose-mortality relationships. In contrast, essential oils contain complex mixtures of terpenoids and phenols. At concentrations below a critical threshold, individual constituents may not penetrate the insect cuticle or disrupt physiological processes sufficiently. Under such conditions, essential oils exert mainly sublethal effects: insect survival is maintained, yet key metabolic-enzyme activities (e.g.,AChE, LOP) are significantly altered. Once this threshold is exceeded, multiple active compounds may act together and cause rapid mortality, producing a threshold-type response. In addition, variation in cuticle thickness and detoxification-enzyme activity among *S. oryzae* individuals may further shape the response curve. Thus, the observed dose-response pattern likely reflects both the chemical complexity of essential oils and the physiological heterogeneity of the test insects.(Zimmermann *et al.*, 2025; Albogami *et al.*, 2025; Yoon and Tak, 2025; Al-Harbi *et al.*, 2021).

Conclusions: This study screened 26 plant essential oils and found that *A. gramineus* oil had the strongest contact toxicity against *S. oryzae*. Among binary oil mixtures, *A. gramineus* oil combined with *R. officinalis* oil produced the strongest synergistic effect. GC-MS analysis showed that the main constituents of *A. gramineus* oil were isoeugenol methyl ether (14.04%), β-asarone (69.94%) and aristolone (5.14%), whereas the main constituents of *R. officinalis* oil were eucalyptol (8.63%), (+)-2-bornanone (5.56%), L(-)-borneol (4.49%) and patchouli alcohol (64.33%). The compound-elimination assay identified β-asarone and patchouli alcohol as the main active constituents of *A. gramineus* and *R. officinalis* oils, respectively. Both compounds showed stronger contact toxicity against *S. oryzae* than their corresponding whole oils. The 1:9 mixture of β-asarone and patchouli alcohol showed an additive effect. These findings suggest that β-asarone is a promising active compound for the botanical control of *S. oryzae*.

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