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Bioactivity of *Pistacia atlantica* desf. Subsp. *Kurdica* (Zohary) Rech. F. and *Pistacia khinjuk* stocks essential oils against *Callosobruchus maculatus* (F, 1775) (Coloeptera: Bruchidae) under laboratory conditions

Maryam Pourya [a], Amin Sadeghi [a, *], Hamed Ghobari [a], Clauvis Nji Tizi Taning [b, **], Guy Smagghe [b, ***]

[a] Department of Crop Protection, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran
[b] Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Gent, Belgium

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ABSTRACT

One of the major storage insect pests of cowpea and many other grains is *Callosobruchus maculatus* (F.) (Col.: Bruchidae). The use of plant materials rather than conventional pesticides to control these pests is a promising alternative to neurotoxic insecticides. In this study, for the first time, the fumigant, contact toxicity and repellent activity of *Pistacia atlantica* Desf. Subsp. *kurdica* (Zohary) Rech. f. (gum, fruit and leaves) and *Pistacia khinjuk* Stocks (fruit and leaf) essential oils were tested against adults of *C. maculatus*. The essential oil from *P. khinjuk* fruit showed a stronger fumigant activity against *C. maculatus* after 24 h exposure ($LC_{50} = 22$ μl/l air) compared to the leaves ($LC_{50} = 29$ μl/l air). As for *P. atlantica* subsp. *kurdica*, the essential oil from the gum showed the highest activity at 24 h ($LC_{50} = 7.0$ μl/l air) compared to the fruit ($LC_{50} = 18$ μl/l air) and leaves ($LC_{50} = 24$ μl/l air). In contact toxicity bioassays, the *P. khinjuk* fruit and leaf oils caused significant mortality to *C. maculatus* adults upon contact for 24 h ($LC_{50} = 0.12$ μl/cm^2 and $LC_{50} = 0.14$ μl/cm^2, respectively), while the *P. atlantica* subsp. *kurdica* gum was the most toxic at 24 h ($LC_{50} = 0.07$ μl/cm^2) compared to the fruit oil ($LC_{50} = 0.11$ μl/cm^2) and leaf oil ($LC_{50} = 0.08$ μl/cm^2). At the highest concentration of 0.0234 μl/cm^3, the respective repellency percentages for *P. atlantica* subsp. *kurdica* gum, leaves and fruit oils at 24 h were 82%, 77% and 70%. For *P. khinjuk*, the respective repellency percentages for the leaf and fruit oils at 24 h were 76% and 67% at the highest concentration (0.0234 μl/cm^3). These results indicate strong fumigant and contact toxicity, and repellent activity for all the tested essential oils against *C. maculatus*. Hence, the data can be exploited further to develop a management strategy against *C. maculatus*.

1. Introduction

Stored-product insects can cause a significant reduction in weight, quality, commercial value and seed viability. More than 600 species of beetle pests and 70 species of moths are responsible for approximately 10–25% annual quantitative and qualitative losses of stored grains worldwide (Pimentel, 1991; Matthews, 1993; White, 1995; Rajendran, 2002). Peas, including cowpea, *Vigna unguiculata* (L.) Walp. (Leguminosae), are an important staple and main source of protein and income for people in developing countries (Rajapakse and van Emden, 1997; Deshpande et al., 2011). However, during the storage period, cowpeas often suffer heavy quantitative and qualitative losses from the attack of *Callosobruchus* species. One of the major storage pests of cowpea is the bean weevil, *Callosobruchus maculatus* (F.) (Col.: Bruchidae) (Epidi et al., 2008), which is also an important insect pest of many other grains, such as chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.) and soybean (*Glycine* max L.) (Mahfuz and Khalequzzaman, 2007). Direct damage is caused when the neonate larvae penetrate the grain leading to grain weight loss and reductions in germination and nutritional

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: a.sadeghi@uok.ac.ir (A. Sadeghi), tiziclauvis.taningnji@ugent.be (C.N.T. Taning), guy.smagghe@ugent.be (G. Smagghe).

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value (Melo et al., 2010; Oke and Akintunde, 2013).

The control of various arthropod pests on stored products has so far relied primarily on the use of synthetic insecticides as fumigants (Damcevski et al., 2003; Ryan et al., 2006). However, these conventional chemical insecticides are expensive for subsistence farmers and may pose potential risks owing to the lack of adequate technical knowledge related to their safe use. Furthermore, insecticides acting as fumigants might be toxic to their users if not carefully handled (Tovignan et al., 2001). They could also have an adverse impact on arthropod predators or parasitoids which attack the pests (Waage, 1989; Van Huis, 1991), and it is well documented that insecticide resistance may rapidly develop following excessive use (Dales, 1996; Sousa et al., 2009). These concerns over the use of synthetic pesticides have recently motivated the need to develop cheaper and safe alternatives for insect pest control, including plant-based products (Papadopoulou-Mourkidou and Tomazou, 1991; Hamacher et al., 2002; Benhalima et al., 2004; Kemabonta and Odebiyi, 2005; Koul et al., 2008; Corrêa and Salgado, 2011; Kim and Lee, 2014).

Farmers in developing countries have for a long time introduced aromatic plants among stored seeds or pods to protect their harvest from insects. These plants release volatile compounds that are believed to have insecticidal properties Golob and Webley, 1980; Don-Pedro, 1996a,b; Keita et al., 2000, 2001; Ketoh et al., 2005; Sanon et al., 2006b; Ngamo et al., 2007). The use of essential oil extracted from these usually native aromatic plants is now perceived as a promising alternative to protect crops in traditional storage systems (Isman, 1999; Regnault-Roger et al., 2002; Ketoh et al., 2005; Sanon et al., 2006a; b; Ngamo et al., 2007). During a preliminary screening program from Iranian medicinal herbs for new agrochemicals against *C. maculatus*, *Pistacia atlantica* subsp. *kurdica* and *Pistacia khinjuk*, were found to possess strong insecticidal and repellent activities against *C. maculatus*.

Pistacia spp. belong to the family Anacardiaceae, order Sapindales. Among the fourteen known species of pistachios, only three species, *P. vera*, *P. khinjuk* and *P. atlantica*, grow in Iran (Khatamsaz, 1989; Karimi et al., 2009). In Iran, *P. vera* mainly grows in the Sarakhs region covering roughly 17,500 ha (Behboodi, 2003). *P. atlantica* subsp. *kurdica* (Zohary) Rech. f. is mainly centered in Iran and Afghanistan, overlapping with *P. vera* in some areas. *P. khinjuk* trees are widely distributed at elevations ranging from 700 to 2000 m on hills and mid-height mountains in Iran (Behboodi, 2003). *Pistacia* spp. essential oils have been reported to possess several beneficial properties ranging from antibacterial, antioxidant and antifungal to acaricide potentials (Benhammou et al., 2008; Kordali et al., 2011; Hesami et al., 2013).

In this study, we aimed to evaluate the insecticidal properties of essential oils from two *Pistacia* spp. (*P. atlantica* and *P. khinjuk*) for the management of *C. maculatus* in stored cowpeas. For the first time, the fumigant and contact toxicity, and repellent activity of *P. atlantica* Desf. subsp. *kurdica* (Zohary) Rech. f. (gum, fruit and leaves) and *P. khinjuk* Stocks (fruit and leaf) essential oils were tested against *C. maculatus* adults. The findings in this study could be exploited in the development of an integrated pest management (IPM) strategy for *C. maculatus* in stored cowpeas.

2. Materials and methods

2.1. Insect culture

C. maculatus were reared on cowpea grains in the laboratory at 25 ± 1 °C and 65 ± 5% relative humidity (r.h.), and a 10:14 h light:-dark lighting regime. All experiments were carried out under the same conditions (Sadeghi et al., 2006).

2.2. Plant materials

Aerial parts of *P. khinjuk* (fruits and leaves) and *P. atlantica* subsp. *kurdica* (fruits, leaves and gum) were collected in July 2014 from the Kurdistan province in Iran (Latitude: 46°, 16′, Longitude: 35°, 22′ and Elevation above sea level: 1264 m). A botany expert (Hosein Maroofi) at the Faculty of Resources Research Center of Sanandaj (Kurdistan, Iran) confirmed the identity of the plant. The plant samples were dried naturally on benches in the laboratory at room temperature (23-24 °C) for 4 days until they became crispy. The dried materials were hydrodistilled to extract the essential oils.

2.3. Extraction and analysis of essential oils

The essential oils were extracted from the plant samples using a Clevenger-type apparatus, wherein the plant material was subjected to hydrodistillation. Conditions of extraction were: 70 g of air-dried sample, 650 ml of water, 3 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. The extracted oil was stored in a refrigerator at 4 °C.

Gas Chromatographic-Mass Spectrometry (GC-MS) analysis was performed with Agilen GC-7890A and helium as a carrier gas. The oven was programmed to rise to 50 °C and then to 290 °C at a rate of 10 °C/min. The ionization energy was 70 eV with a scan time of 1 s. Unknown essential oil components were identified by comparing their GC retention times to those of known compounds and also by comparing their mass spectra to that of either known compounds or published spectra. Essential oil compounds were identified by calculating their retention index (RI) data and also by matching their mass spectrum fragmentation patterns with corresponding data stored in the mass spectra library of the GC-MS data system (Negahban et al., 2007).

2.4. Fumigant toxicity

The experiments were arranged in a completely randomized design. Twenty adults were put into 140 mL-glass bottles with screw lids. Different essential oil concentrations were applied to filter paper pieces (2 cm in diameter). The impregnated filter papers were attached to the screw caps of the glass bottles. Caps were screwed tightly on the bottles, each of which contained 20 adults (1-7 days old) of *C. maculatus*. Based on preliminary tests, essential oils at the following concentrations were used: *P. atlantica* subsp. *kurdica* gum oil (3.55–10.65 μl/l air for 24 h and 1.77–6.95 μl/l air for 48 h), fruit oil (14.2–23 μl/l air for 24 h and 5.32–15.26 μl/l air for 48 h), leaf oil (17.75–30.17 μl/l air for 24 h and 14.2–22.72 μl/l for 48 h) and *P. khinjuk* fruit oil (15.97–24.85 μl/l air for 24 h and 10.65–25.2 μl/l air for 48 h) and leaf oil (23–33.72 μl/l air for 24 h and 14.2–22.74 μl/l air for 48 h). The use of different concentrations at 24 h and 48 h was simply because we could not open the same bottle at 24 h and 48 h without affecting the results of the assay through the escape of some fumes. Hence, we prepared separate concentrations (24 h and 48 h) for the fumigant toxicity tests, which resulted in the observed differences in concentrations. Each treatment was replicated 5 times. The control groups consisted of a similar setup without essential oils. Mortality was evaluated after 24 h and 48 h of exposure at different concentrations. Insects were considered dead when no leg or antenna movements were observed (Robertson et al., 2007). Percentage insect mortality was calculated using the Abbott correction formula for natural mortality (Abbott, 1925).

2.5. Contact toxicity

Filter papers were placed in glass petri dishes (9 cm