The objective of this study was the characterization of nematicidal activity and valorization of hydrolate obtained as a byproduct of the semi industrial vapor-pressure essential oil extraction of a domesticated *Artemisia absinthium* population (Teruel, Spain), in comparison with a population (Sierra Nevada, Spain) undergoing the domestication process. Hydrolates from three crops of these two populations showed strong *in vitro* nematicidal effects against the root knot nematode *Meloidogyne javanica* and similar chemical profiles. The hydrolate of the *A. absinthium* population from Teruel strongly suppressed nematode egg hatching (>95%) after 5 days of incubation. *In vivo* tests on tomato seedlings showed a significant reduction in the of root penetration rate of *M. javanica* J2 treated with a hydrolate sublethal concentration (33%). In pot experiments, the hydrolate of *A. absinthium* tested on tomato plants significantly affected the nematode population, the infection frequency and the reproduction rate. The extraction of the organic fraction of the hydrolate gave a nematicidal extract and an inactive aqueous residue. The chemical profile of the active organic extract showed the presence of (5Z)-2,6-dimethyloct-5,7-diene-2,3-diol, previously described in the hydrodistilled essential oil of this *A. absinthium* population, among other compounds. This study demonstrates that the organic fraction of the *A. absinthium* hydrolate is a potential root-nematode control agent. Therefore, the hydrolate byproduct of the semi industrial vapor-pressure essential oil extraction could be a source of new nematicidal products.
The hydrodistilled and semi industrial vapor-pressure essential oils of these A. absinthium populations are characterized by the presence of (-)-cis-epoxycine, (-)-cis-chrysanthenol, linalool and chrysanthenyl acetate (Martín et al., 2011; Julio et al., 2015). These vapor pressure EOs showed antifungal (Julio et al., 2015), leishmanicidal and trypanocidal activities (Gonzalez-Coloma et al., 2012; Martinez-Díaz et al., 2015) and toxic effects against Trichinella spiralis Owen (García-Rodríguez et al., 2015).

The semi industrial vapor pressure extraction of the A. absinthium essential oil generated a water residue (hydrolate) as byproduct. The aim of this study was to determine the nematicidal activity of A. absinthium hydrolate against Meloidogyne javanica (Treub) Chitwood juveniles, egg hatch, J2s host-plant root penetration and effects on nematode reproduction traits in potted tomato. Additionally, we correlated the nematicidal effects with the chemical profile of the extract.

2. Materials and methods

2.1. Plant material

Pre-domesticated plant material (Teruel population): the seeds originated from thujone-free plants of A. absinthium submitted to a previous domestication period of 6 years and individually selected based on their biomass and essential oil yield at the end of the cycle (T1) (Burillo, 2009; Gonzalez-Coloma et al., 2012) to give the domesticated T2 population.

Wild plant material (Sierra Nevada population): the seeds were collected from individuals grown in a greenhouse and originated from a A. absinthium population donated by the Sierra Nevada National Park (Granada) (SN) (Gonzalez-Coloma et al., 2012) to give the pre-domesticated SN1 population.

The seedlings were planted in 2008 in an experimental field located in Ejea de los Caballeros, Zaragoza, Spain. A detailed description of the field has been reported (Burillo, 2009). Briefly, 6 randomized blocks with 6 rows/block and 30 individuals/row were planted for each population. Flowering plants were harvested in 2008, 2009 and 2010 and processed for agronomic evaluation and extraction. For semi industrial vapor pressure extraction, plants harvested from all the blocks of each population were pooled (1080 plants/population) and extracted.

2.2. Extraction and fractionation

Plant material was extracted by vapor pressure in a semi-industrial plant extraction equipped with two 3000 L vessels (Julio et al., 2015). The hydrolate (aqueous phase) was decanted from the essential oil in a separator funnel and filtered. Finally six hydrolates from two A. absinthium populations (T2 and SN1) of three crops harvested in 2008, 2009 and 2010 were obtained.

One hydrolate (T2 2009) was subjected to extraction with activated carbon (activated charcoal granulate, Scharlau), stirred for 10 min and dried (40 °C, 24 h). The dried adsorbed carbon was extracted in a Soxhlet apparatus with ethanol to give the hydrolate organic fraction (OF).

2.3. HPLC-MS analysis

The hydrolates and the organic fraction were analyzed by HPLC-MS on a Shimadzu LC-20AD HPLC coupled to a LCMS-2020 QP mass spectrometer using an electrospray ionization (ESI) interface and a Teknokroma, Mediterranea Sea 18 column (250 × 4.6 mm, 5 µm particle size) with an ACE 3 C18 analytical guard cartridge. The extracts were eluted with methanol (MeOH): 0.1% acetic acid in milli-Q water 38:100% gradient for 45 min, 100% MeOH for 10 min and 100:38% for 13 min at 0.5 mL/min and 15 L/min nitrogen (drying gas for solvent evaporation) flow rates. The electrospray capillary potential was set to +4.50 kV and ESI was conducted in the Full Scan positive mode (m/z = 145–545) with a potential of 1.30 kV and a capillary temperature of 250 °C. Hydrolate (0.25 µg/µL) and (5Z)-2,6-dimethylocta-5,7-diene-2,3-diol (0.05 µg/µL) (from Bailen et al., 2013) stock solutions were dissolved in MeOH for sample injection (10 µL). All the solvents used were HPLC-MS grade.

2.4. Nematicidal activity evaluation

A field-selected M. javanica population from Barcelona, Spain, was maintained on Solanum lycopersicum L. plants (var. Marmande) in pot cultures at 25 ± 1 °C, >70% relative humidity. Egg masses of M. javanica were handpicked from infected tomato roots two months after inoculation of the seedlings. Second-stage juveniles (J2) were obtained by incubating egg masses in a water suspension at 25 °C for 24 h.

2.4.1. In vitro effect on juveniles

The six hydrolates were tested in vitro on juveniles without and with serial dilutions (50%, 33%, 25% and 10%) in water (V/V) to calculate the active minimum concentration. A nematode inoculum (500 J2 in water) was filtered (25 µm) and the nematodes suspended in 500 µL of A. absinthium hydrolate solutions from plant populations T2 and SN1. Four aliquots (100 µL) of the nematode suspension (approximately 100 J2) and controls (water) were placed in 96-well plates (BD Falcon, San Jose, CA, USA). The organic fraction of the hydrolate (T2 2009) was dissolved in water with 5% of DMSO-Tween solution (0.5% Tween 20 in DMSO) at 20 mg/mL, 5 µL of this solution was added to 95 µL of water containing 90–150 nematodes to obtain a treatment final concentration of 1 mg/mL. Treatment was replicated four times. As a control, four wells were treated with the water/DMSO/Tween 20 in the same volume as the tests. The plates were covered to prevent evaporation and were maintained in the dark at 25 °C. After 72 h, the dead J2 were counted under a binocular microscope. J2 mortality was ascertained by transferring some of treated, apparently dead juveniles to distilled water and examining them after 5–6 h for any revival. The nematicidal activity data are presented as percent dead J2s corrected according to Schneider-Orelli’s formula (Schneider-Orelli, 1947). Effective lethal doses (LC50 and LC90) were calculated by Probit Analysis.

2.4.2. In vitro effect on egg hatching

Three egg masses of uniform size were washed with sterilized distilled water and transferred to a 96-well plate containing 400 µL of hydrolate treatments: undiluted (0.45% OF concentration) and 50% diluted (containing 0.225% OF) from A. absinthium population (T2 2009) selected. Egg masses placed in sterilized distilled water were used as controls. Each experiment was replicated 4 times. The plates were covered to prevent evaporation and incubated in darkness at 25 °C. After 5 days the hatched J2s were counted and the test solutions were replaced with sterilized distilled water. The egg masses were monitored for 4 weeks at weekly intervals, until hatching was complete in the control. Relative hatch suppression rate (compared with the controls) were calculated.

2.4.3. Effect on juvenile infection capacity

This bioassay was performed to evaluate the potential effects of low concentrations of hydrolate (sublethal dose) on nematode