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 μL 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 μM 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.

Keywords: *Rhyzopertha dominica, Sitophilus zeamais, Tribolium confusum,* area preference bioassay, pitfall bioassay, repellence

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.

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 flavouring 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 (Tapondjiou 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

Pistacia 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 Tapondjiou 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 gr 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 insects from escaping and were left for 24 h in the dark at 25 ± 1°C and 65% R. H. Five replicates were performed for each assay, and insects were used only once. The number of insects in the flasks was recorded 24 h from the beginning of the test. The percent repellence (PR) of each volatile was then calculated after 24 h using the formula: PR (%) =[(Nc-Nt)/(Nc+Nt)] × 100 where Nc was the number of insects present in the control flask and Nt the number of insects present in the treated flask. The number of non-choosing insects (Nn), that remained in the arena without choosing any of the two chambers with the food, were recorded.

2.6. Statistics and data analyses

Data were analyzed by one-way ANOVA (insect species or essential oil component as factor) or two-way ANOVA (insect species and essential oil component as fixed factors, essential oil concentration as covariate). Data were arcsine-transformed when needed to fulfill the assumptions of the ANOVA. Means and standard errors (S.E.) given in tables and figures are for untransformed data. In Petri

repellence tests on filter paper the median repellent dose (RD_{50}) was calculated by Log-probit regressions (Finney, 1971). Significant differences between RD_{50} values were determined by estimation of confidence intervals of the relative median potency. Differences among RD_{50} were judged to be statistically significant when values of the 95% confidence interval of relative median potency were $\neq 1.0$.
All the analyses were performed by the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Essential oil extraction and GC-MS analysis

In the essential oil obtained from the flowering aerial parts 52 constituents were identified, accounting for 97.8% of the whole oil. The main components were α-pinene (15.9%), β-caryophyllene (7.0%), α-terpineol (6.9%), and myrcene (6.4%). Other important volatiles were 4-terpineol, β-pinene, and germacrene D (5.4, 5.1 and 5.1%, respectively) (Table 1).
The main represented chemical classes were monoterpene and sesquiterpene hydrocarbons (45.9 and 22.8%, respectively). The corresponding oxygenated derivatives were less represented in this oil (14.9 and 13.3%, respectively) (Table 2).

3.2. Area preference bioassay

The area preference method showed that *P. lentiscus* essential oil (PEO), exerted a clear repellent activity against *R. dominica*, *S. zeamais*, and *T. confusum*

(Table 3). Probit regressions of repellence values showed that *S. zeamais* was the most susceptible species to PEO (RD_{50} = 0.010 µL cm^{-2}) while, the less sensitive species was *R. dominica* (RD_{50} = 0.037 µL cm^{-2}) (Table 3). However, the relative median potency analyses of probits do not detected significant differences among species (Table 4). Such a broad-range activity of PEO was confirmed also by ANOVA that showed no significant differences among species at the end of the bioassays (24 h) ($F_{2, 15}$ = 1.686, P = 0.230). On the contrary, significant differences were detected among species when single compounds were utilized as repellents (Table 5). Relative median potency analyses indicated that β-caryophyllene was the most effective compound against *S. zeamais* and also significantly more effective than α-terpineol against *R. dominica*, while, α-terpineol resulted more effective than α-pinene against *T. confusum* (Table 6). The relative median potency analysis showed also a significant different responsiveness of the three pest species to β-caryophyllene. Such responsiveness was: *S. zeamais* > *T. confusum* > *R. dominica*. *Rhyzophertha dominica* was also the less responsive species to α-terpineol and α-pinene (Table 4). Consistently, two ways ANOVA showed that the repellence after 24 h of PEO main components was significantly different as a function of the species ($F_{2, 122}$ = 9.718, P < 0.001), the compound ($F_{2, 122}$ = 5.928, P = 0.004) and that there was an interaction between the species and the repellent compound ($F_{4, 122}$ = 2.632, P = 0.038).

3.3. Two-choice pitfall bioassay

The effect of the PEO main components in the presence of pasta, was tested by the two-choice pitfall bioassay. The repellency of the PEO main components against *R. dominica* ranged from 4 to 37.4% for α-pinene and β-caryophyllene, respectively, while the activity of the PEO main components against *T. confusum* ranged from 22.2 and 36.0% for α-pinene and α-terpineol, respectively (Fig. 1A). Consistently with the area preference bioassay, the most overall responsive species resulted to be *S. zeamais* with PR values from 23.6 for α-pinene and 58.2% for α-terpineol (Fig. 1A), while the less responsive was *R. dominica* with PR values from 4 for α-pinene and 37.4% for α-terpineol (Fig. 1A). However, no statistically significant differences in repellence rates were found, as a function of species ($F_{2, 45}$ = 2.298, P = 0.115), compound ($F_{2, 45}$ = 2.562, P = 0.091) and no interaction ($F_{4, 45}$ = 1.609, P = 0.193). In the two-choice pitfall we also observed a different percentage of individuals that at the end of the test did no choice and remained in the arena (non-choosing individuals). Such non-choosing individuals ranged in average from 77.9 ± 3.11 to 11.4 ± 3.39% for *R. dominica* and *S. zeamais*, respectively (Fig. 1B). Two-way ANOVA indicates that the differences among the percentages of non-choosing individuals of the three species were statistically significant ($F_{2, 45}$ = 127.856; P < 0.001), while there was no significant effect of the PEO main components ($F_{2, 45}$ = 1.703; P = 0.196) and no interaction ($F_{4, 45}$ = 2.246; P = 0.083). A statistically significant moderate negative relationship between the percentage of non-choosing individuals and the PR (r = 0.334; $F_{1, 45}$ = 5.391; P = 0.025) was also found by regression analysis.

4. Discussion

Results highlighted that PEO and its main chemical components have a clear repellent activity against *R. dominica*, *S. zeamais*, and *T. confusum*, the three pasta pest species tested.

To our knowledge this is the first assessment of *P. lentiscus* essential oil repellency against insects. Overall, our data on the PEO are in accordance with previous studies showing the repellent effect of several plant essential oils on *R. dominica* (Jilani and Malik, 1973), *T. confusum* (Akou-Edi, 1983), and *S. zeamais* (Akou-Edi, 1983; Nerio et al., 2009; Conti et al., 2010). On the base of our data, PEO resulted able to exert an aspecific broad-range repellent activity with no statistically significant differences, in term of effectiveness, among the three insect species. In fact, PEO resulted about 3 to 27 fold more effective against *S. zeamais* than the essential oils extracted from several Colombian aromatic plants and about 30 fold the commercial insect repellent IR3535 (Nerio et al., 2009).

The chemical analysis of the essential oil extracted from Algerian *P. lentiscus* plants indicated that its composition, although consistent, presents some differences, with the composition of other essential oils extracted from *P. lentiscus* plants. Actually, in a previous study about Algerian *P. lentiscus*, Benyoussef and coworkers (2005) found 4-terpineol, α-terpineol, and germacrene D among the principal chemicals. However, such a differences in the chemical composition of *P. lentiscus* essential oil may be due to the different geographical area of growth and period of harvesting of the plant material (Zrira et al., 2003; Aouinti et al., 2013).

Contrary to what observed for the whole PEO, α-terpineol, α-pinene, and β-caryophyllene, the main components of PEO, when tested as single compounds, showed different effectiveness against the three species. These results confirm that the repellent activity of EOs is due to the combined action of its chemical components (Hummelbrunner and Isman, 2001).

Among the tree species studied, *S. zeamais* resulted the overall most sensitive species, while *R. dominica* was the less affected one. Differences in the bioactivity of EOs compounds against stored-product pest species were also observed by Rozman et al. (2007) who found that 1,8-cineole and linalool were insecticidal to *R. dominica* but not effective against *T. castaneum*.

Accordingly to our data, the overall most active compound against the three pasta pests species, was β-caryophyllene. This result is consistent with previous observations by Chaubey (2012) who found that β-caryophyllene was more toxic and with higher anti-feeding activity than α-pinene against *T. castaneum* and *S. oryzae*. On the contrary, Zeng et al. (2010) reported no repellent activity of β-caryophyllene, against *R. dominica* and *S. oryzae*.

For what concern α-pinene and α-terpineol, our results are in agreement with previous reports indicating that α-pinene is effective against *T. confusum* (Ojimelukwe and Alder, 1999), *S. oryzae* (Lee et al., 2001), and *S. zeamais* (Karemu et al., 2013). Actually, in our experiment, the RD_{50} of α-pinene and α-terpineol resulted about five and two fold, respectively, the RD_{50} that can be deduced from the data by Ojimelukwe & Alder (1999) against *T. confusum* while, on the contrary, a much higher repellent activity of α-pinene against *T. confusum* was found by Kim et al. (2010).

In this experiment, to evaluate the bioactivity of the compounds we compared the commonly used area preference method with the pitfall bioassay that is, in theory, more close to a real situation because insects are never in direct contact with the tested compound and because the larger volume of the pitfall chamber allows a better gradient of concentration of the volatiles, whose repulsiveness is counterbalanced by the attractive presence of pasta.

The repellent activities of the PEO compounds assessed by the pitfall bioassay confirmed, overall, the results obtained by the area preference method. However, the pitfall bioassay allowed the detection of a part of the population remaining in the arena at the end of the experiment (non-choosing individuals). Interestingly, the amount of such non-choosing individuals was significantly different among the species and resulted to be negatively correlated to the repellent activity of the tested compounds. Therefore, the possibility that in the area preference method, different species behaviors may introduce undetected biases into the estimates of repellency can not to be excluded.

5. Conclusions

The results obtained in this experiment show that *P. lentiscus* essential oil, is rich in chemical components such as α-terpineol, α-pinene, and β-caryophyllene that resulted very effective as repellents against insect pasta pests. Since, aromatic plants essential oils present a very low toxicity to human beings and pose no significant environmental risk, *P. lentiscus* essential oil represent an appealing sustainable alternative to chemical insects repellents that could be usefully utilized in the reduction of losses caused to stored pasta and other food-stuff by insect pests.

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Figure legends

Fig. 1. Repellent activity of *Pistacia lentiscus* essential oil main components (α-pinene, β-caryophyllene, and α-terpineol) against *Rhyzopertha dominica, Sitophilus zeamais*, and *Tribolium confusum* assessed by the two-choice pitfall bioassay. A) Repellency rates. B) Percentages of individuals who remained in the arena (Non-choosing Individuals). Bars indicate standard error.

Table 1
Chemical composition of the essential oil from *Pistacia lentiscus* aerial parts used in the repellency assays

Constituents[a]	LRI	%
α-pinene	941	15.9
β-caryophyllene	1419	7
α-terpineol	1191	6.9
myrcene	993	6.4
4-terpineol	1179	5.4
β-pinene	982	5.1
germacrene D	1482	5.1
limonene	1032	4.1
α-cadinol	1654	4
δ-cadinene	1524	3.8
camphene	954	3.6
T-cadinol	1641	3.6
p-cymene	1028	2.8
γ-terpinene	1063	1.8
tricyclene	928	1.6
sabinene	978	1.6
bornyl acetate	1287	1.6
terpinolene	1090	1.5
1-*epi*-cubenol	1628	1.4
α-humulene	1455	1.3
γ-muurolene	1476	1.2
α-muurolol	1646	1.2
α-muurolene	1499	1.1
caryophyllene oxide	1582	1.1
α-terpinene	1020	1
α-bisabolol	1684	0.9
(E,E)-α-farnesene	1507	0.7
linalool	1101	0.6
α-copaene	1377	0.5
(Z)-3-hexenyl benzoate	1570	0.5
borneol	1168	0.4
2-undecanone	1293	0.4
β-elemene	1392	0.4
trans-γ-cadinene	1514	0.4
spathulenol	1577	0.4
guaiol	1597	0.4
(E)-β-ocimene	1052	0.3
alloaromadendrene	1461	0.3
epi-bicyclosesquiphellandrene	1476	0.3
valencene	1492	0.3

γ-eudesmol	1631	0.3
α-phellandrene	1006	0.2
δ-elemene	1340	0.2
germacrene B	1557	0.2

[a] Chemical constituents ≥ 0.1%

LRI, linear retention index on DB-5 column

Table 2
Principal chemical classes in the essential oil from *Pistacia lentiscus* aerial parts used in the repellency assays

Chemical classes	%
Monoterpene hydrocarbons	45.9
Oxygenated monoterpenes	14.9
Sesquiterpene hydrocarbons	22.2
Oxygenated sesquiterpene	13.3
Non-terpene derivatives	0.9
Total identified	97.8

Table 3

Repellency of the *Pistacia lentiscus* essential oil (EO) and of its main components (α-pinene, β-caryophyllene, and α-terpineol) against the pasta pests *Rhyzopertha dominica*, *Sitophilus zeamais*, and *Tribolium confusum*.

Repellent	Pest target	RD_{50}	95 % CI	Slope[a]	Intercept[a]	χ2 (df)
	S. zeamais	0.010	0.022-0.920	0.677 ± 0.151	0.882 ± 0.189	2.02* (2)
P. lentiscus EO	*R. dominica*	0.037	0.029-0.046	1.874 ± 0.204	2.677 ± 0.285	3.63* (4)
	T. confusum	0.025	0.291-1.049	1.887 ± 0.202	0.390 ± 0.099	5.49* (3)
	S. zeamais	0.262	0.216-0.324	2.168 ± 0.239	1.260 ± 0.181	1.06* (3)
α-pinene	*R. dominica*	0.706	0.546-0.934	1.375 ± 0.176	0.208 ± 0.087	5.65* (4)
	T. confusum	0.225	0.170-0.337	1.908 ± 0.361	1.236 ± 0.317	2.00* (3)
	S. zeamais	0.046	0.029-0.063	1.277 ± 0.199	1.714 ± 0.232	3.70* (3)
β-caryophyllene	*R. dominica*	0.390	0.274-0.612	1.193 ± 0.255	0.489 ± 0.160	1.81* (2)
	T. confusum	0.153	0.090-0.282	0.746 ± 0.142	0.608± 0.157	0.11* (3)
	S. zeamais	0.099	0.081-0.125	2.289 ± 0.313	2.297 ± 0.356	2.07* (2)
α-terpineol	*R. dominica*	0.949	0.446-4.945	0.945 ± 0.140	0.021 ± 0.103	7.31* (4)
	T. confusum	0.097	0.073-0.128	1.712 ± 0.210	1.739 ± 0.236	3.43* (4)

RD_{50}, dose of repellent that repel 50 % of the exposed insects. Data for *P. lentiscus* EO are expressed as µL cm^{-2}. Data for *P. lentiscus* EO components are expressed as µM cm^{-2};

CI, Confidence Intervall;

[a]Values ± standard error

χ2, chi-square; (df), degrees of freedom.

* indicate $P > 0.05$

Table 4
Comparison of *Rhyzopertha dominica*, *Sitophilus zeamais*, and *Tribolium confusum* susceptibilities to *Pistacia lentiscus* essential oil (PEO) and its main components (α-pinene, β-caryophyllene, and α-terpineol) assessed by the area preference bioassay.

EOc	Species	RMP	95% CI	
			from	to
PEO	*R. dominica vs S. zeamais*	2.668	0.963	12.346
	R. dominica vs T. confusum	1.649	0.639	4.982
	S. zeamais vs T. confusum	0.618	0.157	1704
α-pinene	*R. dominica vs S. zeamais*	2.507*	1.691	4.052
	R. dominica vs T. confusum	3.317*	2.002	6.218
	S. zeamais vs T. confusum	1.323	0.887	2.048
β-cariophyllene	*R. dominica vs S. zeamais*	10.870*	4.727	35.296
	R. dominica vs T. confusum	2.845*	1.486	6.571
	S. zeamais vs T. confusum	0.262*	0.122	0.480
α-terpineol	*R. dominica vs S. zeamais*	6.147*	1.972	43.387
	R. dominica vs T. confusum	7.354*	2.337	52.130
	S. zeamais vs T. confusum	1.196	0.455	3.129

CI, Confidence Intervall
* indicates values statistically significative

Table 5
Pistacia lentiscus essential oil main components repellent activity against *Rhyzopertha dominica*, *Sitophilus zeamais*, and *Tribolium confusum*. Adults exposed to different concentrations of α-pinene, β-cariophyllene, and α-terpineol for different exposure time (1, 3, and 24 h) in the area preference bioassay.

Species	μM cm^{-2}	Exposure (h)	% Repellency		
			α-pinene	β-caryophyllene	α-terpineol
R. dominica	0.2	1	30.00[a] ± 13.04	20.00 ± 13.04	6.00 ± 4.00
		3	32.00 ± 16.25	16.00 ± 13.64	12.00 ± 5.83
		24	18.00 ± 13.19	44.00 ± 13.27	28.00 ± 9.69
	0.4	1	48.00 ± 12.81	42.00 ± 14.28	44.00 ± 10.77
		3	46.00 ± 13.27	46.00 ± 9.27	36.00 ± 12.08
		24	44.00 ± 13.27	48.00 ± 14.97	40.00 ± 15.16
	1	1	28.00 ± 10.68	60.00 ± 13.78	28.00 ± 9.70
		3	36.00 ± 11.22 AB	64.00 ± 9.27 B	22.00 ± 8.00 A
		24	46.00 ± 12.49	68.00 ± 8.00	42.00 ± 15.62
S. zeamais	0.05	1	0.00 ± 0.00 A	56.00 ± 6.78 B	30.00 ± 11.40 B
		3	0.00 ± 0.00 A	60.00 ± 9.49 B	30.00 ± 17.61 AB
		24	8.00 ± 8.00 A	60.00 ± 5.48 B	18.00 ± 15.62 A
	0.1	1	32.00 ± 13.93	48.00 ± 8.00	56.00 ± 8.72
		3	48.00 ± 20.59	56.00 ± 14.35	64.00 ± 5.10
		24	18.00 ± 9.70 A	72.00 ± 6.63 B	54.00 ± 2.45 B
	0.2	1	28.00 ± 13.19	18.00 ± 8.00	47.00 ± 6.63
		3	46.00 ± 25.42	36.00 ± 15.68	56.00 ± 12.88
		24	36.00 ± 17.49	74.00 ± 10.30	76.00 ± 8.71
T. confusum	0.1	1	16.67 ± 12.02 AB	6.00 ± 6.00 A	46.67 ± 8.82 B
		3	26.67 ± 14.53 AB	10.00 ± 10.00 A	66.67 ± 13.33 B
		24	26.67 ± 13.33	44.00 ± 19.13	63.33 ± 8.82
	0.2	1	26.67 ± 21.86 A	4.00 ± 2.45 A	83.33 ± 8.82 B
		3	0.00 ± 0.00 A	0.00 ± 0.00 A	86.67 ± 8.82 B
		24	53.33 ± 13.33	40.00 ± 11.40	73.33 ± 8.82
	0.4	1	56.67 ± 18.56	63.33 ± 14.53	58.00 ± 8.00
		3	76.67 ± 23.33	63.33 ± 23.33	76.00 ± 9.27
		24	66.67 ± 3.33	60.00 ± 23.09	82.00 ± 9.70

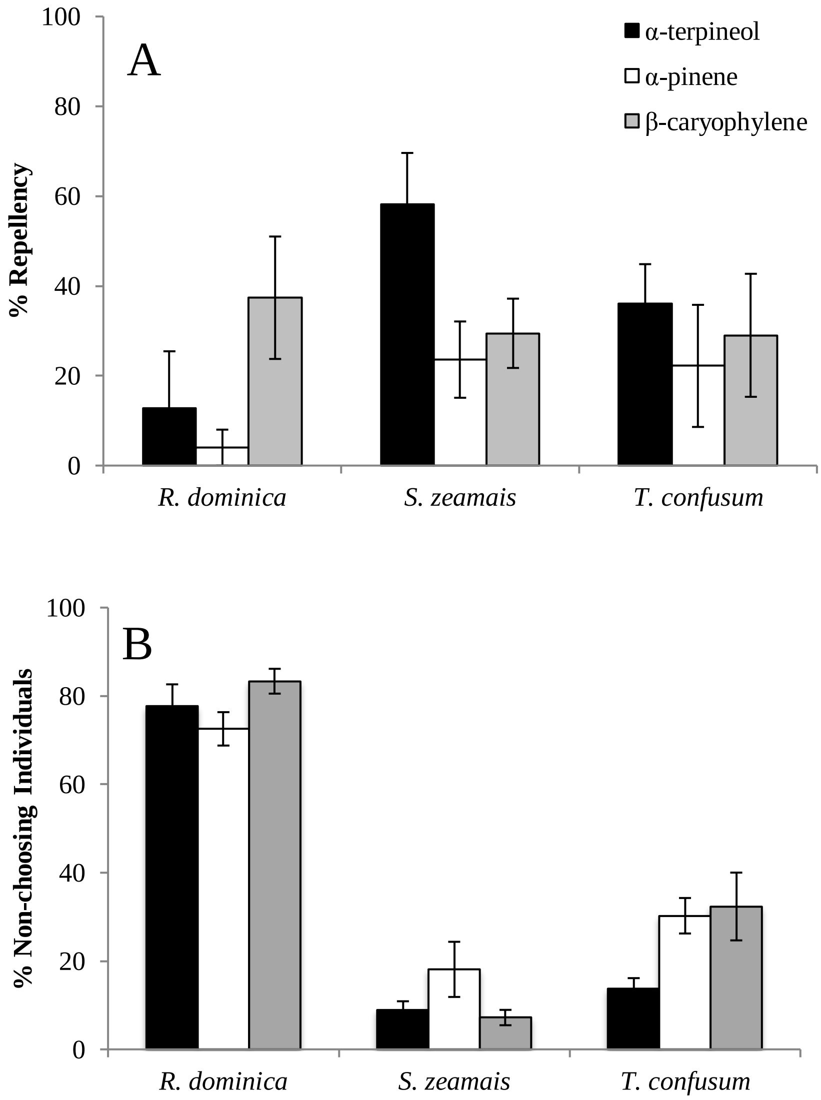
[a] Values are means ± standard error. Values within each species and exposure time followed by different letters are significantly different by Tukey HSD test ($P \leq 0.05$).

Table 6
Comparison of the repellency of *Pistacia lentiscus* essential oil main components (EOCs), α-pinene, β-caryophyllene, and α-terpineol, against the pasta pests *Rhyzopertha dominica*, *Sitophilus zeamais*, and *Tribolium confusum* assessed by the area preference bioassay.

Species	EOCs	RMP	95% CI	
			from	to
R. dominica	α-terpineol *vs* α-pinene	1.105	0.546	2.126
	α-terpineol *vs* β-caryophyllene	2.051*	1.005	4.764
	α-pinene *vs* β-caryophyllene	1.857	0.917	4.494
S. zeamais	α-terpineol *vs* α-pinene	0.389*	0.166	0.721
	α-terpineol *vs* β-caryophyllene	1.950*	1.153	3.552
	α-pinene *vs* β-caryophyllene	5.019*	2.544	13.417
T. confusum	α-terpineol *vs* α-pinene	0.333*	0.097	0.856
	α-terpineol *vs* β-caryophyllene	0.702	0.273	1.603
	α-pinene *vs* β-caryophyllene	2.109	0.865	6.106

CI, Confidence Intervall
* indicates values statistically significative

Fig. 1.


A
B
α-terpineol
α-pinene
β-caryophylene
% Repellency
% Non-choosing Individuals
0
20
40
60
80
100
R. dominica
S. zeamais
T. confusum