Effect of Salinity on the Antiparasitic Activity of Hyssop Essential Oil

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ABSTRACT

The present work reports the effect of salt treatment on *Hyssopus officinalis* growth, essential oil (EO) yield and composition and the antiparasitic effects of the oil against plant (*Phytomonas davidi* and *Meloidogyne javanica*) and human (*Trypanosoma cruzi*) parasites. The plants were grown in hydroponics and treated with different NaCl concentrations (0, 50, 75, and 100 mM NaCl).

Our results showed that salinity decreased plant growth and EO yield of hydroponically cultivated plants of *H. officinalis* with increasing NaCl levels. Salinity also induced changes in the chemical composition of treated hyssop EOs, decreasing the monoterpene and increasing the sesquiterpene groups. The oxygenated monoterpenes, represented primarily by isopinocampone and pinocarvone, were the major chemical class. The EOs from treated *H. officinalis* showed moderate antiparasitic effects against *T. cruzi*, independent of the salt treatment, and stronger salt-dependent effects on *P. davidi*, with the 50 mM NaCl treatment resulting in the strongest phytomonacidal effect. The hyssop EOs tested here were strong nematocidal agents against *M. javanica*, these effects being also stronger for the 50 mM NaCl treatment. Therefore, a moderate salt treatment (50 mM NaCl) reduced plant growth and EO yield but increased its biocidal effects on plant parasites (*P. davidi* and *M. javanica*).

KEYWORDS: Hyssop (*Hyssopus officinalis* L.); Salt stress; Essential oil; *Trypanosoma cruzi*; *Phytomonas davidi*; *Meloidogyne javanica*.

ABBREVIATIONS: AMPs (Aromatic and medicinal plants), EOs (Essential oils), WHO (World health organization), BZ (Benznidazole), NFX (Nifurtimox), GC–MS (Gas chromatography–mass spectrometry), LIT (liver infusion tryptose), FCS (fetal calf serum), GI

(growth inhibition), PMS/MTT (phenazine methosulfate / methylthiazolyldiphenyl-tetrazolium bromide), RH (relative humidity), SDS (sodium dodecyl sulfate).

Introduction

Aromatic and medicinal plants (AMPs) are gaining interest due to the increasing awareness of the harm induced by synthetic chemicals to both human health and environment (1). This prompted many industries to focus on the use of plant bio products as a safe alternative to chemicals (1). Essential oils (EOs) are secondary metabolites synthesized by different organs of APMs with diverse biological activities (2). EOs are considered environmentally friendly products because of their biodegradability, strong efficiency and low level of toxicity to mammals (3). Moreover, in agriculture they can ensure the protection of plants against different diseases and pests without accumulating in the environment and with a lower risk of selection of resistant pathogen strains and pests (3).

Hyssopus officinalis (L.) (*Lamiaceae*) is a perennial herb of Mediterranean origin. It is a well-known medical and culinary herb with therapeutic properties such as spasmolytic, antiviral, sudorific, emmenagogue, carminative, tonic, diuretic, expectorant, antiseptic, antibacterial, antioxidant, anti-inflammatory, cardiovascular and anthelmintic (4,5). There are numerous reports on the chemical composition of *H. officinalis* essential oil (EO), with isopinocampone, pinocampone, and pinenes the most characteristic constituents. However, important qualitative differences in hyssop EO composition due to geographic origin and variety have been reported (6).

Selected hyssop accessions are commercially cultivated. The crop can be productive for approximately 10 years and the plants are preferably harvested during blooming season to produce EOs by steam distillation with yields around 0.5% (6). Furthermore, this plant can grow in semi-arid climatic conditions (7).

67 Drought and salinity are major environmental factors that influence crop productivity
68 worldwide (8, 9). Drought and salinity stress have been shown to trigger various interacting
69 events including the inhibition of enzyme activities in metabolic pathways (10, 11). Drought
70 and saline stress cause changes in essential oil yield and quantitative or qualitative
71 composition of aromatic plants (12, 13, 14, 15 16, 17-18), including *H. officinalis* (19).

72 The increasing demand for bio pesticides, safer antibiotics and drugs (nontoxic to humans or
73 environment and easily biodegradable) have resulted in new medicinal and agricultural
74 applications of hyssop EOs based on their effects on human resistant pathogenic bacteria,
75 food-borne fungal pathogens, insect pests and phytopathogenic fungi (6, 20, 21, 22, 23, 24-
76 25). However, despite its traditional use as an anthelmintic (5), little is known on the
77 antiparasitic effects on hyssop EO except for one record on an EO from *H. officinalis* grown
78 in Spain reported as trypanocidal (26), with moderate effects on the plant parasitic nematode
79 *Meloidogyne javanica* (27).

80 Parasites belonging to *Trypanosomatidae* family are agents of human, animal and plant
81 diseases (28). The genus *Phytomonas* includes all trypanosomatids that parasitise plants (29).
82 About 17 families of plants are infected by these flagellated protozoans which are transmitted
83 through hemipteran insects. They have been detected in latex tubes of laticifers, in phloem of
84 trees, in fruits and also in seeds (30). The most important plant diseases caused by
85 *Phytomonas* are coffee phloem necrosis, oil palm marchitez and coconut hartrot (31).
86 Currently, there are no reports on the effects of EOs or any other plant metabolite on
87 *Phytomonas*. *Trypanosoma cruzi* is a protozoan parasite responsible of Chagas disease which
88 is one of the most serious health problems in Latin America. This pathogenic agent is mostly
89 transmitted to humans by triatomine insects (kissing bugs). Currently, the treatment of this
90 disease is mainly based on two drugs: Benznidazole (BZ) and Nifurtimox (NFX) (32),

however, both have many disadvantages (33). Therefore, the search for more safe, reliable and efficient drugs is necessary.

Root-knot nematodes (*Meloidogyne* spp.) are major threats to agriculture worldwide. In the last decades, environmental and human health concerns have steadily reduced the availability of efficient commercial nematicides. In this context, EOs from AMPs have been proposed as an environmentally friendly alternative to synthetic nematicidal agents (27). Given the potential applications of hyssop EO in the health and agro-food industry and the plant production constraints that saline stress can induce in commercial hyssop, in this report we examine: i) the effect of NaCl concentrations (0, 50, 75 and 100 mM) on EO yield and composition of aerial parts (stems and leaves) of *Hyssopus officinalis* L. plants, from a French population, during vegetative stage and ii) the antiparasitic effects of these EOs against plant (*Meloidogyne javanica* and *Phytophthora davidi*) and human (*Trypanosoma cruzi*) parasites.

Experimental

Plant material and hydroponic cultivation

Hyssopus officinalis L. seeds (organic products certified by ECOCERT FR-BIO-01) were provided by “Graines del País” (Bellegarde-du-Razès, France). First, the seeds were washed thoroughly with distilled water, then placed in Petri dishes, and finally, put for germination in the dark at 25°C. After 7 days, in a controlled growth chamber, seedlings were transplanted into 6-L aerated pots (10 seedling per pot) containing an eightfold diluted nutrient solution of Hoagland and Arnon (34). Four weeks after transplanting, an initial harvest was performed (6 plants) and four NaCl concentrations were applied (0, 50, 75 and 100 mM NaCl). All pots were placed under two luminous ceilings (day/night temperature 23/17°C; relative humidity 80/60%; 16 h photoperiod with 200- $\mu\text{mol.m}^{-2} \text{s}^{-1}$ PAR at the plant level).

Dry weight and water content

After 14 days of treatment, a final harvest was performed and plants were collected and randomly selected (6 plants) from each treatment. Aerial plant parts (leaves and stems) were oven dried (60°C, 72h) to calculate their dry weight (DW) and water content (WC, ml g⁻¹ DW) with the equation: $WC = (FW - DW)/DW$, where FW: fresh weight and DW: dry weight. The rest of the plants were dried at room temperature in an aerated dark environment for essential oil analysis.

Sodium (Na⁺) and potassium (K⁺) analysis

Na⁺ and K⁺ were extracted from 25 mg of oven dried aerial parts using 0.5% HNO₃ and then measured by flame emission photometry (Jenway PFP7) (35).

Essential oil extraction

Air- dried aerial parts were reduced to small pieces, then subjected to hydrodistillation (40 g of each sample) using a Clevenger apparatus for 2 hours (36). The oils were dried over sodium sulfate (Na₂SO₄) and stored in amber glass vials at 4°C until the beginning of analyzes.

Essential oil analysis

Analysis of the studied hyssop EO was carried out using a gas chromatograph Shimadzu, GC-2010 equipped with an auto sampler (auto injector AOC-20i), a FID detector and interfaced with a mass spectrometer QP2010 Ultra and a TRB-5 capillary column (30 m × 0.25 mm x 0.25 µm film thickness). Working conditions were as follows: split ratio (20:1), injector temperature, 300 (260) °C; temperature of the transfer line connected to the mass spectrometer, 220 (280) °C; initial column temperature 70°C, then heated to 290 (270) °C at 6 °C min⁻¹ for 15 min. Scan time and mass range were 0.2 s and 35–450 m/z respectively. The chemical constituents were identified by comparing their relative retention times and mass spectra with those of Wiley 229 library data of the GC–MS system and literature data if needed (37).

In Vitro assay on Trypanosoma cruzi and Phytomonas davidi

The Y strain of *Trypanosoma cruzi* was originally isolated from a human infection (38), and it has been kept in our laboratory, either in culture or frozen in liquid nitrogen (38).

Phytomonas davidi ATCC® 30287™, isolated from *Euphorbia heterophylla* (39), was purchased from the American Type Culture Collection (39).

The bioassays were carried out with epimastigote forms of *T. cruzi*, or promastigotes of *P. davidi* cultured in LIT medium supplemented with 10% heat-inactivated foetal calf serum as described for *T. cruzi* (40). Parasite cultures in logarithmic growth phase ($8-10 \times 10^6$ epimastigotes/mL or $6-7 \times 10^6$ promastigotes/mL) were distributed (90 µL/well) in 96-well flat-bottom plates.

EOs were tested for 72 h at several concentrations (800, 400, 200, 100, 10 and 1 µg/mL) for *T. cruzi* using Nifurtimox as the reference drug. Parasite viability was analyzed by a modified MTT colorimetric assay method (40). After 72 h, 10 µL MTT/PMS were added to each well and incubated for 75 min. For *P. davidi*, EOs were tested for 24 h at the same concentrations (800, 400, 200, 100, 10 and 1 µg/mL). No reference drug was tested because there is no one described for it. After 24 h, 10 µL MTT/menadione (5 mg/mL MTT, 1.6 mg/mL menadione) were added to each well and incubated for 75 min. From here the process was the same for both parasites, 100 µL sodium dodecyl sulfate (SDS) were added to dissolve formazan crystals obtained as a result of the reduction of MTT. After 15-30 min the absorbance was measured at 630 nm. The activity was calculated as percentage growth inhibition (%GI) as follows: $\%GI = 100 - [(At - Ab)/(Ac - Ab)] \times 100$, where At is the absorbance of treated wells, Ac the absorbance of control wells (not treated) and Ab the absorbance of blank wells (culture medium and vehicle only). All assays were carried out in triplicate and were repeated at least three times independently to confirm the results.

In vitro nematocidal effects

The nematodes used for the experiments came from a population of *Meloidogyne javanica* maintained on *Solanum lycopersicum* plants (var. Marmande) in pot cultures at 25 °C, and 70% RH. Egg masses were handpicked from infected tomato roots and second-stage juveniles (J2) were obtained from hatched eggs by incubating handpicked egg masses in a water suspension at 25 °C for 24 h.

The bioassays were carried out as described by Andres et al. (27). The treatments were prepared with EOs diluted in a DMSO-Tween solution (0.5% Tween20 in DMSO). The test solutions (5 µL) were added to of 96-well plates containing 100 nematodes in 95 µL of water per well. The initial concentration tested was 1 mg/mL. Four control wells with water/DMSO-Tween were included in each experiment. All treatments were replicated four times. The plates were covered to prevent evaporation and maintained in darkness at 25 °C. After 72 h, the dead J2 were counted. The nematicidal activity is presented as percent dead J2 corrected according to Schneider-Orelli's formula (41).

Statistics

Data are expressed as mean $\pm$ standard error. The means were compared using the one-way and multivariate analysis of variance (ANOVA) followed by Duncan's multiple range tests. The differences between individual means were deemed to be significant at $P < 0.05$. (Statistica v 5.1" software) (42).

Results and discussion

Effect of salinity on aerial parts growth, water content and ion accumulation

Our results showed that after 2 weeks, salt reduced significantly hyssop plant growth (reduction was about 41, 64 and 67 %, respectively, at 50, 75 and 100 mM NaCl) (Figure 1a). Additionally, Na⁺ content increased within hyssop aerial parts along with NaCl doses to reach 0.288% DW at 100 mM NaCl (Figure 2a). The water (Figure 1b) and K⁺ (Figure 2b) content in the plant increased significantly with respect to the control at 50 mM NaCl. Salt stress

related reduction in plant growth has been previously reported for *H. officinalis* (19). Several authors noted that the salt-induced growth decrease may be linked to osmotic effects, which affect cell turgor and expansion (43). In our case, the reduction of aerial parts growth was not due to an osmotic effect since their water content was not affected under salinity (Figure 1b) suggesting therefore, that plants subjected to salt stress are able to compartmentalize excessive Na⁺ ions into the vacuoles thus contributing to cell osmotic adjustment (44). Several authors found that K⁺ content was higher in salt tolerant plants than in sensitive ones (45, 46). Furthermore, an increase in K⁺ concentrations under high NaCl levels in broad bean and pea plants resulted in an increase in tissue water content (47). Therefore, the increased K⁺ accumulation at 50 mM NaCl could facilitate osmotic adjustment in hyssop plants.

Effect of salinity on essential oil yield and composition

The hydrodistillation of *H. officinalis* aerial parts (control) gave EO with an average yield of 0.48%. The exposure of hyssop plants to increasing NaCl concentrations strongly decreased EO yield (reduction was about 52, 69 and 75%, respectively, at 50, 75 and 100 mM NaCl) (Figure 3).

Average EO yields reported for hyssop range between 0.18- 2.0% (48, 19). Most studies on hyssop EO yields have been carried out on flowering plants, while plants in vegetative stage have been used here. Phenological growth stage and cultivation conditions affect hyssop EO yield. For example, EO yields were the highest in flowering stage (49, 7, 50) and increased with plant age (51), fertilization (52), irrigation (53) and temperature (54).

Contrary to our results, salt stress increased EO yield in field cultivated hyssop plants collected at flowering stage (19). However, in other aromatic species such as *Melissa officinalis* (55), *Mentha x piperita*, *Mentha pulegium*, *M. suaveolens* (56) and *Carthamus tinctorius* (57), EO production was also negatively affected by salt stress. Such decrease in EO content could be due to the degradation of the secretory structures following leaf necrosis

(58). Moreover, in many *Lamiaceae* plants salinity reduced EO yield presumably by inhibiting the supply of cytokinin from roots to shoots and thus altering the ratio between leaf cytokinin and abscisic acid (59).

The analysis of the chemical composition of hyssop EO resulted in the identification of 18 compounds (Table 1). The major components were isopinocampone (26.62-27.70%), pinocarvone (12.45-15.99%), followed by elemol (12.25-14.41%), β -pinene (5.07-8.16%) and germacrene D (7.22-8.12%). Overall, these EOs were composed of oxygenated monoterpenes (40.13-44.18%), sesquiterpene hydrocarbons (18.23-22.11%), monoterpene hydrocarbons (12.33-16.61%) and oxygenated sesquiterpenes (12.25-14.41%). The chemical variations in EO composition were only quantitative. The variance analysis showed that the relative abundance of EO components was affected by salt stress ($P < 0.001$) except isopinocampone ($P < 0.01$) and β -cubebene ($P < 0.05$). Overall, sesquiterpenes (hydrocarbons and oxygenated) increased with salinity; in fact the increase was about 21% at 100 mM NaCl and 18% at 75 mM NaCl. However, monoterpenes (hydrocarbons and oxygenated) decreased with increasing NaCl levels; the reduction was about 26 and 9%, respectively, at 100 mM NaCl (Table 1).

The most characteristic constituents of *H. officinalis* EO are isopinocampone, pinocampone, and pinenes (6). However, other hyssop chemotypes have been described containing 1,8-cineole and β -pinene as major components (25, 60, 48), linalool, 1,8-cineole and limonene (61, 62), linalool and methyl eugenol (63), myrtenol and β -pinene, β -pinene and 1,8-cineole (64), myrtenyl acetate and camphor (4). A similar EO composition to the one described here, with pinocarvone and pinocampone, has been found for different accessions of *H. officinalis* from Turkey (65, 66) and Yugoslavia (67).

The reported effects of salt stress on EO quantitative composition are variable. For example, decreased monoterpenes (hydrocarbon and oxygenated) and increased sesquiterpene hydrocarbons have been reported for marjoram EO (68). In black cumin, monoterpenes

(hydrocarbon and oxygenated) and sesquiterpene hydrocarbons increased in seed EO with saline irrigation water levels (69). Furthermore, saline water affected the quantitative composition of hyssop EO, collected at flowering stage, with *cis*-pinocamphone and β -pinene being the main components and increased the oxygenated monoterpenes and monoterpene hydrocarbons (at 6 and 8 dSm⁻¹ saline water treatments, respectively) (19).

Effect of salinity on the antiparasitic activity of Hyssopus officinalis L. essential oil

The *in vitro* antiprotozoal activity of hyssop EOs is shown in Table 2. All EOs tested were active on *T. cruzi* and *P. davidi* at 200 μ g/mL (100 % inhibition for 800 and 400 μ g/mL, data not shown), there was a reduction in activity at 100 μ g/mL (43-54% growth inhibition) for *T. cruzi*, independent of the salt treatment. In the case of *P. davidi*, the effects of hyssop EO at 100 μ g/mL varied with the salt treatment, with the EOs from the 50 and 100 mM NaCl treatments the most active (97 and 78 % inhibition, respectively), followed by the 75 and 0 mM NaCl EOs (58 and 39 % inhibition).

Guardo et al. (26) reported moderate trypanocidal effects of an *H. officinalis* EO from a Spanish hyssop population on *T. cruzi* with activity levels similar to the ones found here. However, the main EO components were 1,8-cineole and β -pinene which were not active against the parasite, suggesting synergistic effects between the EO components. This is the first report on the phytomonocidal effects of *H. officinalis* EO.

Neither of the major components of the hyssop EO, pinocarvone or isopinocampheol, described here have been reported as being trypanocidal or antiprotozoal. Among other hyssop EO components, α -pinene showed a moderate effect on *T. cruzi* (70) and strong effect on *T. brucei*, while α -phellandrene showed a moderate effect (71). β -Pinene was active against *T. cruzi* and showed synergistic effect with carvacrol (26). Germacrene D inhibited the enzyme cruzain, a cysteine protease from *T. cruzi* (72, 73) and this compound showed moderate activity against trypomastigote forms (74). Therefore, the antitrypanosomal and

phytomonacidal effects observed for hyssop EOs could be related to the presence of these trypanocidal compounds.

Many plant essential oils are known to exhibit nematocidal effect (75). Recently, biocidal (nematicidal, ixodicidal, phytotoxic and insecticidal) activities of hyssop EOs have been reported (76, 77). In our study, the oils showed strong nematocidal activity that negatively correlated with saline treatment. EOs of the control and the 50 mM NaCl treatment show the most nematocidal effects at 1 µg/µL (Table 3).

Previous reports showed a low nematocidal activity for the EO of Spanish *H. officinalis* containing 1,8-cineole and β-pinene as the major components (27). Therefore, this is the first report on the nematocidal effects of hyssop EO containing pinocarvone and isopinocampone and on the negative effect of NaCl treatments such an effect. Neither pinocarvone or isopinocampone, have been reported as being nematocidal while β-pinene was inactive against *M. javanica* (27). Among other hyssop EO components, α-pinene strongly inhibited an acetylcholinesterase from the pinewood nematode *Bursaphelenchus xylophilus* (78), elemol showed *in vitro* larvicidal effects on *Anisakis simplex* (79).

Conclusion

Our results showed that salt stress decreased plant growth and EO yield of hydroponically cultivated plants of *H. officinalis*. Salinity also induced quantitative changes in the chemical composition of treated hyssop EOs, decreasing the monoterpene and increasing the sesquiterpene groups. The oxygenated monoterpenes, represented primarily by isopinocampone and pinocarvone, were the major chemical class. The EOs from treated *H. officinalis* showed moderate antiparasitic effects against *T. cruzi* with independence of the salt treatment and stronger salt-dependent effects on the plant parasite *P. davidi*, with the 50 mM NaCl treatment resulting in the strongest phytomonacidal effect. The hyssop EOs tested here were strong nematocidal agents against *M. javanica*, with these effects also stronger for the 50

290 mM NaCl treatment. Therefore, a moderate salt treatment (50 mM NaCl) reduced plant
291 growth and EO yield but increased its biocidal effects on plant parasites (*P. davidi* and *M.*
292 *javanica*).

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297 **Disclosure statement**

298 No potential conflict of interest was reported by the authors.

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Figure 1. Effect of salinity on dry weight (DW) of aerial parts (a) and water content (b) in hyssop plants grown in the presence of 0, 50, 75 and 100 mM NaCl. Bars represent mean values of six replicates $\pm$ standard error. Different letters indicate significant differences according to Duncan's test at $P < 0.05$.

Figure 2. Effect of salinity on sodium (Na⁺) (a) and potassium (K⁺) (b) contents in the aerial parts of hyssop plants grown in the presence of 0, 50, 75 and 100 mM NaCl. Bars represent mean values of six replicates $\pm$ standard error. Different letters indicate significant differences according to Duncan's test at $P < 0.05$.

Figure 3. Effect of salinity on essential oil yield of hyssop aerial parts in the presence of 0, 50, 75 and 100 mM NaCl. Data with different letters are significantly different according to Duncan's test at $P < 0.05$ (means of three replicates $\pm$ standard deviation).

Table 1. Essential oil composition (% peak area) of *Hyssopus officinalis* L. aerial parts under different NaCl concentrations (0, 50, 75 and 100 mM) at vegetative stage.

Compounds†	RRI	NaCl (mM)				d,f	F	P
		0	50	75	100			
α -Pinene	936	0.36 $\pm$ 0.05 ^a	0.24 $\pm$ 0.01 ^b	0.22 $\pm$ 0.00 ^b	0.09 $\pm$ 0.21 ^c	3	19.84	0.000***
Sabinene	973	1.37 $\pm$ 0.07 ^a	1.04 $\pm$ 0.02 ^b	0.97 $\pm$ 0.01 ^c	0.79 $\pm$ 0.01 ^d	3	684.00	0.000***
β -Pinene	978	8.16 $\pm$ 0.42 ^a	6.5 $\pm$ 0.25 ^b	5.93 $\pm$ 0.02 ^c	5.07 $\pm$ 0.09 ^d	3	508.13	0.000***
β -Myrcene	987	1.34 $\pm$ 0.15 ^a	1.00 $\pm$ 0.01 ^b	0.88 $\pm$ 0.01 ^c	0.86 $\pm$ 0.01 ^c	3	164.29	0.000***
β -Phellandrene	1024	3.08 $\pm$ 0.17 ^a	2.67 $\pm$ 0.07 ^b	2.03 $\pm$ 0.00 ^d	2.58 $\pm$ 0.00 ^c	3	386.34	0.000***
Linalool	1086	0.49 $\pm$ 0.09 ^a	0.34 $\pm$ 0.05 ^b	0.34 $\pm$ 0.02 ^b	0.38 $\pm$ 0.09 ^b	3	19.18	0.000***
Neoolloocimene	1113	2.30 $\pm$ 0.14 ^d	2.67 $\pm$ 0.00 ^c	3.00 $\pm$ 0.02 ^a	2.94 $\pm$ 0.01 ^b	3	390.66	0.000***
Pinocarvone	1137	15.99 $\pm$ 0.55 ^a	14.91 $\pm$ 0.40 ^b	13.86 $\pm$ 0.12 ^c	12.45 $\pm$ 0.05 ^d	3	356.00	0.000***
Isopinocampnone	1151	27.70 $\pm$ 1.08 ^a	27.18 $\pm$ 0.73 ^b	26.62 $\pm$ 0.07 ^c	27.30 $\pm$ 0.4 ^{ab}	3	8.43	0.002**
Myrtenyl acetate	1313	4.70 $\pm$ 0.09 ^b	4.66 $\pm$ 0.10 ^b	4.95 $\pm$ 0.05 ^a	4.66 $\pm$ 0.07 ^b	3	57.13	0.000***
Bicycloelemene	1338	2.44 $\pm$ 0.35 ^c	2.76 $\pm$ 0.21 ^b	3.04 $\pm$ 0.02 ^a	2.95 $\pm$ 0.01 ^a	3	31.19	0.000***
β -Cubebene	1390	0.39 $\pm$ 0.06 ^b	0.40 $\pm$ 0.04 ^{ab}	0.42 $\pm$ 0.00 ^a	0.41 $\pm$ 0.01 ^{ab}	3	3.05	0.004*
α -Gurjunene	1413	0.57 $\pm$ 0.02 ^c	0.61 $\pm$ 0.01 ^b	0.68 $\pm$ 0.00 ^a	0.68 $\pm$ 0.00 ^a	3	293.40	0.000***
<i>trans</i> -Caryophyllene	1422	3.24 $\pm$ 0.25 ^c	3.94 $\pm$ 0.05 ^b	3.94 $\pm$ 0.01 ^b	4.49 $\pm$ 0.05 ^a	3	290.63	0.000***
α -Humulene	1455	0.69 $\pm$ 0.15 ^c	0.84 $\pm$ 0.07 ^b	0.87 $\pm$ 0.01 ^b	0.96 $\pm$ 0.02 ^a	3	32.44	0.000***
Germacrene-D	1479	7.22 $\pm$ 0.26 ^c	7.48 $\pm$ 0.09 ^b	8.12 $\pm$ 0.06 ^a	8.06 $\pm$ 0.06 ^a	3	172.80	0.000***
Bicyclogermacrene	1494	3.68 $\pm$ 0.09 ^c	4.14 $\pm$ 0.11 ^b	4.51 $\pm$ 0.07 ^a	4.56 $\pm$ 0.00 ^a	3	31.19	0.000***
Elemol	1541	12.25 $\pm$ 0.95 ^c	13.13 $\pm$ 0.20 ^b	14.41 $\pm$ 0.16 ^a	13.30 $\pm$ 0.09 ^b	3	58.94	0.000***
Grouped compounds								
Monoterpene hydrocarbons		16.61 $\pm$ 0.73 ^a	14.12 $\pm$ 0.37 ^b	13.03 $\pm$ 0.05 ^c	12.33 $\pm$ 0.09 ^d			
Oxygenated monoterpenes		44.18 $\pm$ 1.71 ^a	42.43 $\pm$ 1.08 ^b	40.82 $\pm$ 0.22 ^c	40.13 $\pm$ 0.10 ^c			
Sesquiterpene hydrocarbons		18.23 $\pm$ 1.18 ^d	20.17 $\pm$ 0.16 ^c	21.58 $\pm$ 0.12 ^b	22.11 $\pm$ 0.14 ^a			
Oxygenated sesquiterpenes		12.25 $\pm$ 0.95 ^c	13.13 $\pm$ 0.20 ^b	14.41 $\pm$ 0.16 ^a	13.30 $\pm$ 0.09 ^b			
Other		4.70 $\pm$ 0.09 ^b	4.66 $\pm$ 0.10 ^b	4.95 $\pm$ 0.05 ^a	4.66 $\pm$ 0.07 ^b			
Total identified		95.97 $\pm$ 8.24^a	94.51 $\pm$ 9.67^c	94.79 $\pm$ 10.25^b	92.53 $\pm$ 9.83^d			

† Order of elution in apolar column (TRB-5). Volatile compounds percentages in the same line with different superscript (a–d) are significantly different at P < 0.05; *P < 0.05; ** P < 0.01; *** P < 0.001

Table 2. Activity of *Hyssopus officinalis* L. essential oils (EO Ho0, EO Ho50, EO Ho75 and EO Ho100) against epimastigotes of *Trypanosoma cruzi* and promastigotes of *Phytomonas davidi*. Nifurtimox was included as reference drug.

EO		Concentration (µg/mL)				EC ₅₀ ^a (µg/mL)
		1	10	100	200	
EO Ho0	<i>T. cruzi</i>	0.6 ± 0.1	0.2 ± 0.1	43.1 ± 4.2	100	110.0
	<i>P. davidi</i>	9.2 ± 0.3	30.4 ± 2.9	39.2 ± 4.2	100	100-160
EO Ho50	<i>T. cruzi</i>	0	0	53.6 ± 13.0	100	~100
	<i>P. davidi</i>	0	0	97.1 ± 0.5	100	10-100
EO Ho75	<i>T. cruzi</i>	0	9.7 ± 0.8	43.7 ± 9.0	100	137.1
	<i>P. davidi</i>	0	0	58.0 ± 6.3	100	~100
EO Ho100	<i>T. cruzi</i>	1.5 ± 0.1	0.2 ± 0.1	45.7 ± 2.2	100	164.4
	<i>P. davidi</i>	0	0	78.1 ± 3.0	100	10-100
Nifurtimox	<i>T. cruzi</i>	7.9 ± 0.7	87.9 ± 4.7	100	100	4.7

Data are expressed as percentage of growth inhibition (means of three replicates ± standard error).

EO Ho0: control, EO Ho50: essential oil treated with 50 mM NaCl, EO Ho75: essential oil treated with 75 mM NaCl, EO Ho100: essential oil treated with 100 mM NaCl.

^a Estimated EC₅₀ values (dose at which the growth of the culture is reduced by 50%).

Table 3. Effects of *Hyssopus officinalis* essential oils (EO Ho0: control, EO Ho50: essential oil treated with 50 mM NaCl, EO Ho75: essential oil treated with 75 mM NaCl, EO Ho100: essential oil treated with 100 mM NaCl) on mortality of *Meloidogyne javanica* second stage juveniles (J2).

EO	µg/µl	J2 mortality (%) ^a
Ho0	1	81.79±3.63
	0.5	0.35±1.05
	0.25	0
Ho50	1	88.73±4.61
	0.5	5.44±1.29
	0.25	1.02±1.0
Ho75	1	73.29±4.09
	0.5	2.76±1.71
	0.25	0
Ho100	1	65.71±1.74
	0.5	9.48±1.64
	0.25	0.75±0.82

^a Values (%) are means of four replicates ± standard error, corrected according to Schneider-Orelli's formula (Schneider-Orelli, 1947).

Table 4. Effects of *Hyssopus officinalis* essential oils (EO Ho0: control, EO Ho50: essential oil treated with 50 mM NaCl, EO Ho75: essential oil treated with 75 mM NaCl, EO Ho100: essential oil treated with 100 mM NaCl) on mortality of *Meloidogyne javanica* second stage juveniles (J2).

EO	µg/µl	J2 mortality (%) ^a
Ho0	1	81.79±3.63
	0.5	0.35±1.05
	0.25	0
Ho50	1	88.73±4.61
	0.5	5.44±1.29
	0.25	1.02±1.0
Ho75	1	73.29±4.09
	0.5	2.76±1,71
	0.25	0
Ho100	1	65.71±1.74
	0.5	9.48±1.64
	0.25	0.75±0.82

^a Corrected according to Scheider–Orelli’s formula. Values are means of four replicates.