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# The essential oil from industrial hemp (*Cannabis sativa* L.) by-products as an effective tool for insect pest management in organic crops

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ABSTRACT

The inflorescences of industrial hemp (*Cannabis sativa* L.) represent a consistent by-product that is underutilized. Moving from the concept that this plant part has evolved as a natural weapon against phytophagous insects, secreting important secondary metabolites such as cannabinoids and volatile terpenes, herein we assayed the potential of its essential oil as a botanical insecticide. For the purpose, the essential oil was obtained by fresh inflorescences of hemp (monoecious cv. Felina 32) by steam-distillation and analysed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC–MS). The oil was tested against the filariasis vector *Culex quinquefasciatus*, the peach-potato aphid *Myzus persicae*, the housefly *Musca domestica* and the tobacco cutworm *Spodoptera littoralis*. To prove its harmlessness on non-target invertebrates, it was tested on the multicolored Asian lady beetle, *Harmonia axyridis*, and *Eisenia fetida* earthworms, and compared with α-cypermethrin as the positive control. The essential oil composition was dominated by monoterpene and sesquiterpene hydrocarbons, with (*E*)-caryophyllene (45.4%), myrcene (25.0%) and α-pinene (17.9%) as the most abundant compounds. Results from insecticidal tests showed that the essential oil from inflorescences of industrial hemp cv Felina 32 was highly toxic to *M. persicae* aphids ($LC_{50}$ of 3.5 mL $L^{-1}$) and *M. domestica* flies (43.3 μg $adult^{-1}$), while toxicity was moderate towards *S. littoralis* larvae (152.3 μg $larva^{-1}$), and scarce against *C. quinquefasciatus* larvae ($LC_{50}$ of 252.5 mL $L^{-1}$) and adults ($LC_{50}$ > 500 μg $cm^{-2}$). Contrary to α-cypermethrin, the hemp cv Felina 32 essential oil was not toxic to non-target invertebrate species, including 3rd instar larvae and adults of *H. axyridis* ladybugs and adults of *E. fetida* earthworms. Taken together our results shed light on the possible utilization of the crop residue of industrial hemp as a source of environmental-friendly botanical insecticides to be used in Integrated Pest Management and organic agriculture, particularly to manage aphid and housefly populations.

## 1. Introduction

In the last years, the market of conventional agrochemical products to combat insects and agricultural pests has experienced a significant decrement due to the development of botanical pesticides that have conquered the trust of farmers and have been increasingly employed in Integrated Pest Management (IPM) programmes (Isman and Machial, 2006; Thakore, 2006; Benelli et al., 2017a, 2018a,b). In this regard, botanical insecticides are favorably accepted by consumers due to their recognized efficacy, eco-friendly impact, low toxicity on mammals and beneficial organisms (Desneux et al., 2007; Benelli et al., 2016; Pavela and Benelli, 2016; Stevenson et al., 2017), and limited possibility to cause resistance in arthropod pests. Thus, this trend is expected to still go up in the next years because of marketing of new products (Isman, 2015; Pavela, 2016) and of the streamlining regulation operated by authorities.

Among various crops having the potential to be employed in IPM programmes, here we focused on industrial hemp (*Cannabis sativa* L.).

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Indeed, a hallmark of hemp (in both var. *indica* and *sativa*) is the presence of glandular hairs concentrated on leaves and, to a major extent, on inflorescences, which secrete a sort of oleoresin functioning as a barrier entrapping and killing plant enemies (Potter, 2009). These parts are normally discharged during the conventional hemp processing, thus representing an underutilized biomass for further application. In particular, they are a rich source of essential oil containing mainly monoterpene and sesquiterpene hydrocarbons (Bertoli et al., 2010).

The exploitation of hemp by-products as a source of botanical insecticides is a matter of interest for farmers, allowing them to maximise the commercial value of hemp cultivation. Our idea is to obtain bioactive essential oils from the inflorescences of industrial hemp that usually remain underutilized, to manufacture natural insecticides to be employed in organic agriculture and IPM programmes. Indeed, research on this issue is still poor.

Cultivation of industrial hemp to produce insecticides displays the following strengths: (i) lack of similar products (i.e. hemp-based insecticides); (ii) low costs of raw material and availability of agricultural lands for its cultivation; (iii) increasing demand for eco-friendly and safe products; (iv) possibility to split the end products in other fields (e.g., cosmetics and pharmaceutics). Supporting literature comes from the recent investigations by Benelli et al. (2018a) and Bedini et al. (2016), who found that the hemp essential oil is effective against larvae of mosquito vectors and moth pests, as well as against flies and snails.

In the present work, we used GC–MS analysis to investigate the chemical composition of the essential oil from the inflorescences of industrial hemp cv. Felina 32 cultivated in central Italy. The quantification of the marker compounds α-pinene, myrcene, terpinolene, (*E*)-caryophyllene and cannabidiol in the essential oil was performed by GC-FID.

Furthermore, we explored the insecticidal effects of industrial hemp cultivated in central Italy on a panel of economically important target insects, including two vectors of public health importance, i.e., the mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae), and the house fly *Musca domestica* L. (Diptera: Muscidae) (WHO, 1991; Benelli and Mehlhorn 2016; Davies et al., 2016), and two insect pests attacking crops of high economic interest, i.e., the aphid *Myzus persicae* (Sulzer) (Rhyncota: Aphididae), and the tobacco cutworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). In particular, *C. quinquefasciatus* is recognized as a vector of lymphatic filariasis, West Nile and Zika virus (Benelli and Romano, 2017; Vadivalagan et al., 2017), while *M. persicae* and *S. littoralis* are able to feed on more than 400 and 80 plant species, respectively (Bass et al., 2014; OEPP/EPPO, 2015), with severe economic damages for farmers.

To prove the safety of hemp essential oil, its toxicity on beneficial organisms such as the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and the earthworm *Eisenia fetida* (Savigny) (Oligochaeta, Lumbricidae), was evaluated and compared with α-cypermethrin as positive control. Based on our results, a future application of this multi-purpose crop as a source of botanical insecticides to combat agricultural pests and vectors of public importance may be possible.

## 2. Materials and methods

### 2.1. Plant material

The inflorescences of industrial hemp cv. Felina 32 (Assocanapa, Torino, Italy) were collected from a cultivated field placed in Fiuminata, central Italy (N 43°11′11", E 12°56′24", 318 m a.s.l.) in August 2017. The crop utilized here was normally employed to produce seed oil. A voucher specimen was archived and deposited in the Herbarium of the Centro Ricerche Floristiche dell'Appennino (APP), Barisciano, L'Aquila, Italy, under the codex APP No. 57789.

### 2.2. Steam distillation

Fresh inflorescences of hemp (2500 g) were inserted in an Albrigi Luigi E0106 (Stallavena di Grezzana-Verona, Italy) stainless steel apparatus (capacity 20 L) and subjected to steam distillation for 3 h. Steam was produced from 2 L of water at the bottom of apparatus. Once obtained, the yellowish oil was decanted, then collected using a funnel and dehydrated with anhydrous $Na_2SO_4$. The oil yield was calculated on a dry weight basis, by calculating the water content of inflorescences prior to distillation. The essential oil was stored in amber glass vials at +4 °C before insecticidal and non-target assays.

### 2.3. GC–MS analysis

Hemp essential oil, diluted 1:100 in *n*-hexane injected into an Agilent 6890N gas chromatograph equipped with a 5973N mass spectrometer. Separation was achieved on a HP-5 MS (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness; J & W Scientific, Folsom) column. As oven temperature programme we used the following operative conditions: 5 min at 60 °C then increase up to 220 °C with a gradient of 4 °C/min, then increase up to 280 °C at 4 °C/min, held for 15 min. The temperature of injector and detector was 280 °C; the carrier gas was helium (He) with a flow rate of 1 mL $min^{-1}$ and using a split ratio of 1:50.

The chromatograms were obtained in full scan using electron-impact (EI, 70 eV) mode. The mass range scanned was 29–400 *m/z*. Data were elaborated by using the MSD ChemStation software (Agilent, Version G1701DA D.01.00) and the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library v. 2.0. The analysis was repeated three times and mean values reported. The peak assignment was achieved by comparison with analytical standards bought from Sigma-Aldrich (Milan, Italy) (see Table 1).

In addition, the combination of the correspondence of the linear retention indices, calculated using a mixture of C*8*-C*30* *n*-alkanes (Supelco, Bellefonte, CA, USA) according to the Van den Dool and Kratz formula (Van den Dool and Kratz, 1963), and mass spectra with respect to those reported in ADAMS, NIST 08 and FFNSC2 libraries (Adams, 2007; NIST 08, 2008; FFNSC 2, 2012), was used as an additional parameter for peak assignment. The percentage values were obtained from the peak areas without calculating the response factors.

### 2.4. Quantification of the marker compounds by GC-FID

Quantification of α-pinene, (*E*)-caryophyllene, terpinolene, cannabidiol, myrcene in the essential oil was performed by means of gas-chromatography coupled with flame ionization detection (GC-FID) using a GC 6850 from Agilent Technologies. Analytical standards of the above compounds were purchased from Sigma-Aldrich (Milan, Italy). The hemp essential oil was diluted with chloroform (10 mg in 1 mL of chloroform) and 0.5 μL injected in split mode (split ratio 1:30) into the GC. The injector temperature was 300 °C. The carrier gas was hydrogen produced by a generator (PGH2-250 from DBS Analytical Instruments, Vigonza, Italy). The initial gas flow in the column was 3.7 mL $min^{-1}$. Chromatographic column coating was a (5%-phenyl)-methylpolysiloxane (HP-5, 30 m, 0.32 mm i.d., 0.25 μm film thickness, Agilent Technologies). The oven temperature was held at 60 °C for 3 min, then raised at 25 °C/min until 350 °C, and held at 350 °C for 1 min, for a total run time of 15.60 min. The FID temperature was set at 360 °C and hydrogen flow was 40 mL $min^{-1}$and air flow was 400 mL $min^{-1}$. The quantification was performed by using the calibration curves obtained for analytes investigated, which were built by preparing stock standard solutions at 6 different concentrations in the range 0.225–14 mg $mL^{-1}$. Correlation coefficients ranged from 0.9944 to 0.9999.