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Pistacia lentiscus essential oil has repellent effect against three major insect pests of pasta

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ABSTRACT

Rhyzopertha dominica, *Sitophilus zeamais*, and *Tribolium confusum* are three of the major food-stuff pests who cause important economic losses of shelved products with special reference to pasta. Due to its long shelf life, pasta is highly exposed to insects that can penetrate into the packaging with consequences economically severe. Eco-friendly strategies to prevent such insect attacks to the final packaged product are therefore highly foreseen by pasta companies. Due to their repellent properties, essential oils, extracted from aromatic plants, could represent a valid, eco-friendly alternative to chemical repellents. In this study, we evaluated the repellent activity of *Pistacia lentiscus* essential oil (PEO) and its main chemical components by two different bioassay with and without the presence of pasta. Results showed that the whole PEO exerts a broad-range aspecific repellency among the target pests with RD_{50} values ranging from 0.010 to 0.037 $\mu L\,cm^{-2}$. On the contrary, the repellence of PEO components resulted to vary depending on the compound and on the pest species. Among the PEO chemical components, relative median potency analyses indicated that β-caryophyllene was able to exert the highest repellency rates against *S. zeamais* (RD_{50} 0.046 $\mu M\,cm^{-2}$). The comparison between the two bioassays, with and without pasta, indicated that the two methodologies gave consistent results. Overall, our research firstly showed that, because of their effectiveness as repellents, PEO and its major constituents could represent valid and safe tools against pasta pests.

1. Introduction

About 13.6 million tons of pasta are produced worldwide (International Pasta Organization, 2012) with a market value that, for the only Italy, the main pasta producer, has been estimated as about 4.6 million Euro (UNIPI, 2012). Due to such a large production, and the particularly long shelf life (about 2–3 years), the control of pests is an important aspect of pasta post-production management. Stored-food insect pests cause severe quantitative and qualitative losses in stored raw materials, such as cereals or stored grains as well as in semi-processed and in final food products, such as pasta (Hou et al., 2004; Germinara et al., 2010; Bachrouch et al., 2010; Trematerra and Süss, 2006).

The Coleoptera *Rhyzopertha dominica* (F.) (Bostrychidae), *Sitophilus zeamais* Motsch. (Dryophthoridae), and *Tribolium confusum* Du Val (Tenebrionidae) are three of the major pasta pests who cause important economic losses of shelved products (Trematerra and Süss, 2006). Actually, due to the long pasta shelf life, insects can easily penetrate into the packaging and reproduce many generations (Locatelli and Süss, 2002). Unfortunately, a single insect occurring in a pasta package, although non compromising the quality of the product, is enough to affect seriously the image of the company manufacturing or distributing the goods (Kim et al., 2010) with consequences that can be economically severe (Hou et al., 2004; Licciardello et al., 2013). For these reasons, strategies to prevent insect attacks to the final packaged product such as the development of new repellent packaging methods are highly foreseen by pasta companies (Cagri et al., 2004; Hou et al., 2004; Germinara et al., 2010). However, because the concerns about their toxicity, chemical insecticides and repellents are not well accepted by costumers. Therefore, alternatives approaches to chemical fumigants that play a major role in insect pest control in stored food are needed.

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In recent years, essential oils (EOs) of aromatic plants, characterized by low toxicity to mammalians and already extensively used in the food industries as supplements, and flavoring compounds, received a great attention as pest control agents due to their insecticidal, repellent, and/or antifeedant properties (Isman, 2006; Nerio et al., 2010; Conti et al., 2010, 2011; Benelli et al., 2012). As a consequence, aromatic plants are studied as potential sources of repellents and insecticides (Nerio et al., 2010; Shaaya and Kostyukovysky, 2006; Conti et al., 2010; Caballero-Gallardo et al., 2012; Olivero-Verbel et al., 2013).

Pistacia lentiscus L. is an aromatic evergreen shrub belonging to the Anacardiaceae family, largely distributed in the Mediterranean basin (Abdelwahed et al., 2007). Although *P. lentiscus* essential oil (PEO) has been showed to have good insecticidal activity (Lamiri et al., 2001; Traboulsi et al., 2002), to the best of our knowledge, no information are available about its properties as insect repellent.

In this research the EO extracted from Algerian *P. lentiscus* plants was analyzed by gas chromatography (GC) and by gas chromatography/electron impact mass spectroscopy (GC-EIMS) and then its repellent activity against adults of *R. dominica, S. zeamais, T. confusum* was evaluated. Since EOs bioactivity is due to the combined action of their chemical compounds (Hummelbrunner and Isman, 2001), in this research we also tested the specific activity of α-terpineol, α-pinene, and β-caryophyllene, three main terpene constituents of PEO. In order to evaluate the repellency also in the presence of pasta, we compared the area preference method (Tapondjou et al., 2005), a commonly used method to assess the repellence with a two choice pitfall method (Germinara et al., 2007), in which the repellent activity of the PEO main constituents was counterbalanced by the attractiveness of the pasta.

2. Materials and methods

2.1. Plant material

P. lentiscus L. leaves were collected from wild plants, at flowering stage, in the locality of Fenaïa, Béjaia (211 km East of Algiers, 481 m above sea level: 36°40′28.08″ N; 4°49′53.97″ E) in June 2012.

2.2. Essential oil extraction and GC–MS analyses

The harvested leaves were dried in the shade, at room temperature (20–25 °C) until constant weight, and then coarsely ground and hydro-distilled in a Clevenger-type apparatus for 4 h. The resulting essential oil was dried over anhydrous sodium sulphate and stored in a glass vial at 4 °C until use.

Gas chromatography (GC) analyses were carried out with an HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60 °C for 10 min, ramp of 3 °C min^{-1} up to 220 °C; injector and detector temperatures 250 °C; carrier gas helium (2 ml min^{-1}); detector dual FID; split ratio 1:30; injection of 0.5 μl (10% hexane solution). Components identification was carried out, for both columns, by comparing their retention times with those of pure authentic samples and by means of their linear retention index (LRI), relative to the series of *n*-hydrocarbons. Gas chromatography-electron impact mass spectroscopy (GC-EIMS) analyses were performed with a Varian CP-3800 gas chromatograph, equipped with a HP-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector with the following analytical conditions: injector and transfer line temperatures 220 °C and 240 °C respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C min^{-1}; carrier gas helium at 1 ml min^{-1}; injection of 0.2 μl (10% hexane solution); split ratio 1:30. Constituents identification was based on the comparison of retention times with those of authentic samples, comparing their LRIs with the series of *n*-hydrocarbons and using computer matching against commercial (Adams, 1995) and home-made library mass spectra (built up from pure substances and components of known oils and MS literature data (Davies, 1990; Adams, 1995). Moreover, molecular weights of all identified substances were confirmed by gas chromatography-chemical ionization mass spectrometry (GC-CIMS), using methanol as the chemical ionizing gas.

2.3. Insect cultures and rearing conditions

Strains of *R. dominica, S. zeamais*, and *T. confusum* were reared at the Department of Agriculture, Food and Environment of the University of Pisa, since 2000. Insects were reared at room temperature, 65% R.H., natural photoperiod, in (20 × 25 × 15 cm) plastic boxes containing grains of maize and wheat and covered by a nylon net allowing air exchange. Since the adults remain until three days into the grain, homogeneous adults (0–3 days old) were obtained by removing adults from the box and the daily newly emerged insects were used for the bioassays.

2.4. Area preference bioassay

The bioassays were conducted following the method described by Tapondjou et al. (2005). Half filter paper disks (8 cm Ø) were treated with 500 μL of PEO as ethanolic solution at doses corresponding from 0.01 to 5 μL cm^2. Single PEO constituents (purchased from Sigma-Aldrich®) were tested as ethanolic solutions at doses ranging from 0.02 to 4 μM cm^{-2}. The treated filter paper disks were dried under a fan. Each Petri dish's bottom (8 cm Ø) was half-covered with half filter paper treated with the PEO or chemical solutions, while the other half, was covered with a half filter paper disk treated with 500 μl of ethanol (control). Twenty unsexed adults were introduced in each Petri dish, and the lid was sealed with Parafilm®. The Petri dishes were maintained at 25 ± 1 °C, 65% R.H., in the dark. Five replicates were performed for each assay, and insects were used only once. The number of insects on the two half of the Petri dish was recorded after 1, 3, and 24 h from the beginning of the test. The percent repellence (PR) of PEO and of each volatile compound was calculated by the formula: PR (%) = [(Nc − Nt)/(Nc + Nt)] × 100 where Nc is the number of insects present in the control half paper and Nt the number of insects present in the treated one.

2.5. Two-choice pitfall bioassay

The repellent activity of the volatile compounds was evaluated against *R. dominica, S. zeamais*, and *T. confusum* adults, using the bioassay described by Germinara et al. (2007). The bioassay was conducted in a steel arena (32 cm Ø × 12 cm high) with, in the bottom, two diametrically opposed holes (3 cm Ø) located 3 cm from the sidewall. 10 μl of chemicals or ethanol (control) were adsorbed onto a filter paper disk (1 cm Ø) suspended at the center of each hole by a cotton thread taped to the lower surface of the arena. Glass flasks (500 ml) filled with 100 g of pasta (spaghetti n. 5, ©Barilla G. e R. Fratelli S.p.A.) were positioned under each hole, and the inside surface of their necks were coated with paraffin oil to prevent insects, that have previously chosen, from returning to the arena. Preliminary trials allowed us to exclude any repellent or attractant effect of paraffin oil. The floor of the arena was covered with filter paper to provide an uniform surface and to facilitate insect movements. Sixty insects, deprived of food for at least 4 hours, were placed under an inverted Petri dish (3 cm Ø × 1.3 cm high) at the center of the arena and allowed to acclimate for 30 min. The arenas were covered with steel lids and sealed with Parafilm® to prevent