Not just for beer: evaluation of spent hops (*Humulus lupulus* L.) as a source of eco-friendly repellents for insect pests of stored foods

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ABSTRACT

Spent hops is a waste produced in large amount by the brewing industry. *Rhyzopertha dominica* and *Sitophilus granarius* are insects that cause important economic losses of stored foods. In this study, for the first time, spent hops has been evaluated as source of essential oil (EO) and chemicals with repellent activity against *R. dominica* and *S. granarius*. Spent hops EO yield was 0.11%. The terpenes myrcene, α-humulene, and β-caryophyllene were its main components (47%). Spent hops EO RD_{50} values were 0.01 and 0.19 µL cm^{-2} for *R. dominica* and *S. granarius*, respectively. Among the chemicals, myrcene was able to exert the highest repellency against *R. dominica* ($RD_{50} = 0.27$ µM cm^{-2}) while limonene was the most effective compound against *S. granarius* ($RD_{50} = 0.89$ µM cm^{-2}). These results indicate spent hops as an excellent source of EO and chemicals to be utilized as low-cost eco-friendly insect pests repellents in the protection of stored food.

Keywords: Spent hops · Essential oil · Terpenes · *Rhyzopertha dominica* · *Sitophilus granarius* · Repellence

Key Message

- No information is available about the bioactivity of extracts from hop or spent hops against stored food insect pests.
- Spent hops EO resulted rich in bio-active substances (myrcene, 24.2%)
- Spent hops EO was strongly repellent activity against *R. dominica* and *S. granarius* (RD_{50} = 0.008 and 0.191 µL cm^{-2}, respectively).
- Myrcene was the most effective compound against *R. dominica* and limonene against *S. granarius*.

- The findings indicate spent hops as a convenient source of eco-friendly chemicals alternative to synthetic repellents.

1. Introduction

Hop (*Humulus lupulus* L.) is a high-climbing, perennial vine, utilized in the brewing industry to add flavour and bitterness to beer (Chadwick et al. 2006) whose production has been estimated at over 100,000 tonnes, worldwide (FAOSTAT 2014). Since only about 15% of the hop constituents end up in the beer, a large amount of residual material, known as “spent hops”, generally considered of no further value, is produced by the brewing industry. Such waste material is usually disposed in agricultural fields or utilized in animal feeding (Davies and Sullivan 1927; Hardwick 1994) and alternative utilizations of spent hops in order to increase its added value are foreseen by industries (Oosterveld et al. 2002).

Insect pests are responsible for the loss of 20% of the world's annual crop production (Sallam 1999) and up to 40% of food grains loss in granaries and storehouses (Matthews 1993). The traditional control of such pests in stored food has relied primarily on synthetic insecticides like methyl bromide and phosphine (Shaaya et al. 1997). However, due to their persistency and neurotoxic, carcinogenic, teratogenic and mutagenic effects in non-target animals, and to the depleting effect on atmospheric ozone, the use of such chemicals is now under increasing restrictions for their environmental and human health hazards (Ayaz et al. 2010; Bakkali et al. 2008; Boyer et al. 2012; Ohr et al. 1996). Besides, several studies indicate an increase of the resistance of stored product insects to conventional synthetic pesticides (Bell and Wilson 1995; Pretheep-Kumar et al. 2010; Kumar et al. 2010; Shukla and Toke, 2013). For these problems the development of alternative strategies to synthetic chemicals is a priority in insect pest control of stored food (González et al. 2014; Saeidi and Moharramipour 2013).

In this view, increased attention had been given to essential oils of aromatic plants as source of natural pesticides (Bougherra et al. 2014; Isman 2006; Nenaah 2013; Zehnder et al. 2007). Essential oils of aromatic plants are among the most promising alternative to synthetic chemicals to be used as pest control agents with no or minimal side effects (Lima et al. 2014; Rajendran and Sriranjini 2008; Regnault- Roger et al. 2012).

Among aromatic plants, hop contains numerous bioactive substances, such as the flavonoid xanthohumol and the flavanone 8-prenylnaringenin, that have been shown to have anti-cancer (Colgate et al. 2007; Drenzek et al. 2011; Okano et al. 2011), antioxidant (Jacob et al. 2011), anti-HIV (Wang et al. 2004) and phyto-estrogen activity (Böttner 2008). Besides, hop also contains α- and *ß*-acids, and terpenes that have been found to be toxic, anti-feeding and repellent for insects (DeGrandi-Hoffman et al. 2012; Gökçe et al. 2009; Powell et al. 1997). Recent investigation performed by means of supercritical CO_2 extraction revealed that spent hops is still rich in bio-active compounds (Aniol et al. 2007). However, for the best of our knowledge no information is available about the bio-activity of extracts from hop or spent hops against stored product insect pests.

The aim of the present study was to evaluate the brewing by-product spent hops as a source of a terpenes-rich essential oil to be utilized as repellent against adults of the lesser grain borer *Rhyzopertha dominica* (F.) (Bostrichidae) and the granary weevil *Sitophilus granarius* (L.) (Curculionidae), two Coleoptera considered among the major stored food pests (Trematerra and Süss, 2006).

2. Materials and Methods

2.1. Plant material

Spent hops, was supplied by the brewery “Opificio Birraio” of Pisa, Italy after utilization of the hop cones (*Humulus lupulus* cv. Northern Brewery) in the brewing process. Spent hops was dried in the shade, at room temperature (20-25°C) until constant weight.

2.2. Essential oil extraction and GC-MS analyses

Dried spent hops was hydro-distilled in a Clevenger-type apparatus for 3 h and stored in a refrigerator 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. Chemicals

Myrcene, *α*-humulene, linalool and *β*-caryophyllene, were purchased from Sigma-Aldrich (Italy). In detail: myrcene with a purity ≥ 90% (prod. # W276200), *α*-humulene with a purity ≥ 96.0% (prod. # 53675), (±)-linalool, with a purity of 97% (prod. # L2602) and *β*-caryophyllene with a purity ≥ 98.5% (prod. # 22075). (+/-)-limonene (with a purity of 96%) was purchased from ChemPur GmbH (Germany).

2.4. Insect cultures and rearing conditions

Strains of *R. dominica* and *S. granarius* were reared at the Department of Agriculture, Food and Environment of the University of Pisa, since 2000. Insects were reared at room temperature (20-25 °C), 65% R.H., with natural photoperiod, in plastic boxes (20×25×15 cm), containing grains of 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.

2.5. Insect pests repellence bioassays

The repellence of the spent hops essential oil and of some of its chemical constituents was evaluated by two methods: the area preference and the two choice pitfall bioassays.

The area preference is, by far, the most common method utilized to assess insect repellency. However, it implies the direct contact of the insects on the filter paper treated with the chemicals and does not allows the presence of food. To test the repellence potential of the chemicals by an assay more close to a real situation we also evaluated the repellence by a two choice pitfall bioassay in which the repellent effect of the tested compound is evaluated in the presence of food. In addition, insects are never in direct contact with the compound.

2.5.1 Area preference bioassay

The bioassays were conducted following the method described by Tapondjiou et al. (2005). Preliminary tests were conducted to determine the appropriate ranges of concentration of spent hops essential oil (EO) and chemicals. For spent hops EO and chemicals the maximum concentration was chose in order to allow the survival of the whole insect population (0% of mortality) after 24h. As regards the spent hops EO, half filter paper disks (Whatman no. 1 filter paper, 8 cm Ø) were treated with 500 μL of spent hops EO as ethanolic solution at 5 doses ranging from 0.002 to 0.3 μL cm^{-2}. Chemicals were tested as ethanolic solutions at the doses of 0.125, 0.25, 0.5, 1, 2 and 3 μM cm^{-2}.

The treated filter paper disks were dried under a fan. In each polystyrene Petri dish (8 cm Ø) were placed two half filter paper disks, one treated with the EO or EO component solutions and the other treated with 500 µl of ethanol (control). Twenty unsexed adults were introduced in each Petri dish, and the lid was sealed with self-sealing film (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 halves of the Petri dish was recorded after 1, 3, and 24 h from the beginning of the test. The percent repellence (PR) of EO and of each volatile compound was calculated by the formula: $PR (\%) = [(Nc-Nt)/(Nc+Nt)] \times 100$ where Nc is the number of insects present in the control half paper and Nt the number of insects present in the treated one.

2.5.2 Two-choice pitfall bioassay

The repellent activity of the spent hops volatile compounds was evaluated against *R. dominica* and *S. granarius* 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 two diametrically opposed holes (3 cm Ø) in the bottom, located 3 cm from the sidewall. The floor of the arena was covered with filter paper to facilitate insect movements. 10 µl of ethanol (control) or chemicals solutions were adsorbed onto a filter paper disk (1 cm Ø). Preliminary tests were conducted to to determine the appropriate range of concentration of spent hops essential oil (EO) and chemicals . The concentrations of chemicals of the treated disks ranged from 0.03 to 0.125 µM cm^{-2}. The paper disks were suspended at the centre of each hole by a cotton thread taped to the outer surface of the arena. Glass flasks (500 ml) filled with 100 gr of pasta (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 from returning to the arena. Preliminary trials allowed us to exclude any repellent or attractant effect of paraffin oil. 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 arena was covered with a steel lid and sealed with Parafilm to prevent insects from escaping and was 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: $PR (\%) = [(Nc-Nt)/(Nc+Nt)] \times 100$ where Nc was the number of insects present in the control and Nt the number of insects present in the treated flask. The number of non-choosing insects (Nn) (individuals that remained in the arena without entering in any of the two chambers with the food) was recorded.

2.6. Statistics and data analyses

Differences among treatments and species were analyzed after data arcsine-transformation 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). Means and standard errors (S.E.) given in tables and figures are for untransformed data. Median repellent dose (RD_{50}) was calculated by Log-probit regressions. Significant differences between RD_{50} values were determined by estimation of confidence intervals of the relative median potency (RMP). Differences among RD_{50} values were judged as statistically significant when values in the 95% confidence interval of relative median potency analyses were ≠ 1.0. All the analyses and RD_{50} determination were performed by the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Essential oil extraction and GC-MS analysis

Essential oil yield from spent hops was 0.11% dry weight. In the spent hops essential oil, 31 constituents were identified, accounting for 94.3% of the whole oil (Table 1). All the components were mono- and sesquiterpenes, both hydrocarbons and oxygenated derivatives, together with some non-terpene compounds such as esters, aldehydes and methylketones. The principal constituents were myrcene (24.2%), *α*-humulene (16.2%), and *β*-caryophyllene (6.6%).

The main chemical class was represented by sesquiterpene hydrocarbons that reached 36.7% followed by monoterpene hydrocarbons (26.4%). Other important classes were non-terpene derivatives and oxygenated sesquiterpenes (Fig. 1).

3.2. Insect pests repellence bioassays

3.2.1 Area preference bioassay

The area preference bioassay showed a strong repellent activity against the two insect pests *R. dominica* and *S. granarius* by spent hops essential oil (SHEO) (Fig. 2). Interestingly, we observed a clear different susceptibility of the two species to SHEO

($F_{1, 15} = 37.563$, $P < 0.001$) (Fig. 2). Actually, according to probit analysis, *R. dominica* resulted about 24-fold more susceptible to SHEO than *S. granarius* (RD_{50} = 0.008 and 0.191 µL cm^{-2}, respectively) (Table 2, see also Table 4).

On the base of our data, the repellent activity of SHEO is consistent with the repellence of the single SHEO compounds (Tab. 3). Two ways ANOVA showed that the repellence after 24 h of SHEO main components was significantly different as a function of the species ($F_{1, 311} = 136.895$, $P < 0.001$), the compound ($F_{3, 311} = 57.517$, $P < 0.001$) and that there was a significant interaction between the species and the repellent compound ($F_{3, 311} = 12.247$, $P < 0.001$).

RMP analyses indicated that the most effective compound against *R. dominica* was myrcene, while limonene was the most effective compound against *S. granarius* (Tab. 5). As regards the activity of *β*-caryophyllene against *R. dominica*, we found that it was significantly higher than the ones of limonene and linalool and similar to the one of myrcene and *α*-humulene, while, the repellency of *β*-caryophyllene against *S. granarius*, was lower than the limonene one but higher than the repellency of linalool and similar to the activity of *β*-caryophyllene and *α*-humulene (Tab. 5). However, albeit limonene was the most repellent compound against *S. granarius*, since SHEO contains 20 fold more myrcene than limonene (Tab. 1), myrcene can be considered the overall most active compound of spent hops EO against the two insect pests species.

2.4.3 Two-choice pitfall bioassay

The repellent effect of the SHEO and of the SHEO main components in the presence of food was tested by the two-choice pitfall bioassay. The repellency of SHEO observed by the pitfall bioassay, varied, from 33.62 to 34.51% for *R. dominica* and *S. granarius*, respectively with no significant differences between the two species ($F_{1,4}$ = 0.009, $P > 0.05$). On the contrary, differences were found in the repellency of the singles SHEO chemical components. Statistically significant differences in repellence rates were found, as a function of species ($F_{1,20}$ = 13.737, P = 0.001), compound ($F_{4,20}$ = 8.433, $P < 0.001$), with significant interaction between species and compound ($F_{4,20}$ = 6.116, P = 0.002). Significant RD_{50} values, consistent with the Probit model, were obtained only for β-caryophyllene and limonene: β-caryophyllene RD_{50} values ranged from 0.074 (95% *CI* = 0.040-0.183; χ^2 = 0.13) to 0.128 (95% *CI* = 0.104-0.188; χ^2 = 1.02) µM cm^{-2}, while, RD_{50} values of limonene were 0.206 (95% *CI* = 0.124-1.452; χ^2 = 0.80) to 0.232 (95% *CI* = 0.168-0.521; χ^2 = 0.78) µM cm^{-2}. RMP analyses of the pitfall bioassay data showed that the most responsive species was *R. dominica*. RMP values (*R. dominica vs*. *S. granarius*) were 0.499 (95% *CI* = 0.259-0.751) and 0.522 (95% *CI* = 0.272-0.776) for β-caryophyllene and limonene, respectively. The two-choice pitfall bioassay also highlight the presence of individuals that did no make a choice remaining in the arena at the end of the experiment (Non-choosing Individuals). The number of such non-choosing individuals was different between the two insect pest species ($F_{1,20}$ = 240.985; $P <$ 0.001), ranging, in average, from 60.00 ± 8.22 to 0.00± 0.00% for *R. dominica* and *S. granarius*, respectively (data not shown). On the contrary, no significant effect of the SHEO compounds was found ($F_{4,20}$ = 1.824; P = 0.164) with an interaction between species and compound ($F_{4,20}$ = 3.380; P = 0.029).

4. Discussion

4.1. Essential oil extraction and GC-MS analysis

To our knowledge this is the first report on the extraction and characterization of essential oil obtained by hydrodistillation from spent hops. Hydrodistillation allowed the extraction of a noteworthy amount of essential from the spent hops. Even if, essential oil yield is quite higher in fresh hops (2.2%, for the Northern Brewer variety) (Davies and Menary 1982) this result showed that a consistent amount of essential oil is still extractable from the spent hops, after the brewing process, In fact, the percentage of essential oil recovered from spent hops is comparable or even higher than that obtained from numerous aromatic and/or officinal plants, i.e. *Salvia officinalis* L. (0.2-2.4%) (Raal et al. 2007), *Rosmarinus officinalis* L. (0.9-1.9%) (Chahboun et al. 2014; Zhang et al. 2012) or *Daucus carota* L. (0.5-0.8%) (Flamini et al. 2014). Moreover, the composition of the spent hops EO resulted not very dissimilar from those reported in literature for the not-spent one: myrcene (52.0%), α-humulene (20.2%), and β-caryophyllene (7.0%) (Davies and Menary 1982). These findings indicate that spent hops could be a convenient low-cost source of essential oil.

4.2. Insect pests repellence bioassays

4.2.1 Area preference bioassay

This work is also the first assessment of spent hops as a source of repellent substances against pest insects. The repellency assays showed a clear repellent activity of spent hops EO against both *R. dominica* and *S. granarius*. SHEO, evaluated by the area preference method, exerted a strong repellent activity against the two insect pests *R. dominica* and *S. granarius*.

Interestingly, we observed a clear different susceptibility of the two species to SHEO. Actually, according to probit analysis, *R. dominica* resulted about 24-fold more susceptible to SHEO than *S. granarius*. This result is consistent with the findings of Bougherra et al. (2014) who observed a higher susceptibility of *R. dominica* respect to the maize weevil *Sitophilus zeamais* (Motsch.), and the confused flour beetle *Tribolium confusum* Du Val to *Pistacia lentiscus* L. essential oil and its main chemical components.

Overall, our data are in accordance with previous studies showing a repellent effect of several plant essential oils on *R. dominica* (Jilani and Malik 1973; Mediouni Ben Jemâa et al. 2012), and *S. granarius* (Benelli et al. 2012; Conti et al. 2011). However, a comparison of the results of this experiment with the data available in literature shows that the SHEO results about 2 to 5 fold more effective against *R. dominica* than what observed by Mediouni Ben Jemâa et al. (2012) for the essential oils of Mediterranean *Laurus nobilis* L. plants and shows about the same percentage of repellency, after 24h against *S. granarius*, of the essential oil of *Hyptis suaveolens* L. (Benelli et al. 2012).

The high repellent activity of SHEO is consistent with the activities of the single SHEO compounds. Chemical analysis and bio-assays indicate that the repellence of the SHEO relies mainly on its high content of myrcene and *β*-caryophyllene. It is noteworthy that myrcene, on the contrary of limonene do not have enantiomers that could exert a different bioactivity. In this regard, Giatropoulos et al. (2012), evaluating the bioefficacy of three *Citrus* essential oils against the Asian tiger mosquito *Aedes albopictus* (Skuse) (Diptera Culicidae) in correlation to their components enantiomeric distribution, found that the two enantiomeric forms of limonene, although similar in the LC_{50}, showed significant differences in their repellent activity. In our experiment, since we have tested the racemic mixture of limonene, its RD_{50} should be considered as the average activity of the two enantiomers. In fact, albeit limonene was the most repellent compound against *S. granarius*, for its much higher content myrcene can be considered the overall most active compound of SHEO against both the two insect pests species.

In previous studies, myrcene has been already found to exert a repellent or toxic activity against insects. A strong larvicidal effect of myrcene against the yellow fever mosquito *Aedes aegypti* L. and *A. albopictus* (Diptera Culicidae) was observed by Cheng et al. (2009). Papachristos et al. (2009) proved that myrcene, together with limonene and terpinene were responsible for the toxic effect of citrus oil in diets of larvae of *Ceratitis capitata* Wiedemann (Diptera Tephritidae) and Karemu et al. (2013) observed that the essential oil of *Eucaliptus camaldulensis* Dehnh., containing myrcene, was more active than DEET (N,N-diethyl-3-methylbenzamide) in repelling *S. zeamais*. In line with this experiment, Kim and Lee (2014) in a study on basil and orange essential oils observed a toxic effect of myrcene against *S. zeamais*. On the contrary, no repellency of myrcene was found against the silverleaf whitefly *Bemisia tabaci* (Gennadius) (Homoptera Aleyrodidae) (Bleeker et al. 2009). As regards to *β*-caryophyllene, we found that its activity was similar to the one of myrcene. Our data confirm a previous work by Bougherra et al. (2014) were *β*-caryophyllene resulted the overall most active compound of *P. lentiscus* essential oil against three pasta pests species, *R. dominica*, *S. zeamais*, and *T. confusum*. Consistently, Chaubey (2012) found that *β*-caryophyllene was more toxic and with higher anti-feeding activity than *α*-pinene against the red flour beetle *Tribolium castaneum* (Herbst) and the lesser rice weevil *Sitophilus oryzae* (L.)

4.2.2 Two-choice pitfall bioassay

When tested in the presence of food by the two-choice pitfall bioassay, no difference in the SHEO repellence between the two species was observed. On the contrary, differences were found in the repellency of the singles SHEO chemical components. Such differences in the insects behavior between the two assays could be due to the different conditions of the test. In fact, in the pitfall bioassay, insects are, in a more close to a real-life situation because they avoid the direct contact with the repellent compound, are in a much larger volume arena than the one of the area preference assay, and for the attractive presence of food (Bougherra et al 2014; Phillips et al 1993). An influence of the presence of food on the efficacy of chemicals such as the synthetic pyrethroid cyfluthrin (Arthur 2000) and the macrocyclic lactone spinetoram (Vassilakos et al 2014) was previously observed. In addition, an interaction between the chemicals and the food such as a differential volatiles sorption cannot be excluded.

Interestingly, the two-choice pitfall bioassay allowed us also to highlight the presence of individuals that did no make a choice remaining in the arena at the end of the experiment (Non-choosing Individuals). Such behavior, that was observed quite exclusively for *R. dominica*, was previously observed also by Bougherra et al. (2014) and is probably a characteristic response of the species to the environmental conditions of the two-choice pitfall bioassay arena.

5. Conclusions

This study, for the first time, provides a scientific rationale for the use of spent hops derivatives in the protection of stored food. The large availability of spent hops as industry by-product and its good content of essential oil with high repellent activity makes spent hops an excellent low-cost resource for the production of eco-friendly alternative to synthetic repellents in the protection of stored food-stuff from insect pests.

Author Contribution Statement

BC conceived and designed research. FC, JG, BC and GF conducted experiments. SB analyzed data. SB, GF and BC wrote the manuscript. All authors read and approved the manuscript.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Acknowledgements

We would like to thanks Mr. Paolo Giannotti for the skilled assistance during the set-up of the experiment, Dr. Riccardo Antonelli for the photographs of the insects and the Brewery “Opificio Birraio” for the supply of the spent hops.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1 Principal chemical classes (%) of the essential oil extracted from spent hops

Fig. 2 Repellence (%) of the spent hops essential oil against the two stored food insect pests *Rizopherta dominica*, black squares, and *Sitophilus granarius*, white squares, assessed by the “Area Preference Method”. Bars indicate standard error

Table 1
Chemical composition (%) of the spent hops essential oil.

Constituents [a]	***LRI***	**%**
Myrcene*	993	24.2
α-Humulene*	1456	16.2
β-Caryophyllene*	1419	6.6
2-Undecanone	1293	4.7
Humulene oxide II	1607	4
2-Methylbutyl isobutyrate	1015	3.6
δ-Cadinene	1524	3.3
Methyl 4-decenoate	1311	3.1
2-Tridecanone	1494	2.4
trans-*γ*-Cadinene	1514	2.4
Caryophyllene oxide	1582	2.2
Methyl geranate	1325	2.1
γ-Muurolene	1479	2.1
Linalool*	1101	1.9
α-Selinene	1495	1.6
1-epi-Cubenol	1628	1.3
Selina-3,7(11)-diene	1544	1.3
Limonene*	1032	1.2
β-Selinene	1487	1.2
β-Pinene	982	1
2-Dodecanone	1393	0.9
Methyl nonanoate	1228	0.8
α-Copaene	1377	0.8
2-Decanone	1194	0.7
Isoamyl 2-methylbutyrate	1105	0.7
Methyl octanoate	1128	0.7
Pentyl propanoate	1008	0.7
Methyl 6-methylheptanoate	1087	0.6
trans-Cadina-1(6),4-diene	1475	0.6
α-Muurolene	1500	0.6
τ -Cadinol	1642	0.8
Total		94.3

[a] Chemical constituents ≥ 0.1%

LRI, linear retention index on DB-5 column

*, chemicals tested for insect pests repellency

Table 2

Repellency, after 24 h, of the spent hops essential oil (EO) and terpene constituents (myrcene, linalool, limonene, *α*-humulene and *β*-caryophyllene) against adults of *Rhyzopertha dominica* and *Sitophilus granarius* assessed by the area preference bioassay.

Repellent	**Pest target**	**RD$_{50}$**	**95 % *CI***	**Slope ± SE**	**Intercept ± SE**	**χ2 (df)**
Spent Hops EO	*R. dominica*	0.01	0.005-0.012	1.269 ± 0.184	2.643 ± 0.345	4.63* (3)
	S. granarius	0.19	0.166-0.224	2.499 ± 0.352	1.797 ± 0.283	2.75* (4)
Myrcene	*R. dominica*	0.27	0.200-0.332	1.944 ± 0.308	1.119 ± 0.172	0.88* (2)
	S. granarius	2.27	1.716-3.376	1.319 ± 0.271	-0.470 ± 0.103	3.57* (3)
Linalool	*R. dominica*	2.04	1.693-2.718	2.137 ± 0.384	-0.663 ± 0.105	1.30* (3)
	S. granarius	2.12	1.847-2.521	2.583 ± 0.364	-0.844 ± 0.118	2.42* (4)
Limonene	*R. dominica*	0.65	0.431-0.887	1.190 ± 0.225	0.224 ± 0.084	3.79* (3)
	S. granarius	0.89	0.434-1.638	0.689 ± 0.211	0.034 ± 0.080	0.63* (3)
α-Humulene	*R. dominica*	0.59	n.d.	2.103 ± 0.3.11	0.486 ± 0.111	6.66* (2)
	S. granarius	2.95	1.839-8.374	0.771 ± 199	-0.362 ± 0.078	1.72* (4)
β-Caryophyllene	*R. dominica*	0.39[a]	0.274-0.612	1.193 ± 0.255	0.489 ± 0.160	1.81* (2)
	S. granarius	2.31	n.d.	2.308 ± 1.432	-0.837 ± 0.570	0.02* (1)

RD$_{50}$, repellency dose for 50% of treated adults. Data are expressed as µL cm^{-2} for spent hops essential oil and as µM cm^{-2} for chemicals.

CI, Confidence Interval;

(df), degrees of freedom;

*, indicate $P > 0.05$;

[a] Data from Bougherra et al. (2014).

Table 3

Spent hops terpene constituens (myrcene, linalol, limonene, *α*-humulene, and *β*-caryophyllene) repellent activity against *Rhyzopertha dominica* and *Sitophilus granarius* adults exposed to different concentrations (1 and 2 µM cm^{-2}) for different exposure time (1, 3, 24 h) in the area preference bioassay.

Species	µM cm^{-2}	h	% Repellency				
			Myrcene	**Linalool**	**Limonene**	***α*-Humulene**	***β*-Caryophyllene**
R. dominica	1	1	32.0[a] ± 8.0	36.0 ± 10.3	60.0 ± 12.3	50.0 ± 27.4	42.0 ± 6.4
		3	46.0 ± 9.3	32.0 ± 8.1	54.0 ± 11.7	48.0 ± 34.2	46.0 ± 4.2
		24	74.0 ± 10.3b	12.0 ± 4.9a	42.0 ± 16.6ab	72.0 ± 22.8b	48.0 ± 6.7ab
	2	1	36.0 ± 20.2	30.0 ± 11.4	52.0 ± 3.7	66.0 ± 15.2	60.0 ± 6.2
		3	42.0 ± 8.0ab	18.0 ± 13.6a	60.0 ± 6.3b	74.0 ± 27.0b	58.0 ± 5.4b
		24	84.0 ± 8.1b	20.0 ± 12.6a	58.0 ± 8.0b	60.0 ± 30.8b	68.0 ± 3.6b
S. granarius	1	1	46.0 ± 2.5	12.0 ± 5.8	24.0 ± 8.1	48.0 ± 14.6	36.0 ± 15.7
		3	40.0 ± 5.5	14.0 ± 6.8	36.00 ± 4.0	52.0 ± 17.4	26.0 ± 6.8
		24	28.0 ± 7.4ab	6.0 ± 4.0a	46.0 ± 2.5b	34.0 ± 11.7ab	48.0 ± 15.6b
	2	1	38.0 ± 8.0	14.0 ± 8.7	14.0 ± 5.1	52.0 ± 12.8	38.0 ± 13.9
		3	24.0 ± 8.2	14.0 ± 5.1	14.0 ± 4.0	62.0 ± 15.9	42.0 ± 2.0
		24	32.0 ± 13.6	14.0 ± 9.8	30.0 ± 8.9	42.0 ± 14.6	48.0 ± 8.0

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

Table 4
Relative susceptibilities of the two insect pests *Rhyzopertha dominica* and *Sitophilus granarius* to the spent hops essential oil (EO) and terpenes constituents (myrcene, linalool, limonene, α-humulene, β-caryophyllene) as assessed by the area preference bioassay.

Repellent	***rmp*** ^{a}
Spent Hops EO	0.045^{b,} *
Myrcene	0.116*
Linalool	0.914
Limonene	0.648
α-Humulene	0.238*
β-Caryophyllene	0.178*

^{a}, relative median potency analyses (rmp) values of the comparison: *Rhyzopertha dominica vs Sitophilus granarius*;
^{b} Values < 1 indicates that *Rhyzopertha dominica* is more susceptible of *Sitophilus granarius*;
*, Indicates significant values (95% CI $\neq$ 1).

Table 5
Relative repellency of spent hops terpenes constituens (myrcene, linalool, limonene, α-humulene, and β-caryophyllene), against *Rhyzopertha dominica* and *Sitophilus granarius* as assessed by the area preference bioassay.

Species	Repellent	Myrcene [a]	Linalool	Limonene	α-Humulene
R. dominica	Linalool	0.108[b], *			
	Limonene	0.357*	3.307*		
	α-Humulene	0.445*	4.118*	1.245	
	β-Caryophyllene	0.679	6.281*	1.899*	0.656
S. granarius	Linalool	0.825*			
	Limonene	2.591*	3.141*		
	α-Humulene	1.042*	1.264*	0.402	
	β-Caryophyllene	1.126	1.365*	0.434*	1.080

[a] Comparison between compounds (row vs column) by relative median potency analysis (rmp) of repellency;

[b] Rmp values < 1 indicates that row compound is more repellent than column compound;

*, Indicates significant values (95% CI $\neq$ 1).

Figures

Fig. 1

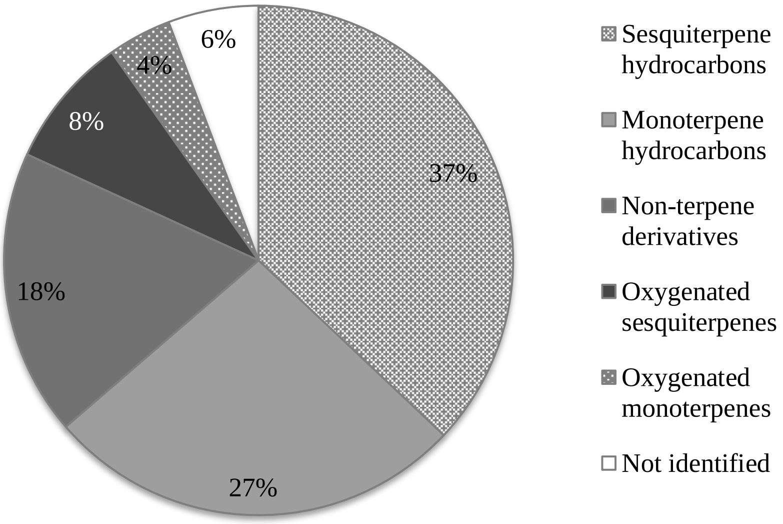
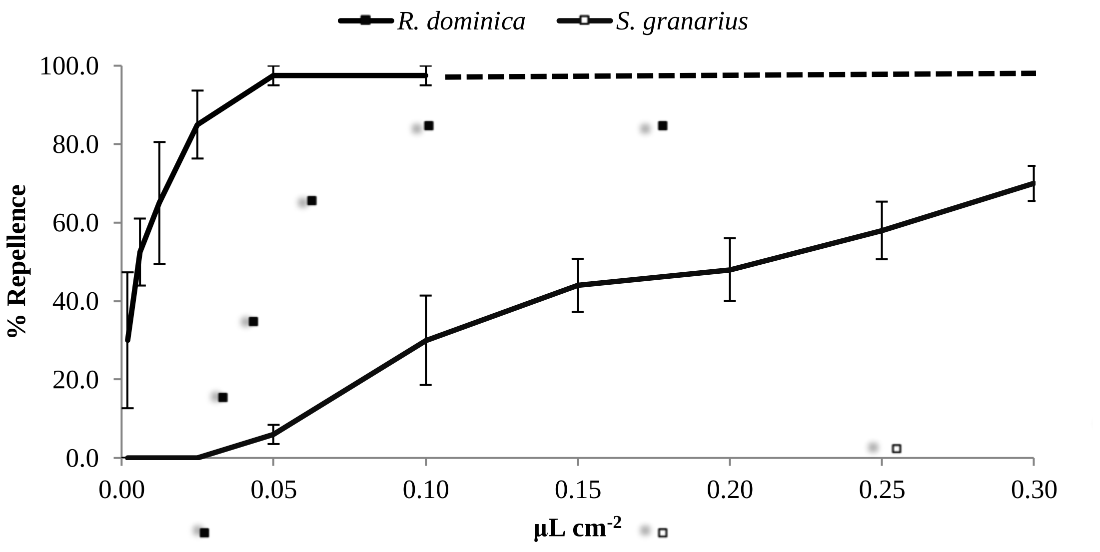


Fig. 2


R. dominica
S. granarius
% Repellence
100.0
80.0
60.0
40.0
20.0
0.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
µL cm-2