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Investigation of insecticidal activity of two *Rhododendron* species on stored-product insects

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Abstract
This study was designed to investigate the insecticidal activity of the essential oils (EOs) and extracts from *Rhododendron rufum* and *Rhododendron przewalskii*. The EOs were extracted from the leaves of *R.* *rufum* and *R.* *przewalskii* by hydrodistillation and their chemical components were analyzed by gas chromatography–mass spectrometry (GC–MS). The repellency, contact toxicity and antifeedant activity of the EOs and extracts were evaluated against *Sitophilus oryzae* and *Tribolium castaneum* along with those of their main components. A total of nine compounds were identified from the EO of *R.* *rufum*, and the most abundant component was myristicin (79.72%). The EO of *R.* *rufum* exhibited repellent activities at different levels and its main compound myristicin showed contact toxicity and repellent effects against *S.* *oryzae* and *T.* *castaneum*. Meanwhile, by bioassay-guided fractionation, four compounds with strong antifeedant activities against *T.* *castaneum*, 24-methylenecycloartanyl-2'E, 4'Z-tetradecadienoate (1), methyl thyrsiflorin B acetate (2), friedelin (3) and Excoecarin R1 methyl ester (4) were separated and identified from the ethanol extract of *R.* *przewalskii* for the first time. Considering the significant anti-insect activities, the EOs and extracts of *R.* *rufum* and *R.* *przewalskii* might be used in integrated pest strategies, establishing a good perspective for the comprehensive use of natural plant resources of *Rhododendron* genus.

Keywords *Rhododendron rufum* · *Rhododendron przewalskii* · Essential oil · Repellent activity · Antifeedant activity

Introduction
The Chinese medicine (TCM) plays an important role not only in the prevention and treatment of conventional diseases, but also effectively in improving the physical conditions of patients during the COVID-19 pandemic (Wang et al. 2021). With the wide application of TCM and fast development of TCM industry, the market demand for Chinese material medica is significantly increasing. As we know most TCM are from natural sources, huge amounts of medicinal materials easily suffer from insect destruction and contamination during transportation and storage every year. At present, there are about more than two hundred kinds of storage pests harmful to TCM in the world, such as the main storage pests *Tribolium castaneum* and *Sitophilus oryzae*, causing serious economic loss and health public concerns (Magan et al. 2003; Wei et al. 2012).

In view of the above situations, a series of measures have been taken to prevent pest risks. The most commonly used method is the application of synthetic chemicals (Liang et al. 2021a, b). For example, phosphine fumigants and pyrethroids contact insecticides have been widely used for controlling insects in stored products worldwide (Nayak et al. 2020; Campolo et al. 2018). Although the conventional insecticides possess remarkable effect against insects, these synthetic products are not easy to degrade, toxic on nontarget animals, affecting pollination and damaging biological control, posing an underlying threat to human health and environment (Okonkwo and Okoye 1996; Isman 2006; Omae 2006).
et al. 2012; Tago et al. 2014; Bedini et al. 2015). Additionally, the overuse of chemical insecticides can enhance insect resistance and result in a vicious circle (Amini et al. 2019). Therefore, the development of insecticides with high efficiency, safety, eco-friendly and great potential to reduce reliance on conventional insecticides has gained increasing attention and has been highly sought after in scientific community (Caballero-Gallardo et al. 2011).

Plant-derived insecticide is recognized as one of the most promising alternatives to synthetic chemical insecticides. It has been reported that the EOs extracted from plants possessed a series of antibacterial, antifungal, antiviral, repellent and insecticidal properties (Abdelgaleil et al. 2016; Hashem et al. 2018; Peixoto et al. 2015; Pavela et al. 2018). In some areas of Asia and Africa, EOs were traditionally applied in preserving crops by the action of fumigant or contact against insects, and the method was quite suitable for protecting grain products in warehouse or some small farms (Shaaya et al. 1997; Mahmoudvand et al. 2011). EOs were extremely abundant in natural aromatic plants and contained a variety of bioactive substances, such as terpenoids, alkaloids, flavonoids and glycosides, which could affect the behavior, growth and development of storage pests in many ways (Mossa 2016; Hikal et al. 2017), so EOs could effectively alleviate the problem of drug resistance. Such natural compounds in EOs were partially biodegradable, such as compound quercetin belongings to flavonoid (Kumar et al. 2015), the capabilities of natural decomposable constituents greatly reduce the environmental and health hazards. In addition, the search for new active substances or precursors from plants, and new targets of action, can provide new ideas for the research of more effective and easily degradable alternatives to synthetic chemical insecticides.

The plants of the family Ericaceae are known for their high ornamental and medicinal values (Jaiswal et al. 2012). There are about 125 genera of Ericaceae worldwide, widely distributed in temperate regions and tropical mountains (Yoichi et al. 2016; Khan et al. 2021). Rhododendron is the largest genus, with about 967 species, mainly located in Asia, Europe and North America, about 700 species growing in China especially in Southwest China (Chang et al. 2021; Yan et al. 2015).

Currently, many kinds of chemical constitute including flavonoids, diterpenoids, triterpenoids, phenols, tannins, volatile oils, coumarins and lignans have been isolated from the different parts of the genus of Rhododendron plants (Jiao et al. 2015; Li and Vederas 2009). Investigations have shown that the genus of Rhododendron plants have pharmacological activities, e.g., expectorant, relieving cough and asthma, anti-inflammatory, analgesic, insecticidal, and effects on cardiovascular system, nervous system, and immune (Innocenti et al. 2010; Popescu and Kopp 2013; Rezk et al. 2015; Grimbs et al. 2017).

Many plants of Rhododendron genus recorded in ancient Chinese classics had insecticidal activities. In recent years, the EOs from the genus Rhododendron plants displayed competence of controlling stored pests because of their highly aromatics and medical properties. For instance, the hexane and methanol extracts of Rhododendron molle flower had significant insecticidal and feeding deterrent activities against S. zeamais and T. castaneum (Liu et al. 2007) and R. molle was widely used as an insecticide (Zong et al. 2021). Moreover, some species of Rhododendron including R. thymifolium, R. anthropogonoides, R. capitatum, R. mucronulatum and R. micrornulatum were confirmed their EOs repellent and insecticidal activities (Liang et al. 2016; Yang et al. 2011; Bai et al. 2019).

Therefore, our team chose R. rufum and R. przewalskii as potential pesticide and investigated the contact toxicity, repellent activities and feeding deterrent activity of the EOs and extracts along with their main compounds of the Rhododendron plants against S. oryzae and T. castaneum for the first time. On the one hand, we compared the chemical composition differences between R. rufum and R. przewalskii with other reported Rhododendrons and expect new findings. On the other hand, we provided scientific data for the application of the species of R. rufum and R. przewalskii in controlling storage insects.

Materials and methods

Plant material and essential oils extraction

Rhododendron rufum and R. przewalskii was collected from Tianzhu County, Gansu Province, in June 2018 and identified by Professor Liu Q.R. (Academy of Life Science, Beijing Normal University). The voucher specimen R. Rufum and R. przewalskii were deposited at the College of Resource, Beijing Normal University.

The fresh leaves (650 g) of the R. rufum were air-dried and ground into powder. The powder was subjected to hydro-distillation for 6 h to yield oil and extracted with the reagent of n-hexane, then the volatile oil was dehydrated by anhydrous sodium sulfate. At last, the pure oil was placed in an appropriate container and stored in a refrigerator at 4 °C.

The sample of R. przewalskii (650 g) was weighted and ground to powder, then shifted into modified volatile oil extractor. The EO was extracted by hydro-distillation with 10 times the amount of water for 6 h. The extracted EO was dehydrated by anhydrous sodium sulfate and stored into brown airtight containers at refrigerator in 4 °C.
Chemicals

The standard sample of myristicin was purchased from Meryer Chemical Technology Co., Ltd (Shanghai China). Pyrethrins (pyrethrin I and II, 37%), as positive control in contact toxicity assay, was purchased from HBCChem Inc. (USA). N, N-diethyl-3-methylbenzamide (DEET), a commercial repellent, was purchased from Service Chemical Inc. (Germany). Other chemical reagents such as n-hexane and anhydrous sodium sulfate were purchased from Shenyang Chemical Reagent Factory (Liaoning, China).

Insects

Two species insects in this experiment were cultured for the last two years in incubator in dark with 30 °C temperature and 70–80% relative humidity. Sitophilus oryzae were reared in glass containers (0.5 L) containing wheat and a small amount of cereal. Tribolium castaneum were raised with wheat flour mixed with yeast (10:1 w/w) in 0.5-L glass containers. The insects of 1–2-week-old adults were used in all experiments.

GC–MS analysis of essential oils

The volatile components from the leaves of R. Rufum were analyzed with Agilent 6890 N gas chromatography (GC) coupled with Agilent 5973 N mass spectrometer (MS), equipped with an FID detector and capillary column with HP-5 MS (30 m × 0.25 mm × 0.25 μm) under the following conditions. The starting temperature was 50 °C for 2 min and then raised at 2 °C/min to 250 °C for keeping 5 min, and the injector temperature was 250 °C. The carrier gas was helium with a flow rate of 1.0 ml/min. The sample of oil (1 μL) was diluted to 1% with hexane was injected with split mode at ratio of 1:10. The relative content (%) of individual compound of EO was obtained by the reports of averaging the GC-FID peak area (%). The retention index (RI) was determined by a series of homologous n-alkanes (C5–C36). The chemical constitutes of EO were characterized by comparing the data of MS and RI with these data in National Institute of Standards and Technology (NIST) 05, Wiley 275 libraries and some reported literatures (Adams 2001; Liang et al. 2016).

The chemical components of R. przewalskii volatile oil were analyzed by GC/MS. Using the above instrument at the same setup parameters, injection volume was 1 ml of 1% solution. An EI ionization source was used, the ion source temperature was 200°C and the electron energy was 70 eV. The mass spectrometer scanning range was 50–550 m/z. Standard control libraries were selected by NIST05 and WILEY275. The above method has been verified in the study of Bai et al (2019).

Preparation and separation of Rhododendron przewalskii extract

R. przewalskii was made into powder and extracted with 95% ethanol at room temperature for 2 h. The extract was concentrated in vacuo on a rotary evaporator to obtain a syrupy gum (972 g). Among them, we chose 500 g that was chromatographed on a silica gel column (160–200 mesh) by gradient elution with various ratios of petroleum ether-ethyl acetate and similar fractions were combined according to thin layer chromatography profiles. After repeated silica gel column chromatography, Sephadex LH-20 gel column, MCI Gel CHP20P column separation, purification, recrystallization, fifteen compounds were identified on basis of nuclear magnetic resonance spectroscopy and high-resolution electrospray ionization mass spectrometry according to the reported data (Rubinstein et al. 1976; Sunnerheim et al. 1988; Kim et al. 1994; Andrade 1998; Konishi et al. 2003; Shahat et al. 2003; Jeong and Shim 2004; González and Zaragozá 2005; Dieskau and Plietker 2011; Hussain et al. 2014; Sukito and Tachibana 2014; Imane et al. 2018).

Assay method of repellent effect

The repellent assay is a method for screening effective components of natural plants against storage pests, which is based on the theory of antagonistic storage (Zhang et al. 2014). In this experiment, the EO and myristicin were separately dissolved in n-hexane to prepare five concentrations (78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm2) to test S. oryzae and T. castaneum. Generally, the filter paper of 9 cm was equally cut into two pieces, one was treated with n-hexane and the other half was treated with test concentrations. After 20 s, two pieces of filter paper were glued carefully and quickly in Petri dishes 9 cm in diameter, then 20 insects were placed in the center of filter paper and quickly covered with the lid of Petri dish. The Petri dishes were placed in incubator in dark, the number of insects present on each strip was counted after 2 h and 4 h, respectively. Additionally, n-hexane was used as negative control, and the commercial repellent N, N-diethyl-3-methylbenzamide (DEET) was used as positive control. All experiments were repeated five times. The percent repellency (PR) was calculated by the following equation.

\[ PR(\%) = \left( \frac{N_c - N_t}{N_c + N_t} \right) \times 100 \]

where \( N_c \) was the number of insects of blank control group and \( N_t \) is the number of insects of treatment group.

The values of PR were recorded by the mean of five replicates of each tested concentration in repellent assay. One-way
Ingestion and T represents the treated group. Feeding deterrence indices are immediately picked out and weighed again. The value of incubation, the total weight was weighed. Finally, all insects were selected and placed in weighed glass bottle, and the insects needed to be starved for 24 h for further weighing. Then, the cookies are placed in bottle and weighed. Five replicates per dose, 10 sects per replicate. The n-hexane solvent was used as negative control and pyrethrins (pyrethrin I and II, 37%) were used as positive controls. The treated insects and control insects were then transferred to glass vials and kept in incubator. After 24 h, the number of insect deaths were recorded. Finally, LD50 values were calculated with Probit analysis (IBM SPSS V 20.0).

The mortality of insects after 24 h were calculated by the mean of five replicates of each tested concentration in contact toxicity assay. The data was analyzed by Probit (IBM SPSS V 20.0) to obtain LD50 (Sakuma 1998).

**Assay method of antifeedant activity**

An antifeedant assay is an improved method from the literature (Du et al. 2011; Wang et al. 2015; Zhang et al. 2018). In this experiment, we choose T. castaneum as targeted insects. The precisely weighed compound (2.0 mg) was dissolved in -hexane solvent and formulated as a 1.0 mg/mL solution. Then, it was diluted with water to obtain a series of concentration gradient solutions of 25, 74, 222, 667 and 2000 mg/kg. After that, the final solution was combined with wheat flour (0.4 g). At the same time, the blank control was prepared with pure water (2.0 mL) and wheat flour (0.4 g). The solution made six small biscuits drop by drop on a clean plate. After air-drying overnight, it was transferred to an incubator and left for 48 h. The incubator was maintained at a temperature of 29–30 °C and a relative humidity of 70–80%. In the experiment, 20 insects were selected and placed in weighed glass bottle, and the insects needed to be starved for 24 h for further weighing. Then, the cookies are placed in bottle and weighed. Five replicates were performed at each concentration. After 72 h of incubation, the total weight was weighed. Finally, all insects were immediately picked out and weighed again. The value of the food deterrence index is calculated as follows:

\[
\text{Ingestion deterrence index} (\%) = \left(\frac{C - T}{C}\right) \times 100
\]

C represents the diet ingested by the blank control group, and T represents the treated group. Feeding deterrence index and EC50 are calculated by SPSS. Statistical analysis was performed using a t-test, and \( p < 0.05 \) indicated significance.

**Results**

**Chemical compositions of essential oil and extract**

**Rhododendron rufum essential oil**

A total of 9 components were identified in the leaves of R. Rufum EO accounted for 98.06% of the EO. The gas chromatogram is shown in Fig. 1. As shown in Table 1, the EO was found to be rich in allylbenzene (> 79%) and terpenoids (> 14%). What is more, the main constituents included myristicin (79.72%), followed by manoyl oxide (6.72%) and 2,2,7,7-tetramethyltricyclo[6.2.1.0 (1,6)]undec-4-en-3-one (4.38%). It was worth noticing that the compound myristicin (79.72%) had an extremely high content in the EO of R. Rufum and had not been reported in the other species of Rhododendron, and thus, it was vitally important to explore the repellent and insecticidal activities of myristicin against stored insects.

**Chemical composition of Rhododendron przewalskii essential oil and ethanol extract**

Thirty compounds were identified in the EOs from R. przewalskii leaves, accounting for 84.94% of the total oil. As shown in Table 2, the main compound is 4-(2,3,4,6-Tetramethylphenyl)-3-buten-2-one (27.74%), followed by bisabolol oxide II (10.39%) and manoyl oxide (10.78%). The gas chromatogram was shown in Fig. 2.

There are fifteen compounds were obtained from the extraction of R. przewalskii by Sephadex LH-20 including Moraxanthin-3-O-β-glucoside, hyperoside, querctin, isoquerctin, 5,7,4-OH-3′-OCH3-3-xylose, 24-methylencyclooctanyl-2'E,4'Z-tetradecadienoate, methyl thyrsiflorin B acetate, friedelin, Excoecarín R1 methyl ester, β-sitosterol, rhododendrin, 2-methyl-1-butanol, menthol, quercetin, avicularin. Among them, the compounds of friedelin, Moraxanthin-3-O-β-glucoside, rhododendrin, 5,7,4-OH-3′-OCH3-3-xylose, methyl thyrsiflorin B acetate and 24-methylencyclooctanyl-2'E,4'Z-tetradecadienoate were the first time separated from the plant. The compounds 24-methylencyclooctanyl-2'E,4'Z-tetradecadienoate (1), methyl thyrsiflorin B acetate (2), friedelin (3) and Excoecarín R1 methyl ester (4) were identified as on basis of nuclear magnetic resonance spectroscopy and high-resolution electrospray ionization mass spectrometry. Their chemical structures are displayed in Fig. 3 and these chemical components exhibited strong feeding deterrent activity against T. castaneum adults.
Results of repellent activity determination

The repellent effects of *R. rufum* EO and myristicin on the adults of *S. oryzae* and *T. castaneum* expressed as PR are shown in Fig. 4. The repellent effects of *R. Rufum* EO and myristicin against *S. oryzae* were increased in a concentration-dependent manner after 2 h and 4 h exposure, and the EO of *R. Rufum* possessed stronger repellent effect against *S. oryzae* than myristicin at all the tested concentrations in the range of 78.63–0.13 nL/cm² after 2 h and 4 h of exposure. At the dose of 78.63 nL/cm², the EO of *R. Rufum* exhibited higher repellency than the positive control of DEET against *S. oryzae* after 2 h exposure. From Fig. 4C, D the *R. Rufum* EO and myristicin revealed significantly repellent activity against *T. castaneum*, and the repellency were increased in dose-dependent manner at the testing concentrations of 78.63–0.13 nL/cm² at 2 h and 4 h exposure. Myristicin showed stronger repellency (PR = 94%, 80%, 50%) than the positive control DEET (78%, 66%, 8%) at the concentrations of 3.15, 0.63 and 0.13 nL/cm² at 2 h after exposure. The PR values of myristicin were higher than that of DEET at all the tested concentrations after 4 h exposure. At the dose of 3.15, 0.63 and 0.13 nL/cm², the EO presented stronger repellent effect than DEET against *T. castaneum* after 4 h exposure.
The above results indicated the EO and myristicin possessed good repellent effect against two stored insects and the repellency was correlated with concentration. Previous reports of our work team (Bai et al. 2019) indicated the EO of *R. przewalskii* exhibited repellent activity against *T. castaneum* in 2- and 4-h exposure.

### Results of Contact toxicity determination

The contact toxicity assay against *S. oryzae* and *T. castaneum* is shown in Table 3. Myristicin had an obviously contact toxicity effect against *S. oryzae*, with the contact toxicity (LD$_{50}$ = 6.13 µg/adult) which was quite closely to the data of the positive control pyrethrins (LD$_{50}$ = 5.27) against *S. oryzae*, while myristicin showed moderate contact toxicity with a LD$_{50}$ value of 37.91 µg/adult against *T. castaneum*. Therefore, the myristicin possessed stronger contact toxicity against *S. oryzae* than against *T. castaneum*. Due to the extremely high content of myristicin and the myristicin had been confirmed the contact toxicity effect against the two stored insects at different levels, thus the existence of myristicin might be one of the most critical factors which affected the contact toxicity of *R. Rufum* EO against *S. oryzae* and *T. castaneum*.  

#### Table 2  The chemical composition of essential oil from the leaves of *R. przewalskii*

| Peak No | RT$^a$ (min) | Compounds                                      | Molecular Formula | Relative content$^b$ (%) | RI$^c$ |
|---------|--------------|------------------------------------------------|-------------------|--------------------------|-------|
| 1       | 11.67        | Linalool                                       | C$_{10}$H$_{16}$O | 0.58                     | 1100  |
| 2       | 13.32        | α-Terpineol                                    | C$_{10}$H$_{16}$O | 1.17                     | 1197  |
| 3       | 14.12        | Geraniol                                       | C$_{10}$H$_{16}$O | 0.55                     | 1253  |
| 4       | 14.29        | 4-Phenyl-2-butanol                             | C$_{10}$H$_{16}$O | 0.48                     | 1259  |
| 5       | 14.35        | Nonanoic acid                                  | C$_{9}$H$_{18}$O$_2$ | 1.9                     | 1278  |
| 6       | 16.89        | Icosapentaenoic acid                           | C$_{20}$H$_{30}$O$_2$ | 0.7                     | 1424  |
| 7       | 17.31        | (E)-(2,4,4-Trimethylcyclohex-1,5-dien-1-yl but-3-en-2-one | C$_{13}$H$_{25}$O$_2$ | 0.53                     | 1432  |
| 8       | 17.35        | β-Ionone                                       | C$_{13}$H$_{20}$O | 0.91                     | 1487  |
| 9       | 17.88        | δ-Cadinene                                     | C$_{15}$H$_{24}$  | 0.99                     | 1543  |
| 10      | 17.93        | Ionene                                         | C$_{15}$H$_{24}$  | 0.77                     | 1556  |
| 11      | 18.33        | E-Nerolidol                                    | C$_{15}$H$_{26}$O | 3.3                      | 1565  |
| 12      | 18.65        | Spathulenol                                    | C$_{15}$H$_{26}$O | 0.71                     | 1572  |
| 13      | 18.74        | Caryophyllene oxide                            | C$_{15}$H$_{28}$O | 1.94                     | 1578  |
| 14      | 18.77        | Globulol                                       | C$_{12}$H$_{20}$O | 0.99                     | 1585  |
| 15      | 18.96        | Aromadendrene oxide-(2)                       | C$_{15}$H$_{24}$O | 0.84                     | 1601  |
| 16      | 19.09        | Cedrenol                                       | C$_{15}$H$_{26}$O | 2.13                     | 1606  |
| 17      | 19.21        | β-Guaiene                                      | C$_{15}$H$_{24}$  | 1.5                      | 1611  |
| 18      | 19.42        | γ-Cadinol                                      | C$_{15}$H$_{26}$O | 1.86                     | 1641  |
| 19      | 19.44        | α-Murolol                                      | C$_{15}$H$_{26}$O | 0.74                     | 1656  |
| 20      | 19.54        | Bisabolol oxide II                            | C$_{12}$H$_{20}$O$_2$ | 10.39                  | 1658  |
| 21      | 19.85        | α-Bisabolol                                    | C$_{12}$H$_{20}$O$_2$ | 2.04                  | 1685  |
| 22      | 20.54        | 4-(2,3,4,6-Tetramethylphenyl)-3-buten-2-one    | C$_{14}$H$_{20}$O | 27.74                    | 1740  |
| 23      | 20.61        | Bisabolol oxide A                             | C$_{12}$H$_{20}$O$_2$ | 0.57                  | 1745  |
| 24      | 21.04        | Phenantherene                                  | C$_{14}$H$_{10}$  | 0.61                     | 1778  |
| 25      | 21.48        | Hexhydrofarnesyl acetone                      | C$_{18}$H$_{30}$O | 2.12                     | 1836  |
| 26      | 22.66        | trans-Nuciferol                                | C$_{12}$H$_{20}$O | 4.09                     | 1897  |
| 27      | 23.45        | Manoyl oxide                                   | C$_{20}$H$_{32}$O | 10.78                    | 1989  |
| 28      | 23.8         | Kaurene                                        | C$_{20}$H$_{32}$O | 2.12                     | 2045  |
| 29      | 24.13        | Phytol                                         | C$_{20}$H$_{40}$O | 1.39                     | 2112  |
| 30      | 25.83        | Eicosane                                       | C$_{20}$H$_{42}$O | 0.5                      | 2123  |
| Total   |              |                                                |                   |                          | 84.94 |

$^a$ RT: relative time

$^b$ Relative content (%): peak area relative to the total peak area

$^c$ RI: Retention indices relative to the homologous series of n-hydrocarbons on the HP-5MS capillary column
Fig. 2  Gas chromatogram plot of the leaves of *R. przewalskii* essential oil

Fig. 3  Chemical structures of compounds 1–4
Results of antifeedant effect determination

The 4 triterpene compounds isolated from *R. przewalskii*, 24-methylenecycloartanyl-2'E,4'Z-tetradecadienoate (1), methyl thrysiflorin B acetate (2), friedelin (3) and Excoecarin R1 methyl ester (4), all had strong antifeedant effects (Table 4). At the lowest concentration of 25 mg/kg, friedelin (3) exhibited the strong antifeedant activity against *T. castaneum* with an antifeedant index of 98.09%. 24-methylenecycloartanyl-2'E,4'Z-tetradecadienoate (1), methyl thrysiflorin B acetate (2) and excoecarin R1 methyl ester (4) also showed higher antifeedant activity against *T. castaneum*.

Table 3  Contact toxicity of myristicin against *S. oryzae* and *T. castaneum*

| Treatment    | LD50 (μg/adult) | 95% FL (μg/adult) | Slope ± SE | P-value | Chi-Square ($\chi^2$) |
|--------------|-----------------|-------------------|------------|---------|-----------------------|
| *S. oryzae*  | 6.13            | 5.69–6.62         | 2.87 ± 0.51| 0.996   | 2.49                  |
| Pyrethrins   | 5.27            | 3.45–5.27         | 0.76 ± 0.19| 1.000   | 1.01                  |
| *T. castaneum* | 37.91         | 34.12–42.29       | 0.37 ± 0.05| 0.918   | 6.68                  |
| Pyrethrins*  | 0.26            | 0.22–0.30         | 3.34 ± 0.32| 13.11   | 0.950                 |

*All values of Pyrethrins against *T. castaneum* from You et al (2014)

Table 4  Antifeedant activities of the isolated compounds from ethanol extract of *R. przewalskii* against *T. castaneum*

| Compound                                           | Antifeedant Index (%) |
|----------------------------------------------------|-----------------------|
|                                                    | 25 mg/kg | 74 mg/kg | 222 mg/kg | 667 mg/kg | 2000 mg/kg |
| 24-methylenecycloartanyl-2'E,4'Z-tetradecadienoate (1) | 92.65 ± 11.50 | 100.1 ± 1.73 | 99.05 ± 3.78 | 90.56 ± 5.00 | 100.14 ± 2.41 |
| Methyl thrysiflorin B acetate (2)                  | 90.73 ± 5.76 | 94.78 ± 2.56 | 99.23 ± 4.68 | 83.08 ± 5.10 | 84.34 ± 3.42 |
| Friedelin (3)                                      | 98.09 ± 3.04 | 90.64 ± 19.47 | 104.19 ± 4.77 | 101.59 ± 4.73 | 102.38 ± 1.44 |
| Excoecarin R1 methyl ester (4)                     | 95.46 ± 3.05 | 98.92 ± 3.61 | 101.88 ± 1.68 | 104.39 ± 1.34 | 104.66 ± 1.25 |
| Toosendanin*                                       | 32.32 ± 2.18 | 52.45 ± 3.27 | 69.52 ± 2.47 | 76.54 ± 3.62 | 86.27 ± 3.51 |

*represents the positive control. The antifeedant index of the blank control is 0
than the positive control, toosendanin, at the concentration of 25 mg/kg, with antifeedant index of 92.65%, 90.73% and 95.46%, respectively. Among them, excoecarin R1 methyl ester (4) showed the highest antifeedant index of 104.66% at the concentration of 2000 mg/kg. Additionally, all monomeric compounds exhibited higher antifeedant index than the positive control except methyl thrysiflorin B acetate (2) at the concentration of 2000 mg/kg. Therefore, these four terpenoids possessed significant antifeedant activity against T. castaneum, establishing a very good perspective of novel application of R. przewalskii to control stored-product insects.

As for these terpenoids, some special chemical groups such as carbonyl might be the functional group or main factor that made the chemicals display the obvious antifeedant activities. The specific reasons need to be further explored with the help of synthetic chemistry by structure comparison.

**Discussions**

At present, the chemical composition of EO in R. Rufum has never been reported. Similarly, the chemical analysis of the EO in R. przewalskii is very rare. Most of the chemical constituents detected in R. przewalskii EO reported in this paper were consistent with the previous studies (Bai et al. 2019). Compared with the other Rhododendron plants, these two EOs were also found rich in terpenoids, while the types and contents of terpenoids were different; for example, the content of β-Elemenone was 0.84% in the specie of R. Rufum in this experiment, whereas β-Elemenone (35.17%) had a high content in the specie of R. thymifoliu (Liang et al. 2021a, b). Spathulenol (14.4%), one of the main components isolated in the EO of R. albiflorum leaves, is only 0.71% in the specie of R. przewalskii (Schepetkin et al. 2021). This is normal, even if the percentage of chemical components detected in the same plant essential oil is different (Saroukolai et al. 2014). The growing environment of the plants and harvest season might be the reasons that resulted in the chemical differences of the species in the same genus. As reported (Faloum 2007; Gašić and Lukić, 1990), the contents of chamomile essential oil components were influenced by light, temperature, nutrient supply, sowing and harvesting time, etc. Firstly, light affects photosynthesis, and when irradiance, a control factor in photosynthesis, was reduced, the chamazulene content in the chamomile EO decreased significantly. Secondly, the proportion of chamazulene in the EO was higher at a temperature of 15 °C than at other temperatures. The next, increased nitrogen and phosphorus supply resulted in lower chamazulene content, but higher nitrogen supply had a positive effect on the (−)-α-bisabolol content in the oil. Last, different sowing seasons and harvest dates led to great fluctuations in the chamazulene and (−)-α-bisabolol content of many chamomile varieties.

According to Lu et al. (2021) myristicin (75%) as the main component of Clerodendrum bungei Steud. (C. bungei) EO had significant repellent activity against T. castaneum. At the concentrations of 78.63, 15.73 and 3.15 mL/cm², the PR values of myristicin were more than 80% after 2 h and 4 h exposure, which was basically identical with our finding. Compared with myristicin, the EO in R. Rufum possessed a stronger repellent active against S. oryzae after 2 h and 4 h exposure, which might be related to other chemical compounds in EO. According to previous research in the literature (Guo et al. 2016; Guerreiro et al. 2018), some terpenoids from natural plants played an important role in controlling stored-product pests. Therefore, the terpenoids in EO of R. Rufum including manoloyl oxide (6.72%), kaur-16-ene (2.21%), tricyclo[5.2.2.0(1,6)]undecan-3-ol,2-methylene-6,8,8-trimethyl (0.58%) and 2,2,7,7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one (1.66%) might exert synergism effect with myristicin (79.72%) against S. oryzae. On the contrary, the myristicin in EO exhibited higher repellent effect than EO against T. castaneum at 2 h and 4 h exposure, which showed the other chemicals in EO might exert antagonism effect with myristicin, or the two above insects maybe had different sensitivity to various repellent active ingredients.

The species of R. Rufum and R. przewalskii have been confirmed insecticidal activity against S. oryzae and T. castaneum, and myristicin showed obvious contact toxicity against S. oryzae in the study. Meanwhile, there were also slight differences in antifeedant activities of four compounds isolated from R. przewalskii. These results might be due to differences in anti-insect mechanisms between different compounds and insects, however, the specific anti-insect mechanisms of EOs and compounds from the Rhododendron plants were still not clear. The relevant literature indicated that the mode of action of repellency, contact toxicity and antifeedant activity might be related to three enzymes including glutathione s-transferase (GST), carboxylesterase (CarE), acetyl cholinesterase (AChE) and mixed-functional oxidase (MFO) in vivo of insects (Pan et al. 2016; Gao et al. 2019). The GST, CarE and MFO are important detoxification enzyme, and AChE is an important hydrolase in the insect nervous system. Therefore, the death of insects might be caused by the inhibition of detoxification activities mediated by detoxification enzyme. On the other hand, AChE can inhibit nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. For future research, the mechanism of insect resistance can be further revealed by detecting the contents variation of these four enzymes in vivo of insects.
Conclusions

In this study, insecticidal activity of *R. Rufum* and *R. przewalskii* against two stored-product insects were evaluated. All results clearly indicated that the EO of *R. Rufum* showed the contact toxicity and repellent activities against *S. oryzae* and *T. castaneum*, and the existence of high content myristicin (79.72%) might be the most key element which affected the contact toxicity and repellent effects of *R. Rufum* EO against two stored insects. Four compounds with significant antifeedant effect were isolated by bioassay-guided fractionation from the ethanol extract of *R. przewalskii* and their antifeedant activity were tested against *T. castaneum* for the first time.

Collectively, we provided scientific data for the application of the species of *R. rufum* and *R. przewalskii* in controlling storage insects and the extracts of *R. rufum* and *R. przewalskii* might be introduced as effective alternatives of synthetic insecticides against two stored insects.

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Data availability All data generated or analyzed during this study are included in this published article and also available from the corresponding author.

Conflict of interests The authors declare that they have no competing interests.

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