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Evaluation of two invasive plant invaders in Europe (*Solidago canadensis* and *Solidago gigantea*) as possible sources of botanical insecticides

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Abstract

Solidago gigantea and *Solidago canadensis* (Asteraceae) are two invasive weeds native to North America and introduced in Europe and Asia, where they are spreading quickly threatening the stability of native ecosystems. These two plant invaders may represent an ideal bioresource to be exploited for production of green pesticides. Therefore, herein we evaluated the efficacy of the essential oils (EOs) obtained from their different parts, i.e., leaves, inflorescences and roots, against *Culex quinquefasciatus*, *Spodoptera littoralis*, and *Musca domestica*. The essential oil composition was investigated by gas chromatographic-mass spectrometry (GC-MS) analysis. *Solidago canadensis* leaf EO was the most toxic to *C. quinquefasciatus*, with a LC_{50} of 89.3 µl L^{-1}. The two most effective oils against *M. domestica* adults were *S. canadensis* leaf and flower EOs, with LD_{50} values of 206.9 and 207.1 µg $adult^{-1}$, respectively. Three EOs highly toxic to *S. littoralis* were also identified, namely *S. gigantea* leaf EO, *S. canadensis* leaf EO and *S. gigantea* flower EO, with LD_{50} values of 84.5, 98.9 and 107.4 µg $larva^{-1}$, respectively. Since the *S. canadensis* leaf EO was the only green product effective against all the tested insect pests, we selected it for non-target toxicity assays on *E. fetida* earthworms, along with the leaf EO from *S. gigantea*. Both the *S. canadensis* and *S. gigantea* leaf EOs did not led to mortality on *E. fetida* adult earthworms, at variance with the positive control *α*-cypermethrin, allowing us to propose them for pest control purposes in IPM and organic farming.

Keywords: essential oil; *Culex quinquefasciatus*; insect pest; mosquito vector control; *Musca domestica; Spodoptera littoralis*

Key message

- *Solidago* invasive species may represent an ideal green resource to be exploited for production of green pesticides.
- *Solidago gigantea* and *S*. *canadensis* essential oils from various plant parts was tested on 3 insect pests.
- *Solidago canadensis* leaf oil was the most toxic to *Culex quinquefasciatus* and *Musca domestica*
- *Solidago gigantea* leaf oil was the most toxic to *Spodoptera littoralis* larvae.
- *Solidago* essential oils were not toxic to non-target earthworms, *Eisenia fetida*.

Introduction

The eco-friendly management of insect pests is a timely challenge nowadays (Isman 2006; Desneux et al. 2007; Benelli 2015, 2018a,b; Athanassiou et al. 2018). In this framework, essential oils extracted from plants may represent a promising reservoir of effective products for pesticide development (Pavela 2016; Stevenson et al. 2017; Benelli and Pavela 2018a,b; Pavela et al. 2018), due to a wide number of favourable characteristics that are compatible with well Integrated Pest Management (IPM) criteria, including multiple mechanisms of action and low toxicity to vertebrates (Isman 2000, 2015; Pavela and Benelli 2016a,b).

Solidago canadensis L. (Canada goldenrod) and *Solidago gigantea* Aiton (giant goldenrod) are rhizomatous, long-lived, perennial herbs native to North America. When introduced to Europe and Asia, they became invasive and, by their increased dominance, threatened the stability of native ecosystems (Ledger et al. 2015; Pal et al. 2015). *Solidago canadensis* and *S. gigantea* are generally described as having a broad tolerance with respect to soil moisture, light, nutrient contents, temperature or pH range, although they prefer ruderal habitats, where they are dominant (Werner et al. 1980; Weber and Jakobs 2004). However, their ecological needs overlap and regularly co-exist both in their native and introduced range: *S. canadensis* prefers loose and drier soils than *S. gigantea*, hence *S. canadensis* occurs near to urban areas, roadsides and railways more often and *S. gigantea* occurs mainly on riverside and embankments (Botta-Dukát and Dancza 2004).

Solidago species (both the two-aforementioned species and *S. virgaurea* L., which is native to Europe) are well-known for their medicinal use in Europe: They are ingredients of the so-called *Herba Solidaginis* included in the ESCOP publication (Kalemba and Thiem 2004). This preparation is used to treat disorders of urinary tract, prostate and kidney. Regarding the

83 secondary metabolites, several groups are reported in the two species, mainly flavonoids,
84 phenolic acids, diterpenes, saponosides and essential oils (Apáti et al. 2003; Kołodziej et al.
85 2011; Kraujalienė et al. 2017; Zihare and Blumberga 2017). These compounds have been shown
86 to exert anti-inflammatory, antimicrobial, antioxidant, antispasmodic and diuretic properties (Liu
87 et al. 2016).
88 Although these species are close relatives, they have distinct chemical profiles suggesting
89 a possible influence of the geographic origin, genetics (e.g., polyploidy level) and plant part
90 investigated (Radusiene et al. 2018; Kalemba and Thiem 2004; Gruľová et al. 2016; Shelepova et
91 al. 2018; Kalemba et al. 2001; Hull-Sanders et al. 2009).
92 *Solidago gigantea* and *S. canadensis* are consumed by many specialist herbivores in their
93 native range (Pilson and Rausher 1995; Carson and Root 2000; Meyer et al. 2005). On the other
94 hand, in their introduced ranges there are only few generalist insects consuming them (Botta-
95 Dukát and Dancza 2004; Jakobs et al. 2004) suggesting there are no specialist herbivores in the
96 place of introduction. However, Hull-Sanders et al. (2009a) reported lower foliar concentrations
97 of monoterpenes and diterpenes in the introduced *S. gigantea* populations, than in the native
98 populations. The same authors found a higher growth rate of a generalist herbivore, *Spodoptera*
99 *exigua* (Hubner), fed on introduced plants than on native ones, while the specialist *Trirhabda*
100 *virgata* LeConte was not influenced (Hull-Sanders et al. (2009b). In contrast, in a common
101 garden experiment, Nagy et al. (2017) found a higher insect resistance of *S. gigantea* populations
102 introduced in Europe compared with native ones. This might support the potential of introduced
103 *Solidago* populations under natural conditions as a source of insecticidal compounds.
104 Since *S. gigantea* and *S. canadensis* may represent an ideal bioresource to be exploited
105 for production of highly-valued products, in the present work we evaluated the efficacy of the
106 EOs obtained from their different parts (i.e. leaves, inflorescences and roots), whose

compositions were analysed by gas chromatography-mass spectrometry (GC-MS), as insecticidal agents. For the purpose, we assayed them on larvae of the filariasis vector and Zika virus vector *Culex quinquefasciatus* Say (Benelli and Romano 2017) and the tobacco cutworm *Spodoptera littoralis* (Boisduval), as well as against adults of the housefly, *Musca domestica* L. The most effective essential oils were tested to evaluate potential non-target effects on adult earthworms, *Eisenia fetida* (Savigny). The insecticidal effects of *Solidago* EOs from different plant parts of the two studied species were compared, linking their bioactivity against insects to the chemical profiles obtained.

Materials and methods

Plant material and sample preparation

The sample collection was performed in the flowering phenophase of *S*. *canadensis* and *S*. *gigantea*, during a three-week period in August 2017 (Fig. 1). Weather conditions were sunny, slightly windy and there was no rainfall for 48 h before each sampling day. Sample collection took place in the introduced range of both species, i.e., a semi-humid meadow close to an agricultural field and a canal in Szentlőrinc, Hungary (46°02'47.3"N; 17°58'37.4"E; elevation: 114.5 m above sea level). The selection of goldenrod populations was based on the high dominance of both species (alone or together at least 70 % vegetation cover), open, unshaded vegetation and the co-occurrence of the investigated species to exclude the effect of different environmental conditions on the overall chemical composition. An area of 400 x 500 m was sampled randomly throughout its entire range. For the analyses, young and intact (without any injury or infection) materials were collected from around 50-100 individuals of both species,

which were located at least 5 m apart from another, to reduce the risk of resampling the same clone. Individuals were removed, using a hand shovel; rhizomes, leaves and inflorescences were separated immediately with secateurs and placed separately into plastic bags. Collection continued until 2 kg fresh mass were reached from all organs except for roots of *S*. *canadensis* (1 kg). After collections, samples were air-dried separately, at 24-28 °C in a storage room, without direct light, for one month. The herbarium specimens of the two species were deposited in the Herbarium of the University of Pécs, Hungary, under the codes JPU 82/3630 (*S*. *gigantea*) and JPU 82/3631 (*S*. *canadensis*).

Chemicals

Analytical standards of some essential oil constituents (Table 1) were purchased from Sigma-Aldrich (Milan, Italy) and used for GC-MS peak assignment. Viridiflorol was kindly furnished by Michael Russell, Department of Primary Industries, Industry and Investment NSW, Wollongbar, NSW, Australia. A mix of *n*-alkanes, ranging from octane (C_8) to triacontane (C_{30}) was obtained from Supelco (Bellefonte, CA, USA) and injected using the analytical conditions reported below to determine the temperature-programmed retention index (RI) according to the following formula:

$$RI_x=100_n+100(t_x-t_n)/(t_{n+1}-t_n),$$

Where n is the number of carbon atoms of the alkane eluting before the compound x, tn and tn+1 are retention times of the reference alkanes eluting before and after compound x, and tx is the retention time of the compound x (Van den Dool and Kratz 1963). All compounds were of analytical standard grade. Analytical grade *n*-hexane solvent was bought from Carlo Erba (Milan, Italy) and distilled by a Vigreux column before use.

155

156 Isolation of *Solidago* essential oils

157

158 Different amounts of dry plant organs of *S*. *gigantea* and *S*. *canadensis*, namely roots (700
159 and 625 g, respectively), leaves (650 and 500 g, respectively) and inflorescences (200 and 300 g,
160 respectively) were reduced into small pieces and inserted in 10 L flasks filled with 5-6 L of
161 deionized water, then subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The
162 EOs were decanted, separated from water and dehydrated using anhydrous Na_2SO_4. They were
163 stored in amber vials capped with PTFE-faced silicon septa at 4°C until analysed. The yield was
164 calculated as g of EO/100 g of dry matter.

165

166 GC-MS analysis

167

168 Chemical analysis of the EOs from various plant parts of the two *Solidago* species was
169 performed by using an Agilent 6890N gas chromatograph coupled to a single quadrupole 5973N
170 mass spectrometer. Separation was achieved on a HP-5 MS (5% phenylmethylpolysiloxane, 30
171 m, 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific, Folsom) capillary column. The
172 temperature programme used was as follows: 5 min at 60 °C then 4°C min^{-1} up to 220 °C, then
173 11°C min^{-1} up to 280 °C, held for 15 min. Injector and detector temperatures: 280 °C; carrier gas:
174 He; flow rate: 1 ml min^{-1}; split ratio: 1:50; acquisition mass range: 29–400 *m*/*z*; mode: electron-
175 impact (EI, 70 eV). The EO was diluted 1:100 in *n*-hexane, and 2 μl of the solution were injected
176 into the GC-MS system twice. The MSD ChemStation software (Agilent, Version G1701DA
177 D.01.00) and the NIST Mass Spectral Search Program for the NIST/EPA/NIH EI and NIST
178 Tandem Mass Spectral Library v. 2.3 were used to analyze data. For identification of EO

components, co-injection with the above standards was used, together with correspondence of retention indices and mass spectra with those of ADAMS, NIST 17 and FFNSC2 libraries (Adams 2007, NIST 17 2017, FFNSC2 2012). Some oxygenated sesquiterpenes were identified by comparison of RI and MS with those reported by Kalemba et al. (2001). Semi-quantification of EO components was made by peak area normalisation considering the same response factor for all volatile components. Percentages values were the mean of two independent chromatographic analyses.

Insect and earthworm rearing

Culex quinquefasciatus 3rd instar larvae and *M. domestica* adult females were reared as reported by Benelli et al. (2018a,b). *Spodoptera littoralis* early 3rd instar larvae were reared following Sut et al. (2017). Insects were maintained at 25±1 °C, 70±3 % R.H. and 16:8 h (L:D). *Eisenia fetida* adults (weight 350–500 mg) were reared as reported by Pavela (2018) in artificial soil (OECD 1984). Room temperature was 20±1 °C. Soil maximum water-holding capacity (35 %) was monitored weekly.

Toxicity on *Culex quinquefasciatus* larvae

In insecticidal assays, we tested the EOs extracted from various plant parts of *S. canadensis* and *S. gigantea*, except for the root EO of *S. canadensis*, since the yield of this one was too scarce to be considered in insecticidal assays (see paragraph 3.1). The 5 *Solidago* EOs were diluted in dimethyl sulfoxide (DMSO), formulated at the concentrations of 100 ml L^{-1}, then tested on *C. quinquefasciatus* 3rd instar larvae following Benelli et al. (2017). Based on

preliminary assays, we tested dilution series ranging from 50 to 200 ml L^{-1} to estimate the EO lethal concentration values. For each concentration, we conducted 4 duplicate trials. Negative control was distilled water with the same amount of DMSO used testing *S*. *canadensis and S*. *gigantea* EOs. α-cypermethrin (Vaztak®) was tested as positive control (Benelli et al. 2018c). Larval mortality was noted after 24 h.

Toxicity on *Musca domestica* adults

Topical application tests were conducted to evaluate the acute toxicity of 5 EOs extracted from various plant parts of *S*. *canadensis* and *S*. *gigantea* on *M*. *domestica* adult females (3–6 days old). According to Benelli et al. (2018b), 1 μL of acetone (Sigma-Aldrich, Germany) carrying a given *Solidago* EO at the dose of 200 μg $adult^{-1}$ (each replicated at least 4 times), was applied through a microelectric applicator on the pronotum of fly adults anesthetized using CO_2. Acetone without the *Solidago* EO served as negative control. α-cypermethrin (Vaztak®) was tested as positive control (Benelli et al. 2018c). Houseflies were then moved to a recovery box (10×10×12 cm, 26±1 °C 16:9 L:D) for 24 h, before checking mortality rates. We tested the following dilution series ranging from 50 to 400 μg $adult^{-1}$ to estimate the lethal doses.

Toxicity on *Spodoptera littoralis* larvae

Toxicity of the 5 EOs extracted from various plant parts of *S*. *canadensis* and *S*. *gigantea* on 3rd instar larvae of *S*. *littoralis* was evaluated through topical application of the EO diluted in acetone, as detailed by Sut et al. (2017). Larvae were treated on the dorsum with 1 μL of acetone containing of the selected *Solidago* EO at dose of 150 μg $larva^{-1}$. We did four duplicate replicates

(n=20 larvae per replicate) for each tested *Solidago* EO concentration. Acetone without EO served as negative control. α-cypermethrin (Vaztak®) was tested as positive control (Benelli et al. 2018c). Then, *S. littoralis* larvae were moved to a recovery box (10×10×7 cm, with thin holes on each wall to avoid fumigation effects, 26±1 °C, 70±3% R.H., and 16:8 L:D) for 24 h, before checking mortality. We tested the following dilution series ranging from 30 to 250 μg larva^{-1} to estimate the lethal doses.

Toxicity on non-target earthworms

Since the *S. canadensis* leaf EO was the only tested product effective against the three selected insect pests, it was selected for non-target tests, along with the leaf EO from *S. gigantea*. The standard OECD (1984) method was followed to test the *Solidago* leaf EO toxicity on *E. fetida* adult earthworms. The artificial soil had the same composition and pH as described for *E. fetida* rearing; the soil was prepared by adding the *Solidago* EOs at concentrations of 200, 100 and 50 mg kg^{-1}, mixed with Tween 80 (ratio 1:1 v:v), equivalent to 100, 50 and 25 mg EO a.i. per kg of dry weight basis soil. *α*-cypermethrin at 50.0, 25.0 and 12.5 mg kg^{-1} of dry soil [i.e., Vaztak® at 1000, 500 and 250 μL kg^{-1} (v/v)] was the positive control. Distilled water with Tween 80 at concentration of 100 mg kg^{-1} of dry soil was used as negative control. An aqueous formulation containing the leaf EO from the two studied *Solidago* species, pure water or *α*-cypermethrin was mixed into the soil (650 g) and 10 *E. fetida* adults were added. Treated and control soil samples were stored in glass pots (1 L) covered with gauze to ensure aeration. *Eisenia fetida* mortality was noted 7 and 14 days post-exposure to the treatments at 20±1 °C, R.H. 80-85%, 16:8 (L:D) and 600 lux (Pavela, 2018).

Statistical analysis

If control mortality was >20%, the treatment mortality rates were corrected by the Abbott's formula (Abbott 1925). Lethal dose $LD_{50(90)}$ or concentration $LC_{50(90)}$ values, with associated 95% LCL and UCL, were estimated by probit analysis (Finney 1971) using BioStat version 5.

Results

Chemical analysis of *Solidago* essential oils

The hydrodistillation of leaves, inflorescences and roots of *S*. *gigantea* and *S*. *canadensis* gave similar EO yields, with leaf and flower being richer (0.15-0.16 and 0.18-0.20 %, respectively) than root (0.06 and 0.04 %, respectively). The GC analysis performed by using a combination of MS and RI and, whenever possible, co-elution with available standards, allowed us to identify 121 volatile compounds in the six EOs from the two *Solidago* species (Table 1). Overall, the chemical profiles of leaves of *S*. *gigantea* and *S*. *canadensis* species were quite similar, whereas those of inflorescences (Fig. 2 a,b) and, to a major extent, roots exhibited noteworthy differences (Fig. 2 c-f).

A total of 80 volatile components were identified in the leaf EO from *S*. *gigantea*, accounting for 83.3% of the total. This EO was dominated by oxygenated sesquiterpenes (45.1%), followed by sesquiterpene hydrocarbons (19.5%) and oxygenated monoterpenes (15.1%), with cyclocolorenone (15.6%), bornyl acetate (13.7%) and germacrene D (6.3%) as the major compounds. Other components occurring at noteworthy levels were the sesquiterpenes

275 eudesma-4(15),7-dien-1β-ol (4.4%), spathulenol (4.3%) epoxysalvial-4(14)-ene (4.1%) and
276 isospathulenol (3.0%). A total of 43 compounds were detected in percentages below 1% and 19 at
277 trace levels (<0.1%).
278 *Solidago canadensis* leaf EO yielded a total of 66 components, corresponding to 85.5% of
279 the total composition, were identified. The oxygenated sesquiterpenes 42.1%) were still the major
280 fraction of this oil, along with similar levels of sesquiterpene hydrocarbons (17.9%) and
281 oxygenated monoterpenes (17.2%), and minor amounts of monoterpene hydrocarbons (8.3%).
282 The most abundant components were again bornyl acetate (13.4%), germacrene D (11.0%) and
283 cyclocolorenone (8.8%), accompanied by minor components like eudesma-4(15),7-dien-1 β-ol
284 (7.1%), α-pinene (4.6%), torilenol (4.1%), and salvial-4(14)-en-1-one (3.0%). Thirty-two
285 compounds were present in percentages lower than 1% and 13 at trace levels.
286 The EO from inflorescences of *S. gigantea* showed a chemical profile (84 identified
287 components accounting for 88.1% of the EO) similar to that of leaf EO, of the same species with
288 oxygenated sesquiterpenes (34.5%), sesquiterpene hydrocarbons (19.1%) and oxygenated
289 monoterpenes (17.7%), and an additional occurrence of monoterpene hydrocarbons (16.0%).
290 Here, the major components were bornyl acetate (11.4%), germacrene D (9.0%), α-pinene
291 (8.1%) and cyclocolorenone (6.4%). Minor contributions derived from eudesma-4(15),7-dien-
292 1 β-ol (4.6%), *p*-cymene (3.5%), spathulenol (3.4%) and epoxysalvial-4(14)-ene (3.0%). A total
293 of 56 components were present in percentages below 1% and 6 at trace levels.
294 A different profile was found in the EO from inflorescences of *S. canadensis*, where a
295 total of 71 compounds, accounting for 94.3% of the total, was identified. Here, monoterpenoids
296 (monoterpene hydrocarbons 42.3%, oxygenated monoterpenes 30.8%) dominated over
297 sesquiterpenes (oxygenated sesquiterpenes 13.6%, sesquiterpene hydrocarbons 5.9%). The major

298 compounds were α-pinene (29.5%) and bornyl acetate (12.2%), with minor contributions of
299 limonene (5.1%), *trans*-verbenol (3.9%) and *p*-mentha-1,5-dien-8-ol (3.8%). Main leaf volatile
300 components such as cyclocolorenone and germacrene D were here poorer (2.9 and 1.0%,
301 respectively). A total of 34 components was present in percentages lower than 1.0% and 14 at
302 trace levels.
303 The chemical profiles of the two *Solidago* root EOs differed considerably from each
304 other. In *S. gigantea* EO, we identified 88 compounds accounting for 83.5% of the total
305 composition. Sesquiterpene hydrocarbons (29.2%) were the most abundant fraction, followed by
306 oxygenated sesquiterpenes (23.1%), alkenes (14.5%) and monoterpene hydrocarbons (12.9%).
307 Germacrene D (14.4%) and 1-nonene (13.1%) were the most abundant constituents, with minor
308 amounts of β-pinene (4.6%), spathulenol (4.6%), isospathulenol (3.6%), limonene (3.1%) and
309 α-gurjunene (3.0%). A total of 53 constituents were present in percentages below 1% and 12 at
310 trace levels. *Solidago canadensis* EO showed a different profile, with a total of 69 constituents,
311 corresponding to 96.2% of the oil. The EO was dominated by monoterpene hydrocarbons
312 accounting for 74.0% of the total composition. The remaining compounds comprised
313 sesquiterpene hydrocarbons (9.0%), oxygenated monoterpenes (6.2%) and alkenes (4.9%). The
314 oil composition was dominated by two components, namely limonene (32.7%) and β–pinene
315 (31.3%), whereas germacrene D (3.9%), β-elemene (3.4%), methylcamphenoate (3.2%) and 2,6-
316 dimethyl-1,3,6-heptatriene (3.0%) were present in low concentrations. Thirty-eight constituents
317 were below 1% and 19 at trace levels. 1-nonene, i.e., one of the major volatile constituents in the
318 roots of *S. gigantea*, was here present at scant amounts (1.6%).
319
320 Insecticidal activity and toxicity on non-target earthworms

321

322 The acute toxicity of the EOs extracted from various plant parts of *S*. *canadensis* and *S*.
323 *gigantea* varied consistently among the tested insect pests. Tables 2, 3 and 4 show the bioactivity
324 of the tested five EOs on *C*. *quinquefasciatus*, *M*. *domestica* and *S*. *littoralis*, respectively. At the
325 maximum tested concentration, i.e., 100 µl L^{-1}, mortality rates on *C*. *quinquefasciatus* 3rd instar
326 larvae varied from 22.0 % (*S*. *gigantea* root EO) to 61.0 % (*S*. *canadensis* leaf EO). According to
327 the criteria exposed by Pavela (2015), *Solidago* EOs achieving mortality rates lower than 50 %
328 when tested at the highest concentration of 100 µl L^{-1}, were excluded from probit analysis.
329 Therefore, the only *Solidago* EO of interest for developing *C*. *quinquefasciatus* larvicides was
330 from *S*. *canadensis* leaves, with a LC_{50} of 89.3 µl L^{-1} and a LC_{90} of 189.6 µl L^{-1} (Table 2).
331 Concerning toxicity assays on *M*. *domestica* adults, *Solidago* EOs tested at the maximum
332 dose of 200 µg $adult^{-1}$ led to fly mortality rates ranging from 30 % (*S*. *gigantea* flower EO) to
333 67.5 % (*S*. *canadensis* flower EO) (Table 3). The two most effective EOs were those from *S*.
334 *canadensis* leaf and flowers, with LD_{50} values of 206.9 and 207.1 µg $adult^{-1}$, respectively. LD_{90}
335 values were 355.6 and 426.4 µg $adult^{-1}$, respectively (Table 3).
336 Furthermore, three out of the five tested *Solidago* EOs showed relevant toxicity against
337 3rd instar larvae of *S*. *littoralis*. EOs tested at the highest dose of 150 µg $larva^{-1}$ led to caterpillar
338 mortality rates ranging from 33.3 % (*S*. *canadensis* flower EO) to 93.5 % (*S*. *gigantea* leaf EO)
339 (Table 4). Three highly effective EOs were identified, including *S*. *gigantea* leaf EO, *S*.
340 *canadensis* leaf EO and *S*. *gigantea* flower EO, with LD_{50} values of 84.5, 98.9 and 107.4 µg
341 $larva^{-1}$, respectively. LD_{90} values were 149.4, 200.4 and 264.6 µg $larva^{-1}$, respectively (Table 4).
342 For all the tested insect pests, the toxicity results achieved testing α-cypermethrin as positive
343 control are provided in Tables 2, 3 and 4.

344 Since the *S. canadensis* leaf EO was the only tested bioproduct effective against the three
345 selected tested pests, we selected it for non-target toxicity tests on *E. fetida* earthworms, along
346 with the leaf EO from *S. gigantea*. Results, given in comparison with the positive control α-
347 cypermethrin, are provided in Table 5. Notably, neither of the EOs produced any earthworm
348 mortality on adults of the *E. fetida* earthworms, at variance with the positive control *α*-
349 cypermethrin, which led to 100% mortality when applied at 25 and 50 $mg.kg^{-1}$ in the soil (Table
350 5).
351
352 **Discussion**
353
354 Chemical analysis of *Solidago* essential oils
355
356 Results highlighted a chemical polymorphism in the vegetative and reproductive organs
357 of the two *Solidago* species, with bornyl acetate, germacrene D and cyclocolorenone as marker
358 compounds of the leaves, α-pinene, bornyl acetate and germacrene D characterizing the
359 inflorescence EOs, and 1-nonene, germacrene D, β-pinene and limonene as markers of the root
360 EOs (Fig. 3).
361 Germacrene D is a ubiquitous sesquiterpene occurring in many plant EOs (Casiglia et al.
362 2017). It is a chiral compound arising from the methylerythritol phosphate pathway and playing
363 an important role in the plant cell metabolism as the precursor of many sesquiterpenes
364 (Steliopoulos et al. 2002). In addition, it has been recognized as an important pheromone for the
365 males of *Periplaneta americana* L. (Kitamura et al. 1996), and has been indicated as a useful
366 compound for pest control (Stranden et al., 2002; Zihare and Blumberga 2017). Bornyl acetate is

367 an ester of the monoterpenoid borneol having camphoraceous smell and occurring in many EOs
368 such as those of conifers and valerian (Matsubara et al. 2011). This compound has been proved to
369 exert anti-inflammatory activity (Tung et al. 2008). Interestingly, bornyl acetate is used by some
370 insects, such as *Corythucha marmorata* (Uhler) (Hemiptera: Tingidae), as a source of sex
371 pheromones (Watanabe and Shimizu 2017). 1-Nonene is a linear alkene occurring in the
372 defensive secretions of tenebrionid beetles (Tschinkel 1975). Cyclocolorenone is a tricyclic
373 sesquiterpene ketone occurring also in other species, namely *Pseudowintera colorata* (Raoul)
374 Dandy, *Ledum palustre* L., *Magnolia grandiflora* L. and *S*. *canadensis* (Kalemba et al. 2001).
375 This compound has been also reported as an allopathic and antimicrobial agent (Jacyno et al.
376 1991).
377 When comparing our data on Hungarian *Solidago* species with those of previously
378 published reports, we found both similarity and differences. For instance, Kalemba et al. (2001)
379 examined a population of *S*. *gigantea* growing in Poland and reported germacrene D (23.5%) and
380 cyclocolorenone (32.4%) as the major essential oil constituents of aerial parts. The same authors
381 examined the chemical profile of the EO from inflorescences of Polish *S*. *canadensis* and
382 reported α−pinene (13.0%), limonene (12.0%) and γ-cadinene (27.1%) as the most abundant
383 constituents (Kalemba et al. 1990). The same group also analysed the volatile fraction of
384 micropropagated plants of *S*. *gigantea* and *S*. *canadensis* and found α-gurjunene (16.6%),
385 germacrene D (12.8%) and cyclocolorenone (32.8%) as the major compounds in the former, and
386 α-pinene (59.5%), limonene (9.7%) and germacrene D (15.2%) in the latter (Kalemba and Thiem,
387 2004). Fujita (1980) reported germacrene D (66-77%) and bornyl acetate (5-7%) as the major
388 components of S. *gigantea* EO. Weyerstahl et al. (1993) studied the chemical profile of the EO
389 from *S*. *canadensis* growing in Poland and found α-pinene (14.7%), germacrene D (19.8%) and

390 β-sesquiphellandrene (10.4%) as the most abundant constituents. Synowec et al. (2017) reported
391 α-pinene (26.0%), limonene (11.5%) and germacrene D (27.5%) as the major EO constituents of
392 Polish *S*. *canadensis*. Gruľová et al. (2016) analysed Slovak populations of *S*. *gigantea* and *S*.
393 *canadensis* and found a significant chemical polymorphism depending on the collection site and
394 species. *S*. *gigantea* was found rich in sesquiterpenes, namely curlone (14.4%), tumerone (14.0%)
395 and δ-cadinene (5.4%); on the other hand, *S*. *canadensis* contained α-pinene (36.3%), limonene
396 (7.8%) and germacrene D (9.9%) as the main EO constituents. Shelepova et al. (2018) studied
397 different populations of *S*. *canadensis* growing in Europe (i.e. Austria, Ukraine, Kazakhstan and
398 Russia) and found α-pinene (12.6-52.4%), germacrene D (2.9-36.2%), bornyl acetate (3.4-26.3%)
399 and limonene (6.4-22.5%) as the major EO components. Watanabe and Shimizu (2017) reported
400 bornyl acetate (20.2%) and germacrene D (54.0%) as the major EO components of *S*. *canadensis*
401 growing in Japan. This oil was slightly phytotoxic against four common weeds (Synowec et al.
402 2017). Chanotiya and Yadav (2008) analyzed Indian *S*. *canadensis* and found limonene (0.2-
403 12.5%) and germacrene D (56.7-75.5%) as the main EO constituents. Liu et al. (2016) examined
404 the EO from leaves of Chinese *S*. *canadensis* and found α-pinene (53.6%) as the major
405 compound followed by germacrene, limonene and β-pinene.
406 In conclusion, EOs from these two invasive species show significant variability that can
407 be linked to several factors, such as the geographic origin of samples, together with the cytotype,
408 phenological stage and part studied.
409
410 Insecticidal activity and toxicity on non-target earthworms
411

412 The insecticidal efficacy of botanical insecticides based on EOs depends on
413 multiple factors, such as the size and species of target organisms, mode of application, post-
414 application temperature and, in particular, chemical composition and mutual ratios of major
415 substances, which may exhibit both synergistic and antagonistic relationships (Pavela
416 2015a,b; Pavela and Benelli 2016b; Pavela and Sedlák 2018). Herein, the efficacies of the
417 five tested *Solidago* EOs were different. *Solidago canadensis* leaf EO was most toxic to *C*.
418 *quinquefasciatus*, with an LC_{50} of 89.3 μl L^{-1}. Therefore, it can be viewed as promising for
419 the development of botanical larvicides given that EOs are generally considered as
420 prospective if their LC_{50} is lower than 100 ppm (Pavela 2015a). The two most effective
421 EOs against *M*. *domestica* adults were *S*. *canadensis* leaf EO and *S*. *canadensis* flower EO,
422 with LD_{50} values of 206.9 and 207.1 μg $adult^{-1}$, respectively. Three EOs highly toxic to *S*.
423 *littoralis* were also identified, namely *S*. *gigantea* leaf EO, *S*. *canadensis* leaf EO and *S*.
424 *gigantea* flower EO, with LD_{50} values of 84.5, 98.9 and 107.4 μg $larva^{-1}$, respectively.
425 Although these lethal concentrations were relatively higher compared to other EOs
426 or plant extracts (Pavela et al. 2008 and 2017; Benelli at al. 2018b), they can still be
427 considered as suitable for the development of botanical insecticides, particularly the *S*.
428 *canadensis* leaf EO, which showed efficacy against all three tested insect species.
429 The *Solidago* EOs studied here contained a high number of various substances of
430 which none exhibited a major share exceeding 50% (Table 1). No major constituents can
431 thus be identified, which could be believed to be responsible for the insecticidal efficacy.
432 However, it can be noted that the efficacy was related to the overall amount of oxygenated
433 sesquiterpenes, where the EO efficacy rose correspondingly with increasing amounts of
434 these substances. The closest relationship between oxygenated sesquiterpenes and achieved
435 mortality rate was found for the EOs applied in the dose of 150 μg $larva^{-1}$ against *S*.

436 *littoralis* larvae, while a significant linear relationship was observed (Fig. 4). Based on this
437 finding, it is likely that oxygenated terpenes are substances with a significantly higher
438 insecticidal efficacy compared to non-oxygenated terpenes, which agrees with earlier
439 research (Bakkali et al. 2008; Pavela 2014, 2015b). The position of the functional group in
440 the molecule and the shape of the molecule both result in diverse mechanisms of action.
441 The compounds exert their activities on insects through neurotoxic effects involving several
442 mechanisms, notably through GABA, octopamine synapses, and the inhibition of
443 acetylcholinesterase (Pavela and Benelli 2016; Jankowska et al. 2017).

444 Developing eco-friendly pesticides is important in Integrated Pest Management
445 (Lucchi and Benelli 2018), as well as a One Health perspective (Benelli and Duggan 2018).
446 Herein, since the *S. canadensis* leaf EO was the only tested bioproduct effective against the
447 three selected tested pests, we selected it for non-target toxicity tests on *E. fetida*
448 earthworms, along with the leaf EO from *S. gigantea*. Both the *S. canadensis* and *S.*
449 *gigantea* leaf EOs did not led to mortality when used to treat *E. fetida* adult earthworms, at
450 variance with the positive control α-cypermethrin. This fact is very important given that
451 earthworms rank among significant soil organisms. Earthworms are necessary for the
452 development and maintenance of the nutritional value and structure of soil (Datta et al.
453 2016), and they play an important role in the conversion of biodegradable materials and
454 organic waste to vermicast, which is rich in nutrients (Jansirani et al. 2012). Protection of
455 these organisms is thus clearly important.

456 Even though earthworms can consume a wide range of contaminated organic
457 materials including sewage sludge and industrial waste (Lim et al. 2016), they are very
458 sensitive to insecticides (Datta et al. 2016; Vasantha-Srinivasan et al. 2017). Generally,

459 insecticides exhibit a negative effect on the survival of earthworms, especially in
460 concentrations over 25 $mg.kg^{-1}$ (Datta et al. 2016).

461 More in general, it is expected that *S*. *gigantea* and *S*. *canadensis* EOs are harmless
462 against pollinators and natural predators such as honeybees and ladybird beetles,
463 respectively. In this regard, it has been reported that goldenrod is an important source of
464 nectar for honeybees (Stefanic et al. 2003). Besides, the fact that some major leaf volatile
465 constituents of *S*. *gigantea* and *S*. *canadensis* EOs, such as germacrene D and bornyl
466 acetate, are pheromones within species belonging to cockroaches and lacewings (Kitamura
467 et al. 1996; Watanabe and Shimizu 2017), should give a low risk from an ecotoxicological
468 standpoint. Notably, *Solidago* spp. have are used as feed for cattle and other mammalian
469 herbivores (Botta-Dukát and Dancza 2004; Werner et al. 1980). Furthermore, *Solidago* spp.
470 host beneficial invertebrates, such as aphid predators, e.g. *Harmonia axyridis* (Pallas)
471 (Genung et al. 2012; Kamo et al. 2010). Regarding the impact on aquatic ecosystems, it has
472 been reported that the *S*. *canadensis* extracts exert low toxicity on *Daphnia magna* Straus
473 and zebrafish, *Danio rerio* Hamilton (Huang et al. 2014).

474 In a broader perspective, the relatively high tolerance of insect pollinators, including
475 social bees, to plant EOs used for pest management purposes has been confirmed by several
476 researches (Umpierrez et al, 2017, Ribeiro et al., 2018, Palmer-Young et al., 2018). In
477 addition, this is also substantiated by the fact that EOs are used at relatively high
478 concentrations to protect bees against *Varroa destructor* (Andreson and Trueman) (Acari:
479 Varroidae) (Ramzi et al., 2017). Besides, the selectivity of EOs was also determined against
480 other non-target organisms including native predators of pests (Castilhos et al 2018; Pavela
481 2018) or larvivorous fish (Govindarajan et al. 2016a,b; AlShebly et al. 2017; Pavela and
482 Govindarajan 2017).

It has been earlier noted that *S*. *gigantea* and *S*. *canadensis* can represent a serious threat for the preservation of autochthonous ecosystems. However, the high biomass produced by them may be a resource to be exploited for fair purposes. Indeed, they are extremely common in Europe, North America and Asia so that they can satisfy a huge demand for the manufacture of insecticides. In this regard, their distribution throughout several regions, namely British Isles, Germany, North America and Europe is mapped on several websites (http://www.floraweb.de/webkarten/karte.html?taxnr=5680; http://www.brc.ac.uk/plantatlas/plant/solidago-gigantea; http://www.brc.ac.uk/plantatlas/plant/solidago-canadensis; https://www.cabi.org/isc/datasheet/50575#toDistributionMaps; https://www.cabi.org/isc/datasheet/50599). Therefore, we believe that the production of botanical insecticides from these two plant invaders may be scalable since both species are renewable resources being able to easily regenerate from their rhizomes. Cooperation among agrochemical industry and landscape managers will be a key point to make the production of botanical insecticides from goldenrod sustainable through a correct management of mowing.

Overall, from a natural product research standpoint, herein we have succeeded in finding these two *Solidago* EOs as prospective, environmentally acceptable and active ingredients utilizable in botanical insecticides to be employed in Integrated Pest Management.

Acknowledgments

The authors are grateful to University of Camerino (Fondo di Ateneo per la Ricerca, FAR 2014/2015, FPI 000044) for financial support. Dr. R. Pavela would like to thank the Ministry of Agriculture of the Czech Republic for its financial support concerning botanical pesticide and basic substances research (Project MZE-RO0418).

Conflict of Interest

The authors declare no conflict of interest.

Author contributions

GB, RP and FM conceived and designed research. All authors conducted experiments and/or analysed data. GB, RP and FM wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Animals and human rights

All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Ethical approval

530

531 This article does not contain any studies with human participants performed by any of the
532 authors.

533

534 **References**

535

536 Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol
537 18:265–267

538 Adams R (2007) Identification of Essential Oil Components by Gas Chromatography/Mass
539 Spectrometry, fourth ed. Allured Publishing Corp., Carol Stream, IL, USA

540 AlShebly MM, AlQahtani FS, Govindarajan M, Gopinath K, Vijayan P, Benelli G (2017)
541 Toxicity of ar-curcumene and epi-β-bisabolol from *Hedychium larsenii* (Zingiberaceae)
542 essential oil on malaria, chikungunya and St. Louis encephalitis mosquito vectors.
543 Ecotoxicol Environ Saf 137:149-157

544 Apáti P, Szentmihályi K, Kristó SzT, Papp I, Vinkler P, Szőke É, Kéry Á (2003) Herbal remedies
545 of *Solidago*—correlation of phytochemical characteristics and antioxidative properties. J
546 Pharm Biom Anal 32:1045-1053

547 Athanassiou CG, Kavallieratos NG, Benelli G, Losic D, Rani PU, Desneux N (2018)
548 Nanoparticles for pest control: current status and future perspectives. J Pest Sci 91:1-15

549 Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils –a
550 review. Food Chem Toxicol 46:446–475

551 Benelli G (2018a) Plant-borne compounds and nanoparticles: challenges for medicine,
552 parasitology and entomology. Environ Sci Pollut Res 25:10149–10150

553 Benelli G (2018b) Mode of action of nanoparticles against insects. Environ Sci Pollut Res doi:
554 10.1007/s11356-018-1850-4
555 Benelli G, Duggan MF (2018) Management of arthropod vector data – Social and ecological
556 dynamics facing the One Health perspective. Acta Trop 182:80-91
557 Benelli G, Romano D (2017) Mosquito vectors of Zika virus. Entomol Gen 36:309–318
558 Benelli G, Pavela R (2018) Repellence of essential oils and selected compounds against ticks – a
559 systematic review. Acta Trop 179:47–54
560 Benelli G, Pavela R (2018) Beyond mosquitoes – Essential oil toxicity and repellency against
561 bloodsucking insects. Ind Crops Prod 117:382–392
562 Benelli G, Pavela R, Iannarelli R, Petrelli R, Cappellacci L, Cianfaglione K, Afshar FH, Nicoletti
563 M, Canale A, Maggi F (2017) Synergized mixtures of Apiaceae essential oils and related
564 plant-borne compounds: larvicidal effectiveness on the filariasis vector *Culex*
565 *quinquefasciatus* Say. Ind Crops Prod 96:186-195
566 Benelli G, Pavela R, Giordani C, Casettari L, Curzi G, Cappellacci L, Petrelli R, Maggi F
567 (2018a) Acute and sub-lethal toxicity of eight essential oils of commercial interest against
568 the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. Ind
569 Crops Prod 112:668–680
570 Benelli G, Pavela R, Lupidi G, Nabissi M, Petrelli R, Ngahang Kamte SL, Cappellacci L, Fiorini
571 D, Sut S, Dall'Acqua S, Maggi F (2018b) The crop-residue of fiber hemp cv. Futura 75:
572 from a waste product to a source of botanical insecticides. Environ Sci Pollut Res
573 25:10515-10525
574 Benelli G, Pavela R, Petrelli R, Cappellacci L, Santini S, Fiorini D, Sut S, Dall'Acqua S, Canale
575 A, Maggi F (2018c) The essential oil from industrial hemp (*Cannabis sativa* L.) by-

products as an effective tool for insect pest management in organic crops. Ind Crops Prod doi: 10.1016/j.indcrop.2018.05.032

Botta-Dukát Z, Dancza I (2004) Magas aranyvessző és kanadai aranyvessző. In: Mihály B, Botta-Dukát Z (eds.) Özönnövények. A KvVM Természetvédelmi Hivatalának Tanulmánykötetei 9. Természet Búvár Alapítvány Kiadó, Budapest, pp. 289–314

Carson WP, Root RB (2000) Herbivory and plant species coexistence: community regulation by an outbreaking phytophagous insect. Ecol Monogr 70:73-99

Castilhos RV, Grutzmacher AD, Coats JR (2018) Acute toxicity and sublethal effects of terpenoids and essential oils on the predator *Chrysoperla externa* (Neuroptera: Chrysopidae). Neotrop Entomol 47:311-317

Casiglia S, Bruno M, Bramucci M, Quassinti L, Lupidi G, Fiorini D, Maggi F (2017) *Kundmannia sicula* (L.) DC: a rich source of germacrene D. J Essent Oil Res 29:437-442

Chanotiya CS, Yadav A (2008) Natural variability in enantiomeric composition of bioactive chiral terpenoids in the essential oil of *Solidago canadensis* L. from Uttarakhand, India. Nat Prod Comm 3:263-266

Datta S, Singh J, Singh S, Singh J (2016) Earthworms, pesticides and sustainable agriculture: a review. Environ Sci Pollut Res 23:8227–8243

Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81-106

FFNSC 2 (2012) Flavors and fragrances of natural and synthetic compounds. Mass spectral database. Shimadzu Corps, Kyoto

Finney DJ (1971) Probit Analysis. Cambridge University, London, pp. 68–78

Fujita S (1980) Koen Yoshishu-Koryo, Terupen oyobi Seiyu Kagaku ni Konsuru Toronkai. Chem. Abstr. 92, 143284f

600 Genung MA, Crutsinger GM, Bailey JK, Schweitzer JA, Sanders NJ (2012) Aphid and ladybird
601 beetle abundance depend on the interaction of spatial effects and genotypic diversity.
602 Oecologia 168:167-174.
603 Govindarajan M, Rajeswary M, Benelli G (2016a) Chemical composition, toxicity and non-target
604 effects of *Pinus kesiya* essential oil: an eco-friendly and novel larvicide against malaria,
605 dengue and lymphatic filariasis mosquito vectors. Ecotoxicol Environ Saf 129:85-90
606 Govindarajan M, Kadaikunnan S, Alharbi NS, Benelli G (2016b) Acute toxicity and repellent
607 activity of the *Origanum scabrum* Boiss. & Heldr. (Lamiaceae) essential oil against four
608 mosquito vectors of public health importance and its biosafety on non-target aquatic
609 organisms. Environ Sci Poll Res 23(22):23228-23238
610 Gruľová D, Baranová B, Ivanova V, De Martino L, Mancini E, De Feo V (2016) Composition
611 and bio activity of essential oils of *Solidago* spp. and their impact on radish and garden
612 cress. Allelopathy J 39:129-142
613 Huang Y, Bai Y, Wang Y, Kong H (2014) *Solidago canadensis* L. extracts to control algal
614 (*Microcystis*) blooms in ponds. Ecol Eng 70:263-267
615 Hull-Sanders HM, Johnson RH, Owen HA, Meyer GA (2009a) Effects of polyploidy on
616 secondary chemistry, physiology, and performance of native and invasive genotypes of
617 *Solidago gigantea* (Asteraceae). Am J Bot 96:762–770
618 Hull-Sanders HM, Johnson RH, Owen HA, Meyer GA (2009b) Influence of polyploidy on insect
619 herbivores of native and invasive genotypes of *Solidago gigantea* (Asteraceae). Plant
620 Signal Behav 4:893-895
621 Isman MB (2000) Plant essential oils for pest and disease management. Crop Protection 19(8-
622 10):603-608

623 Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an
624 increasingly regulated world. Annu Rev Entomol 51:45-66
625 Isman MB (2015) A renaissance for botanical insecticides? Pest Manag Sci 71(12):1587-1590
626 Jacyno JM, Montemurro N, Bates AD, Cutler HG (1991) Phytotoxic and antimicrobial properties
627 of cyclocolorenone from *Magnolia grandiflora* L. J Agric Food Chem 39:1166-1168
628 Jakobs G, Weber E, Edwards PJ (2004) Introduced plants of the invasive *Solidago gigantea*
629 (Asteraceae) are larger and grow denser than conspecifics in the native range. Divers
630 Distrib 10:11-19
631 Jankowska M, Rogalska J, Wyszkowska J, Stankiewicz M (2017) Molecular Targets for
632 Components of Essential Oils in the Insect Nervous System—A Review. Molecules 23:34
633 Jansirani D, Nivethitha S, Singh MVP (2012) Production and utilization of vermicast using
634 organic wastes and its impact on *Trigonella foenum* and *Phaseolus aureus*. Int J Res Biol
635 Sci 2:187–189
636 Kalemba D, Thiem B (2004) Constituents of the essential oils of four micropropagated *Solidago*
637 species. Flavour Fragr J 19:40-43
638 Kalemba D, Góra J, Kurowska A (1990) Analysis of the essential oil of *Solidago canadensis*.
639 Planta Med 56:222-223
640 Kalemba D, Marschall H, Bradesi P (2001) Constituents of the essential oil of *Solidago gigantea*
641 Ait. (giant goldenrod). Flavour Fragr J 16:19-26
642 Kamo T, Tokuoka Y, Miyazaki M (2010) Influence of aphid–host plant pairs on the survivorship
643 and development of the multicolored Asian ladybird beetle: implications for the
644 management of vegetation in rural landscapes. Ecol Res 25:1141-1149

645 Kołodziej B, Kowalski R, Kędzia B (2011) Antibacterial and antimutagenic activity of extracts
646 aboveground parts of three *Solidago* species: *Solidago virgaurea* L., *Solidago canadensis*
647 L. and *Solidago gigantea* Ait. J Med Plant Res 5:6770-6779
648 Kraujalienė V, Pukalskas A, Venskutonis PR (2017) Biorefining of goldenrod (*Solidago*
649 *virgaurea* L.) leaves by supercritical fluid and pressurized liquid extraction and evaluation
650 of antioxidant properties and main phytochemicals in the fractions and plant material. J
651 Funct Foods 37:200-208
652 Ledger KJ, Pal RW, Murphy P, Nagy DU, Filep R, Callaway RM (2015) Impact of an invader on
653 species diversity is stronger in the non-native range than in the native range. Plant Ecol
654 216:1285-1295
655 Lim SL, Lee LH, Wu TY (2016) Sustainability of using composting and vermicomposting
656 technologies for organic solid waste biotransformation: recent overview, greenhouse gases
657 emissions and economic analysis. J Clean Prod 111:262–278
658 Liu S, Shao X, Wei Y, Li Y, Xu F, Wang H (2016) *Solidago canadensis* L. essential oil vapor
659 effectively inhibits *Botrytis cinerea* growth and preserves postharvest quality of strawberry
660 as a food model system. Front Microbiol 7:1179
661 Lucchi A, Benelli G (2018) Towards pesticide-free farming? Sharing needs and knowledge
662 promotes Integrated Pest Management. Environ Sci Poll Res 25:13439–13445
663 Matsubara E, Fukagawa M, Okamoto T, Ohnuki K, Shimizu K, Kondo R (2011) (-)-Bornyl
664 acetate induces autonomic relaxation and reduces arousal level after visual display terminal
665 work without any influences of task performance in low-dose condition. Biomed Res
666 32:151-157
667 Meyer G, Clare R, Weber E (2005) An experimental test of the evolution of increased
668 competitive ability hypothesis in goldenrod, *Solidago gigantea*. Oecologia 144:299-307

669 Nagy DU, Stranczinger Sz, Godi A, Weisz A, Rosche C, Suda J, Mariano M, Pal RW (2017)
670 Does higher ploidy level increase the risk of invasion? A case study with two geo-cytotypes
671 of *Solidago gigantea* Aiton (*Asteraceae*). J Plant Ecol 11:317–327
672 NIST 17 (2017) Mass Spectral Library (NIST/EPA/NIH). Gaithersburg, USA: National Institute
673 of Standards and Technology.
674 Pal RW, Chen S, Nagy DU, Callaway RM (2015) Impacts of *Solidago gigantea* on other species
675 at home and away. Biol Invasions 17:3317-3325
676 Palmer-Young EC, Calhoun AC, Mirzayeva A, Sadd B (2018) Effects of the floral
677 phytochemical eugenol on parasite evolution and bumble bee infection and preference. Sci
678 Reports 8:2074
679 Pavela R, Sajfrtová M, Sovova H, Bárnet M (2008) The insecticidal activity of *Satureja hortensis*
680 L. extracts obtained by supercritical fluid extraction and traditional extraction techniques.
681 Appl Entomol Zool 43:377–382
682 Pavela R (2014) Acute, synergistic and antagonistic effects of some aromatic compounds on the
683 *Spodoptera littoralis* Boisd. (Lep., Noctuidae). Ind Crops Prod 60:247–258
684 Pavela R (2015a) Essential oils for the development of eco-friendly mosquito larvicides: a
685 review. Ind Crops Prod 76:174-187
686 Pavela R (2015b) Acute toxicity and synergistic and antagonistic effects of the aromatic
687 compounds of some essential oils against *Culex quinquefasciatus* Say larvae. Parasitol
688 Res 114:3835–3853
689 Pavela R (2016) History, presence and perspective of using plant extracts as commercial
690 botanical insecticides and farm products for protection against insects–a review. Plant
691 Prot Sci 52:229–241

692 Pavela R (2018) Essential oils from *Foeniculum vulgare* Miller as a safe environmental
693 insecticide against the aphid *Myzus persicae* Sulzer. Environ Sci Pollut Res 25:10909-
694 10910
695 Pavela R, Benelli G (2016a) Ethnobotanical knowledge on botanical repellents employed in the
696 African region against mosquito vectors - a review. Exp Parasitol 167:103–108
697 Pavela R, Benelli G (2016b) Essential oils as eco-friendly biopesticides? Challenges and
698 constraints. Trends Plant Sci 21:1000–1007
699 Pavela R, Govindarajan M (2017) The essential oil from *Zanthoxylum monophyllum* a potential
700 mosquito larvicide with low toxicity to the non-target fish *Gambusia affinis*. J Pest Sci
701 90:369-378
702 Pavela R, Sedlák P (2018) Post-application temperature as a factor influencing the insecticidal
703 activity of essential oil from *Thymus vulgaris*. Ind Crops Prod 113:46-49
704 Pavela R, Maggi F, Lupidi G, Cianfaglione K, Dauvergne X, Bruno M, Benelli G (2017)
705 Efficacy of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils against *Culex*
706 *quinquefasciatus* Say and *Spodoptera littoralis* (Boisd.). Ind Crops Prod 109:603-610
707 Pavela R, Maggi F, Lupidi G, Mbuntcha H, Woguem V, Womeni HM, Barboni L, Tapondjou
708 LA, Benelli G (2018) *Clausena anisata* and *Dysphania ambrosioides* essential oils: from
709 ethno-medicine to modern uses as effective insecticides. Environ Sci Pollut Res 25:10493-
710 10503
711 Pilson D, Rausher MD (1995) Clumped distribution patterns in goldenrod aphids: genetic and
712 ecological mechanisms. Ecol Entomol 20:75-83
713 OECD (1984) Guideline for testing of chemicals no. 207. Earthworm, acute toxicity tests,
714 OECD—guideline for testing chemicals. Paris, France

715 Radusiene J, Marksa M, Karpaviciene B (2018) Assessment of *Solidago×niederederi* origin
716 based on the accumulation of phenolic compounds in plant raw materials. Weed Sci doi:
717 10.1017/wsc.2018.8
718 Ramzi H, Ismaili MR, Aberchane M, Zaanoun S (2017) Chemical characterization and acaricidal
719 activity of *Thymus satureioides* C. & B. and *Origanum elongatum* E. & M. (Lamiaceae)
720 essential oils against *Varroa destructor* Anderson & Trueman (Acari: Varroidae). Ind Crop
721 Prod 108:201-207
722 Ribeiro AV, Farias ED, Santos AA, Filomeno CA, dos Santos IB, Barbosa LCA, Picanco MC
723 (2018) Selection of an essential oil from *Corymbia* and *Eucalyptus* plants against *Ascia*
724 *monuste* and its selectivity to two non-target organisms. Crop Prot 110:207-213
725 Shelepova O, Vinogradova Y, Zaitchik B, Ruzhitsky A, Grygorieva O, Brindza J (2018)
726 Constituents of the essential oil in *Solidago canadensis* L. from Eurasia. Potravinarstvo
727 Slovak Journal of Food Sciences 12:20-25
728 Stefanic E, Puskadija Z, Stefanic I, Bubalo D (2003) Goldenrod: a valuable plant for beekeeping
729 in north-eastern Croatia. Bee World 84:86-90
730 Steliopoulos P, Wüst M, Adam KP, Mosandl A (2002) Biosynthesis of the sesquiterpene
731 germacrene D in *Solidago canadensis*: 13C and 2H labeling studies. Phytochemistry 60:13-
732 20
733 Stevenson PC, Isman MB, Belmain SR (2017) Pesticidal plants in Africa: a global vision of new
734 biological control products from local uses. Ind Crop Prod 110:2-9
735 Stranden M, Borg-Karlson AK, Mustaparta H (2002) Receptor neuron discrimination of the
736 germacrene D enantiomers in the moth *Helicoverpa armigera*. Chem Senses 27:143-152

737 Sut S, Pavela R, Kolarčik V, Lupidi G, Maggi F, Dall'Acqua S, Benelli G (2017)
738 Isobutyrylshikonin and isovalerylshikonin from the roots of *Onosma visianii* inhibit larval
739 growth of the tobacco cutworm *Spodoptera littoralis*. Ind Crops Prod 109:266–273
740 Synowiec A, Kalemba D, Drozdek E, Bocianowski J (2017) Phytotoxic potential of essential oils
741 from temperate climate plants against the germination of selected weeds and crops. J Pest
742 Sci 90:407-419
743 Tschinkel WR (1975) A comparative study of the chemical defensive system of tenebrionid
744 beetles: chemistry of the secretions. J Insect Physiol 21:753-783
745 Tung YT, Chua MT, Wang SY, Chang ST (2008) Anti-inflammation activities of essential oil
746 and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs.
747 Bioresour Technol 99:3908-3913
748 Umpierrez ML, Paullier J, Porrini M, Garrido M, Santos E, Rossini C (2017) Potential botanical
749 pesticides from Asteraceae essential oils for tomato production: activity against whiteflies,
750 plants and bees. Ind Crop Prod 109:686-692
751 Van den Dool H, Kratz PD (1963) A generalization of the retention index system including linear
752 temperature programmed gas—liquid partition chromatography. J Chromatogr A 11:463-
753 471
754 Vasantha-Srinivasan P, Senthil-Nathan S, Ponsankar A, Thanigaivel A, Chellappandian M,
755 Edwin ES, Selin-Rani S, Kalaivani K, Hunter WB, Duraipandiyan V, Al-Dhabi NA (2017)
756 Acute toxicity of chemical pesticides and plant-derived essential oil on the behavior and
757 development of earthworms, *Eudrilus eugeniae* (Kinberg) and *Eisenia fetida* (Savigny).
758 Environ Sci Pollut Res 25:10371-10382
759 Watanabe K, Shimizu N (2017) Identification of a sex pheromone of the chrysanthemum lace
760 bug *Corythucha marmorata* (Hemiptera: Tingidae). Sci Rep 7:7302

761 Weber E, Jakobs G (2005) Biological flora of central Europe: *Solidago gigantea* Aiton. Flora
762 200:109–118
763 Werner PA, Gross RS, Bradbury IK (1980) The biology of Canadian weeds: 45. *Solidago*
764 *canadensis* L. Can J Plant Sci 60:1393-1409.
765 Weyerstahl P, Marschall H, Christiansen C, Kalemba D, Góra J (1993) Constituents of the
766 essential oil of *Solidago canadensis* ("Goldenrod") from Poland-A Correction. Planta Med
767 59:281-282
768 Zihare L, Blumberga D (2017) Insight into bioeconomy. *Solidago canadensis* as a valid resource.
769 Brief review. Energy Procedia 128:275-280
770

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Figure 1. *Solidago gigantea* (a) and *S. canadensis* (b) in the collection site (Szentlőrinc, Hungary).

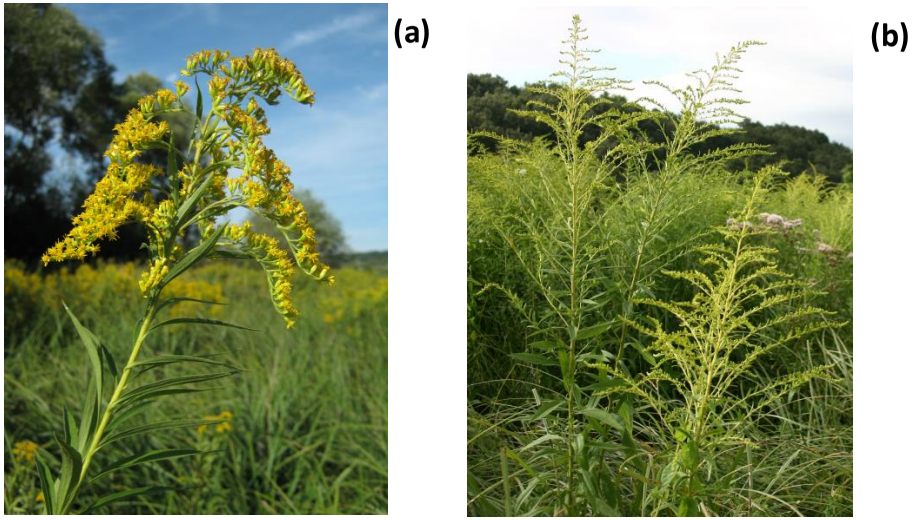

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Figure 2. TIC-GC/MS chromatograms of the essential oils extracted from leaves, inflorescences and roots of *Solidago gigantea* (a,c,e, respectively) and *Solidago canadensis* (b,d,f, respectively). Numbers of main peaks refer to those reported in Table 1.

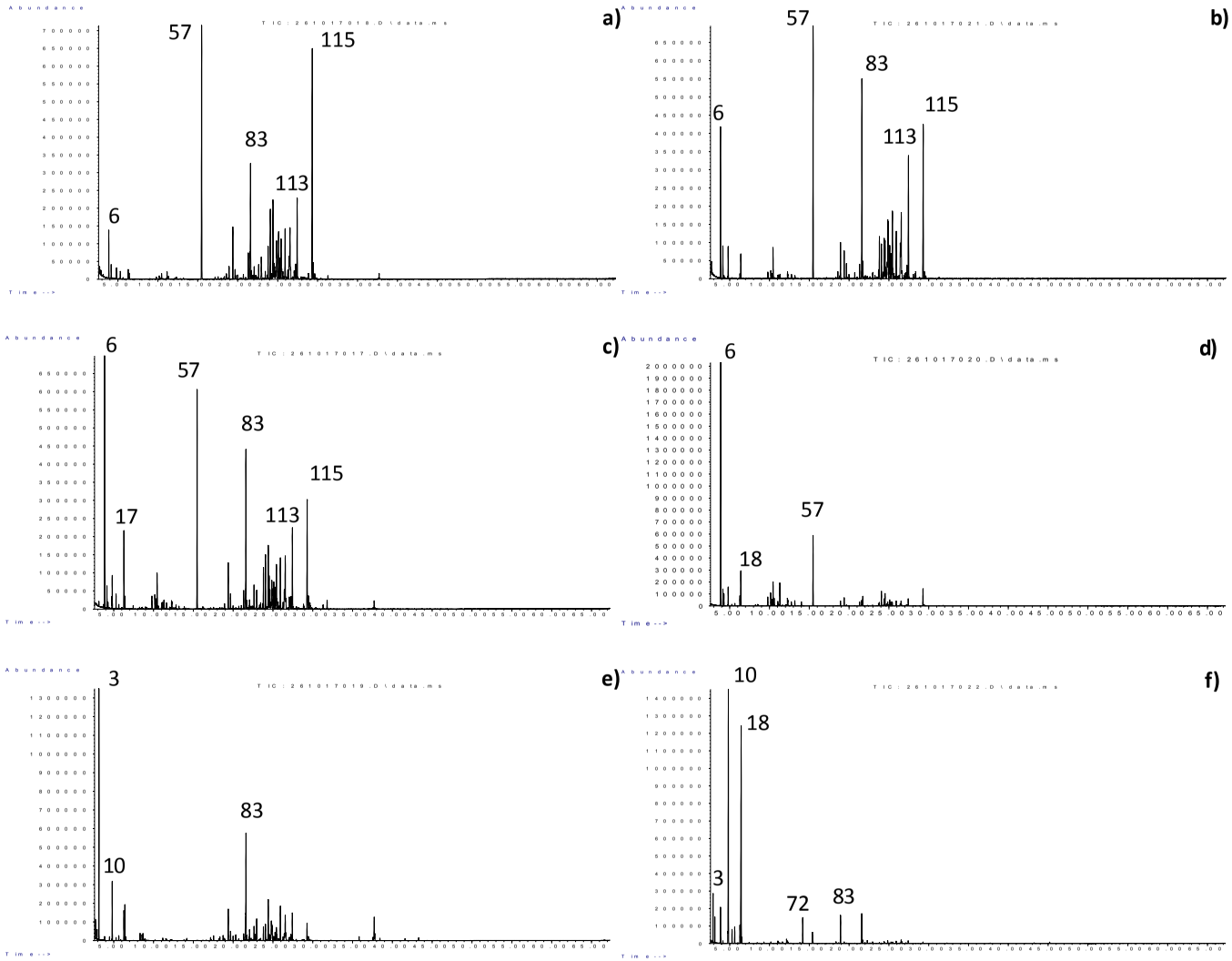

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Figure 3. Marker volatile compounds in the essential oils extracted from different plant parts of *Solidago gigantea* and *Solidago canadensis*.

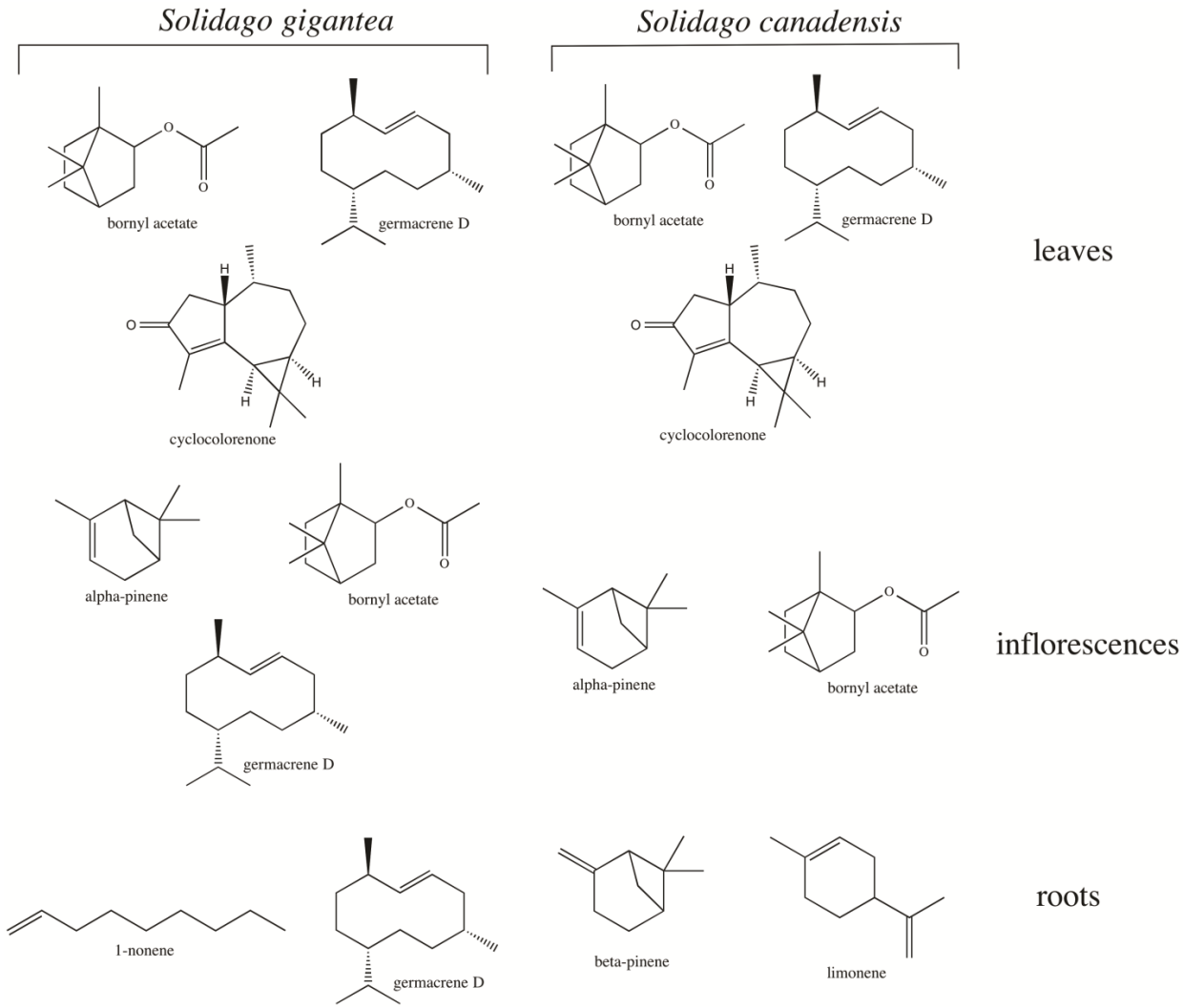

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Figure 4. A relationship between *Spodoptera littoralis* larval mortality and the oxygenated sesquiterpene content characterizing the five tested *Solidago* essential oils (all at 150 µg larva^{-1}) was observed. A significant linear relationship was noted (P=0.001). The same was not observed analysing *C. quinquefasciatus* data.

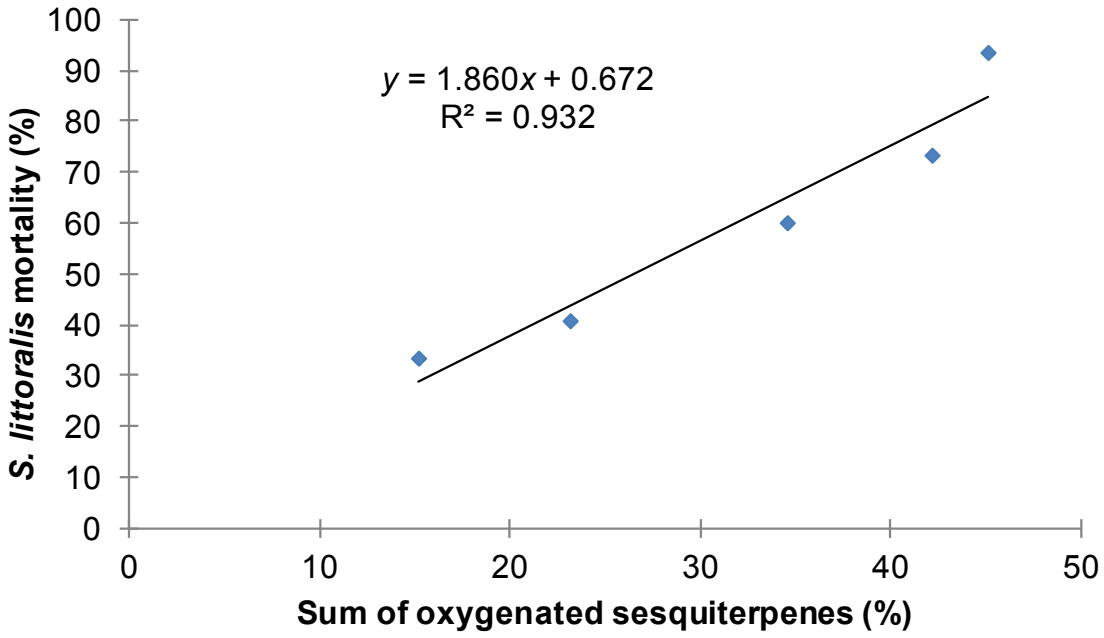

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Table 1. Chemical composition of the essential oils obtained from leaves, inflorescences and roots of *Solidago gigantea* and *Solidago canadensis*.

No	Component[a]	RI exp.[b]	RI Lit.[c]		*Solidago gigantea* (%)[d]			*Solidago canadensis* (%)[d]			ID[e]
			ADAMS	NIST 17	Leaves	Flowers	Roots	Leaves	Flowers	Roots	
1	1-octene,7-methyl	847		847			0.9±0.2			0.2±0.0	b,c
2	2,6-dimethyl-1,3,6-heptatriene	861		858			0.6±0.1			3.0±0.6	b,c
3	1-nonene	888		889	0.1±0.0	0.1±0.0	13.1±2.5			1.6±0.3	b,c
4	tricyclene	916	921	921					0.1±0.0		b,c
5	α-thujene	921	924	922		0.1±0.0		tr[f]	tr	tr	b,c
6	α-pinene	926	932	925	1.5±0.3	8.1±1.5	0.2±0.0	4.6±0.9	29.5±4.5	2.9±0.6	a,b,c
7	camphene	939	946	940	0.5±0.1	0.7±0.2		1.0±0.2	1.9±0.5	0.2±0.0	a,b,c
8	thuja-2,4(10)-diene	945	953	945	tr	0.2±0.0		0.1±0.0	1.4±0.4		b,c
9	sabinene	967	969	968	tr	0.4±0.1	0.1±0.0	0.1±0.0	0.1±0.0	1.0±0.2	a,b,c
10	β-pinene	968	974	968	0.4±0.1	1.3±0.3	4.6±0.9	1.2±0.3	2.4±0.5	31.3±4.0	a,b,c
11	1-octen-3-ol	977	974	978			tr				a,b,c
12	2-pentyl-furan	987	984	990			0.1±0.0	tr	0.1±0.0		b,c
13	myrcene	989	988	988	0.3±0.0	0.6±0.2	2.1±0.4	tr		1.5±0.3	a,b,c
14	α-phellandrene	1003	1002	1003	0.1±0.0	0.2±0.0	0.4±0.1	tr		1.8±0.4	a,b,c
15	*p*-methyl-anisole	1009	1015	1009			0.1±0.0			tr	b,c
16	α-terpinene	1014	1014	1014		0.1±0.0	tr		tr	0.1±0.0	b,c
17	*p*-cymene	1022	1020	1020	0.4±0.1	3.5±0.7	2.5±0.5	0.2±0.0	1.5±0.3	2.3±0.4	a,b,c
18	limonene	1025	1024	1025	0.2±0.0	0.6±0.2	3.1±0.6	1.0±0.2	5.1±1.1	32.7±5.0	a,b,c
19	1,8-cineole	1027	1026	1027			0.3±0.1			0.6±0.2	a,b,c
20	2-ethyl-hexanol	1030		1031			0.1±0.0			tr	b,c
21	benzene acetaldehyde	1043	1036	1042	tr						a,b,c
22	(*E*)-β-ocimene	1047	1044	1047					tr		a,b,c
23	γ-terpinene	1055	1054	1055		0.1±0.0	tr		0.1±0.0	0.1±0.0	a,b,c
24	1-nonen-3-ol	1080		1081			0.7±0.2				b,c
25	terpinolene	1085	1086	1085		tr	tr		tr	0.1±0.0	a,b,c
26	*p*-cymenene	1087	1089	1089	tr	0.1±0.0		tr	0.2±0.0	tr	b,c
27	3-nonanone	1087		1089			0.6±0.1			tr	b,c
28	1-undecene	1091		1093			0.6±0.1				b,c
29	3-nonanol	1098		1099			0.2±0.0				b,c

30	linalool	1101	1095	1100	tr	0.1±0.0			0.1±0.0		a,b,c
31	*endo*-fenchol	1109	1114	1110						0.1±0.0	b,c
32	*trans*-*p*-mentha-2,8-dien-1-ol	1118	1119	1117					0.1±0.0		b,c
33	α-campholenal	1123	1122	1123	0.1±0.0	0.6±0.2		0.3±0.0	1.5±0.3	tr	b,c
34	*trans*-pinocarveol	1133	1135	1133	0.1±0.0	0.7±0.2	tr	0.4±0.1	2.1±0.4	0.2±0.0	a,b,c
35	*cis*-verbenol	1138	1137	1139	tr	0.5±0.1		0.2±0.0	1.1±0.3		b,c
36	*trans*-verbenol	1141	1140	1142	0.2±0.0	1.7±0.4		1.4±0.3	3.9±0.8		b,c
37	isoborneol	1151	1155	1151						tr	a,b,c
38	*trans*-pinocamphone	1155	1158	1156		0.1±0.0			0.1±0.0		b,c
39	pinocarvone	1157	1160	1164	tr	0.3±0.0	tr	0.2±0.0	0.8±0.2	0.3±0.1	b,c
40	borneol	1160	1165	1160	0.4±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.3±0.0	0.2±0.0	a,b,c
41	*p*-mentha-1,5-dien-8-ol	1164	1166	1165	0.1±0.0	0.5±0.1		0.2±0.0	3.8±0.7		b,c
42	(2*E*)-nonenol	1170	1163	1171			0.2±0.0				b,c
43	terpinen-4-ol	1173	1174	1174	tr	0.3±0.0	0.1±0.0		0.2±0.0	0.2±0.0	a,b,c
44	*n*-nonanol	1174	1165	1174			0.1±0.0				b,c
45	*p*-cymen-8-ol	1184	1179	1184	tr	0.1±0.0	tr	tr	0.3±0.1	tr	b,c
46	α-terpineol	1187	1186	1186	tr	0.1±0.0	0.1±0.0		0.1±0.0	0.5±0.1	a,b,c
47	myrtenal	1190	1195	1190	0.1±0.0	0.3±0.1	tr	0.3±0.1	1.1±0.3	0.3±0.0	a,b,c
48	myrtenol	1191	1194	1191	0.1±0.0	0.4±0.1	tr	0.2±0.0	1.1±0.2	0.1±0.0	a,b,c
49	verbenone	1205	1204	1205	tr	0.1±0.0		0.2±0.0	0.7±0.2		a,b,c
50	*trans*-carveol	1216	1215	1216	tr	0.1±0.0		0.1±0.0	0.9±0.2		b,c
51	*cis*-carveol	1228	1226	1229					tr		b,c
52	thymol, methyl ether	1234	1232	1235			tr			0.1±0.0	b,c
53	cumin aldehyde	1235	1238	1235		0.1±0.0	tr		tr		b,c
54	carvacrol, methyl ether	1243	1242	1244			0.1±0.0			0.1±0.0	b,c
55	carvone	1241	1239	1241	tr	tr		tr	0.7±0.1	tr	a,b,c
56	methylcamphenoate	1248		1250			0.2±0.0			3.2±0.7	b,c
57	bornyl acetate	1282	1287	1281	13.7±2.5	11.4±2.4	tr	13.4±2.8	12.2±2.0	0.2±0.0	a,b,c
58	lavandulyl acetate	1293	1288	1292		tr					b,c
59	carvacrol	1302	1298	1302	tr	0.1±0.0	tr		tr	tr	a,b,c
60	silphiperfol-5-ene	1325	1326	1330			0.1±0.0				b,c
61	methyl decanoate	1327	1323	1328			tr				b,c

62	δ-elemene	1332	1335		0.1±0.0	0.1±0.0	0.3±0.1	tr		tr	b,c
63	7-*epi*-silphiperfol-5-ene	1343	1345	1348			0.1±0.0				b,c
64	α-cubebene	1344	1345	1345	0.1±0.0	0.1±0.0	0.4±0.1	tr	tr	tr	b,c
65	eugenol	1355	1356	1355	tr	tr		tr	tr		a,b,c
66	α-ylangene	1363	1373	1364	0.1±0.0	tr	0.2±0.0				b,c
67	α-copaene	1368	1376	1367	0.1±0.0	0.1±0.0	0.4±0.1	0.1±0.0	0.1±0.0		a,b,c
68	modheph-2-ene	1374	1383	1376						0.2±0.0	b,c
69	α-isocomene	1376	1387	1376			tr				b,c
70	β-bourbonene	1376	1387	1376	0.2±0.0	0.1±0.0		0.3±0.1	0.1±0.0		b,c
71	β-cubebene	1383	1387	1383	0.1±0.0	tr	0.2±0.0	0.1±0.0	tr		b,c
72	β-elemene	1386	1389	1387	0.6±0.2	0.2±0.0	0.1±0.0	1.7±0.4	0.7±0.2	3.4±0.7	a,b,c
73	α-gurjunene	1400	1409	1400	2.6±0.5	2.3±0.4	3.0±0.7	1.3±0.3	1.3±0.4	0.2±0.0	a,b,c
74	(*E*)-caryophyllene	1409	1417	1412	0.5±0.1	0.8±0.2	1.1±0.2	0.8±0.2	0.2±0.0	0.1±0.0	a,b,c
75	β-copaene	1420	1430	1424	0.2±0.0	0.2±0.0	0.4±0.1	0.2±0.0	tr		b,c
76	α-*trans*-bergamotene	1431	1432		0.1±0.0	0.1±0.0	0.5±0.1	tr	tr	tr	b,c
77	6,9-guaiadiene	1436	1442		tr		tr	tr			b,c
78	α-humulene	1443	1452	1444	0.2±0.0	0.1±0.0	0.2±0.0	0.3±0.0	0.1±0.0	tr	a,b,c
79	geranyl acetone	1453	1453	1453	0.1±0.0			0.1±0.0			b,c
80	*cis*-cadina-1(6),4-diene	1453	1461			0.2±0.0					b,c
81	γ-gurjunene	1463	1475	1465	1.3±0.3	0.9±0.2	0.6±0.2	0.6±0.2	0.6±0.2		b,c
82	γ-muurolene	1470	1478	1469	1.2±0.2	0.8±0.2	0.1±0.0			0.1±0.0	b,c
83	germacrene D	1472	1484	1473	6.3±1.1	9.0±1.6	14.4±2.8	11.0±2.2	1.0±0.2	3.9±0.8	b,c
84	β-selinene	1476	1489	1476	0.4±0.1	0.4±0.1	0.4±0.1	0.8±0.0	1.6±0.3	0.4±0.1	b,c
85	*epi*-cubebol	1487	1493	1488				0.2±0.0			b,c
86	α-selinene	1487	1498	1488	0.2±0.0	0.1±0.0	0.3±0.0			0.1±0.0	b,c
87	bicyclogermacrene	1488	1500	1490	0.7±0.2	0.5±0.1	1.2±0.3				b,c
88	α-muurolene	1494	1500	1494	0.2±0.0	0.1±0.0	0.3±0.1	0.1±0.0	tr		b,c
89	δ-amorphene	1500	1511		0.1±0.0	0.1±0.0	0.1±0.0				b,c
90	β-bisabolene	1505	1505	1505					tr		b,c
91	γ-cadinene	1506	1513	1507	0.7±0.2	1.2±0.3	1.5±0.3	0.1±0.0		0.1±0.0	b,c
92	*trans*-calamenene	1517	1521	1517	1.5±0.3	0.1±0.0	0.2±0.0			0.1±0.0	b,c
93	δ-cadinene	1518	1522	1520	1.5±0.4	1.2±0.3	2.6±0.5	0.3±0.1	0.2±0.0	0.2±0.0	b,c

94	β-sesquiphellandrene	1519	1521	1520						0.1±0.0	b,c
95	*trans*-cadina-1,4-diene	1524	1533	1525	tr		tr				b,c
96	α-cadinene	1530	1537	1533	tr	0.2±0.0	0.1±0.0				b,c
97	α-calacorene	1534	1542	1535	0.4±0.1	0.3±0.1	0.2±0.0	0.1±0.0	0.1±0.0	tr	b,c
98	*epi*-torilenol[g]	1546	1546		1.7±0.3	2.1±0.3	1.3±0.3	2.1±0.4	0.5±0.1		b,c
99	epoxysalvial-4(14)-ene	1556		1557	4.1±0.7	3.0±0.6	1.5±0.3	1.8±0.4	2.5±0.5	0.1±0.0	b,c
100	β-calacorene	1556	1564	1555			0.2±0.0				b,c
101	(*E*)-nerolidol	1562	1561	1562	0.3±0.0	0.1±0.0		0.3±0.0	0.1±0.0		a,b,c
102	spathulenol	1568	1576	1570	4.3±0.8	3.4±0.7	4.6±0.9	2.1±0.4	1.1±0.3	tr	b,c
103	caryophyllene oxide	1571	1583	1571	0.9±0.2	1.8±0.4	0.6±0.2	2.1±0.5	2.1±0.5		a,b,c
104	eudesm-4(15)-en-1-one[g]	1575	1574		0.7±0.1	1.0±0.2	0.6±0.1	0.6±0.1	0.1±0.0		b,c
105	viridiflorol	1581	1592	1583	0.4±0.1	0.2±0.0	2.0±0.4	0.8±0.2	0.4±0.1	tr	a,b,c
106	salvial-4(14)-en-1-one	1583	1594	1584	2.0±0.4	1.4±0.3	1.5±0.3	3.0±0.6	0.7±0.1	0.3±0.0	b,c
107	ledol	1592	1602	1593	2.9±0.6	1.7±0.4	0.5±0.1	2.6±0.5	1.1±0.3	0.1±0.0	b,c
108	rosifoliol	1597	1600	1595			0.5±0.1			0.2±0.0	b,c
109	humulene epoxide II	1597	1608	1596	1.3±0.3	1.3±0.3		1.7±0.4	0.7±0.1		a,b,c
110	torilenol[g]	1601	1599		0.5±0.1	2.5±0.4	1.4±0.3	4.1±0.7	0.6±0.1	0.1±0.0	b,c
111	isospathulenol	1621	1622	1621	3.0±0.6	2.8±0.6	3.6±0.7	2.4±0.5	0.8±0.2	0.3±0.1	b,c
112	*epi*-α-cadinol	1633	1640	1633	0.3±0.1	0.2±0.0	0.2±0.0	0.1±0.0		tr	b,c
113	*epi*-α-muurolol	1634	1640	1635	0.3±0.0	0.2±0.0	0.2±0.0	0.1±0.0		tr	b,c
114	opposita-4(15),7(11)-dien-1β-ol[g]	1636	1633		1.4±0.3	1.2±0.3	0.8±0.2	2.1±0.4	0.3±0.0	0.1±0.0	b,c
115	α-cadinol	1646	1652	1646	0.7±0.2	0.6±0.1	0.8±0.2			0.3±0.1	a,b,c
116	cadalene	1666	1675	1665	0.2±0.0	0.2±0.0	0.3±0.0			0.1±0.0	b,c
117	eudesma-4(15),7-dien-1β-ol	1676	1687	1674	4.4±0.9	4.6±0.9	2.8±0.6	7.1±1.5	1.2±0.3	0.3±0.0	b,c
118	10-nor-calamenen-10-one	1692		1702	0.1±0.0	0.1±0.0					b,c
119	cyclocolorenone	1748		1748	15.6±3.1	6.4±1.3	0.2±0.0	8.8±1.6	2.9±0.6±0.0	0.2±0.0	b,c
120	phytone	1844		1845	tr	0.4±0.1		tr	0.1		b,c
121	kaurene	2028	2025	2030			0.3±0.1				b,c
	Oil yield (%)				0.16	0.15	0.06	0.20	0.18	0.04	
	Total identified (%)				83.3	88.1	83.5	85.5	94.3	96.2	

Grouped compounds (%)						
Monoterpene hydrocarbons	3.5	16.0	12.9	8.3	42.3	74.0
Oxygenated monoterpenes	15.1	17.7	1.2	17.2	30.8	6.2
Sesquitepene hydrocarbons	19.5	19.1	29.2	17.9	5.9	9.0
Oxygenated sesquiterpenes	45.1	34.5	23.1	42.1	15.2	2.0
Others	0.1	0.5	17.4	tr	0.1	5.0

[a] Compounds are listed in order of their elution from a HP-5MS column. [b] Linear retention index on HP-5MS column, calculated according to Van den Dool and Kratz equation (1963) using homologous series of C_8-C_{30} alkanes. [c] Linear retention index taken from Adams (2007) and NIST 17 (2017). [d] Relative percentage values are means of three determinations ± SD. [e] Identification methods: a, based on comparison with authentic compounds; b, based on comparison with WILEY, ADAMS, FFNSC2 and NIST 17 MS databases; c, based on comparison of calculated RI with those reported in ADAMS, FFNSC 2 and NIST 17. [f] tr, % below 0.1%. [g] RI and MS taken from Kalemba et al. (2001)

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Table 2. Acute toxicity of the essential oils from various plant parts of *Solidago canadensis* and *Solidago gigantea* on *Culex quinquefasciatus* 3rd instar larvae.

Treatment	Mortality at 100 µl.L^{-1}	LC_{50} µl.L^{-1}	CI95	LC_{90} µl.L^{-1}	CI95	Chi square
Solidago gigantea flowers	28.0±3.3	ND	-	-	-	-
Solidago gigantea leaves	44.8±3.3	ND	-	-	-	-
Solidago gigantea roots	22.0±2.3	ND	-	-	-	-
Solidago canadensis flowers	38.9±2.8	ND	-	-	-	-
Solidago canadensis leaves	61.0±8.2	89.3	72.9-92.3	189.6	172.3-199.8	3.256 ns
Negative control	0.0±0.0	-	-	-	-	-
Positive control, α-cypermethrin	100.0±0.0	0.0005	0.0003-0.0007	0.0018	0.0009-0.0023	2.756 ns

ND = not determined.

ns = not significant (P>0.05).

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Table 3. Acute toxicity of the essential oils from various plant parts of *Solidago canadensis* and *Solidago gigantea* on *Musca domestica* adult females.

Treatment	Mortality at 200 µg.adult^{-1}	LC_{50} µg.adult^{-1}	CI95	LC_{90} µg.adult^{-1}	CI95	Chi square
Solidago gigantea flowers	32.5±2.5	ND	-	-	-	-
Solidago gigantea leaves	42.5±7.5	ND	-	-	-	-
Solidago gigantea roots	30.0±0.0	ND	-	-	-	-
Solidago canadensis flowers	67.5±12.5	207.1	191.3-226.2	355.6	310.1-369.8	1.718 ns
Solidago canadensis leaves	57.8±12.5	206.9	187.5-232.4	426.4	401.8-471.5	5.246 ns
Negative control	0.0±0.0	-	-	-	-	-
Positive control, *α*-cypermethrin	100.0±0.0	0.19	0.16-0.35	0.85	0.78-1.15	3.121 ns

ND = not determined.

ns = not significant (P>0.05).

Click here to download Table R2 Table 4.docx

Table 4. Acute toxicity of the essential oils from various plant parts of *Solidago canadensis* and *Solidago gigantea* on *Spodoptera littoralis* 3rd instar larvae.

Treatment	Mortality at 150 µg.larva^{-1}	LC_{50} µg.larva^{-1}	CI95	LC_{90} µg.larva^{-1}	CI95	Chi square
Solidago gigantea flowers	60.0±8.2	107.4	94.6-118.9	264.6	173-7-316.1	0.044 ns
Solidago gigantea leaves	93.5±2.5	84.5	72.9-89.5	149.4	122.7-178.5	1.787 ns
Solidago gigantea roots	40.8±8.2	ND	-	-	-	-
Solidago canadensis flowers	33.3±12.5	ND	-	-	-	-
Solidago canadensis leaves	73.3±2.5	98.9	83.4-124.1	200.4	180.4-256.7	2.517 ns
Negative control	0.0±0.0	-	-	-	-	-
Positive control, α-cypermethrin	-	0.0032	0.0022-0-0039	0.0082	0.0057-0.0105	2.482 ns

ND = not determined.

ns = not significant (P>0.05).

Click here to download Table R2 Table 5.docx

Table 5. Toxicity of the essential oils extracted from *Solidago canadensis* and *Solidago gigantea* leaves, and α-cypermethrin on *Eisenia fetida* earthworms. Herein, the *S. canadensis* leaf essential oil was the only tested product effective against the three selected tested pests, therefore it was selected for non-target tests, along with the leaf essential oil from *S. gigantea*.

Concentration ($mg.kg^{-1}$)	7^{th} day* (%±SD)	14^{th} day* (%±SD)
Solidago canadensis 200.0	$0.0±0.0^{a}$	$0.0±0.0^{b}$
Solidago gigantea 200.0	$0.0±0.0^{a}$	$0.0±0.0^{a}$
α-Cypermethrin 50.0	$100.0±0.0^{c}$	$100.0±0.0^{c}$
α-Cypermethrin 25.0	$100.0±0.0^{c}$	$100.0±0.0^{c}$
α-Cypermethrin 12.5	$75.5±2.5^{b}$	$95.5±2.5^{b}$
Control	$0.0±0.0^{a}$	$5.0±2.5^{a}$
ANOVA $F_{5,18}$, P	358.15; 0.001	459.22; 0.001

* *E. fetida* mortality (±SD) achieved on the 7^{th} and 14^{th} day post-application of *Solidago canadensis* and *Solidago gigantea* essential oils. Numbers within a column follower by the same letter do not differ significantly according to ANOVA, Tukey's HSD test at $P<0.05$.