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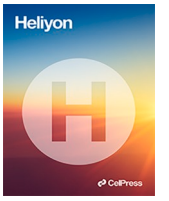

Research article

Evaluation of *Solanum linnaeanum* and *S. sisymbriifolium* extracts for the management of *Meloidogyne chitwoodi*

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ABSTRACT

Meloidogyne chitwoodi causes significant yield losses in many crops and the chemical control measures currently used are less effective for this nematode. The activity of aqueous extracts (0.8 mg/mL) of one-month-old (R1M) and two-months-old roots and immature fruits (F) of *Solanum linnaeanum* (Sl) and *S. sisymbriifolium* cv. Sis 6001 (Ss) were tested on hatching, mortality, infectivity and reproduction of *M. chitwoodi*. The extracts selected reduced the hatching of second-stage juveniles (J2) (cumulative hatching of 40% for Sl R1M and 24% for Ss F) but did not affect J2 mortality. However, infectivity of J2 exposed to the selected extracts, during 4 and 7 days, was lower (3% and 0% for Sl R1M and 0% and 0% for Ss F) compared to the control (23% and 3%). Reproduction was affected only after 7 days of exposure (reproduction factor (RF) was 7 for Sl R1M and 3 for Ss F) compared to the control (RF = 11). The results suggest that the selected *Solanum* extracts are effective and can be a useful tool in sustainable *M. chitwoodi* management. This is the first report on the efficacy of *S. linnaeanum* and *S. sisymbriifolium* extracts against root-knot nematodes.

1. Introduction

Root-knot nematodes (RKN, *Meloidogyne* spp.) are considered among the "top ten" plant parasitic nematodes (PPN) of phytosanitary importance, causing an estimated $100 billion loss/year worldwide and affecting vegetable crops in tropical and subtropical regions of the world [1–4]. They feed on the roots of infected plants and induce abnormal growth of the root systems characterized by galls, which limit the uptake of nutrients and water and inhibit mineral translocation [4]. In addition, they also form disease-complexes with other micro-organisms that increase crop losses [5]. Some species are considered by the European and Mediterranean Plant Protection Organization (EPPO) as quarantine pests interfering with international trade [6,7].

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Meloidogyne chitwoodi, the Columbia RKN, has quarantine status under the EPPO (EPPO A2 list: No. 227) and has already been detected in Portugal, where it causes significant yield losses in potato (*Solanum tuberosum*) and tomato (*S. lycopersicum*), both of which are crucial crops in the country [3,4,8]. When 5% or more of the tubers are affected, the crop is usually unmarketable [9]. Poor cropping practices and climate changes result in increase of nematode populations.

The control measures currently used for suppressing RKN infestation, including clean nematode-free planting material, cultural practices (such as crop rotation), mixed cropping and resistant cultivars, combined with chemical nematicide application, have proved to be less effective than desired against *M. chitwoodi* [10–12]. The high price and the adverse side effects of commercial synthetic pesticides, including impact on the environment, toxicity to non-target organisms (including humans) and the development of resistance in nematode populations, increase the interest in developing pesticides from natural sources [13]. Both the development of chemical pesticides and the development of natural controls for PPN are difficult challenges because the outer surface of nematodes is a poor biochemical target and is impermeable to many organic molecules. Also, the delivery of a toxic compound by an oral route is nearly impossible because most PPN species ingest material only when feeding on plant roots [14]. Several plant species have proved to release natural phytochemicals that have nematicidal properties and that can be found in their root exudates and extracts. The plant root exudates may act as stimulants or inhibitors of second-stage juveniles (J2) hatching [15]. Based on the complex chemical interactions between plants and nematodes and considering that members of the plant kingdom produce a variety of secondary metabolites, several research groups are attempting to develop phytochemical-based strategies for nematode control [13,16]. In recent years, a rich assortment of over 100 different secondary metabolites have been identified as being responsible for plant-mediated nematotoxicity, and various plant-based products have appeared with putative nematicidal activity. Most of these products have not been available long enough to permit satisfactory evaluation by agricultural researchers and rather than acting directly on the nematodes but may limit nematode damage simply by stimulating plant growth [14,17].

The plant secondary metabolites that are crucial for defense and stress responses (e.g., alkaloids, diterpenes, phenols, polyacetylenes, sesquiterpenes and thienyl derivatives) can have nematicidal or nematostatic activity. Some phytochemicals induce immobilization, incapacitation, mortality, or poor penetration of plant roots by the J2 of PPN, thereby reducing the numbers of some RKN species [18,19]. Renčo and collaborators (2014) gathered crucial information about plant secondary metabolites, herbal powders, aqueous extracts, essential oils and green manure that had nematicidal effects against several PPN [20–24].

The plant family Solanaceae contains species of economic importance and many of them contain powerful alkaloids [12]. Some of these compounds, such as the steroidal glycoalkaloids (SGAs), are phytoanticipins that act as the first line of chemical defense against pathogen attacks [25]. Many wild *Solanum* species display resistance to *Meloidogyne* spp. [26–29]. *Solanum sisymbriifolium*, originating from South America, with effects on several PPN species, and *S. linnaeanum*, native to South Africa and present in the South of Portugal, are two wild *Solanum* species [29–35]. The use of *S. sisymbriifolium* as a trap crop and its resistance to PCN is already known, as well as its resistance to *M. chitwoodi* and its antagonistic effects on the development and reproduction of root-lesion nematodes (RLN), *Pratylenchus* spp. [4,26,29,34,36,37]. The alkaloid solasodine, which is present in *S. sisymbriifolium*, confers resistance to some pests and diseases [4]. Recently, nematicidal properties have been recognized in some SGAs present in *S. sisymbriifolium*, demonstrating deleterious effects on *G. pallida* hatch, infection and reproduction [25].

In this context, the present study evaluates the nematicidal potential of two wild *Solanum* plants, *S. linnaeanum* and *S. sisymbriifolium* cv. Sis 6001, against *M. chitwoodi*, in relation to J2 hatching, mortality, infectivity and nematode reproduction. In this way, it assists in the development of sustainable and ecofriendly management methods of key enemies in agriculture, reducing dependence on chemical pesticides and improving crop productivity. Therefore, extracts from these plants with nematicidal activities may provide alternative sources of botanical nematicides. This work aimed to propose alternative and effective tools that could be used in nematode Integrated Pest Management (IPM) programs, as a method safe for both human health and the surrounding environment.

2. Material and methods

2.1. Nematode isolates

An isolate of *M. chitwoodi* was obtained from a potato field in Porto, Portugal, and currently kept at the NEMATO-lab, University of Coimbra. It was maintained and multiplied in tomato plants, *S. lycopersicum* (cv. Coração de Boi), susceptible to RKN, and inoculated with 20 egg masses/plant [8]. The plants were grown in pots with 500 g of a homogeneous steam-sterilized soil of sand, soil and peat (1:1:1 v/v) mixture, and kept at 25 ± 2 °C, in a growth chamber, with a 12 h photoperiod. Ninety days after inoculation (DAI), plants were carefully uprooted and the nematode eggs were extracted with a 0.52% sodium hypochlorite (NaOCl) solution, according to Hussey and Barker (1973). At the beginning of each assay, identification of *M. chitwoodi* was confirmed through esterase phenotype analysis, following the methodology described by Maleita et al. (2012) [38].

2.2. Plant material

The seeds of *S. linnaeanum* were kindly provided by Prof. Óscar Machado and Dr. Filipe Melo (Agriculture School, Polytechnic Institute of Coimbra, Portugal) and *S. sisymbriifolium* cv. Sis 6001 seeds by Vandinter Semo. *Solanum linnaeanum*, *S. sisymbriifolium* (cv. Sis 6001) and *S. lycopersicum* (cv. Coração de Boi) were grown from seeds. Before germination, *S. linnaeanum* seeds were sterilized with 5 drops of a solution of Tween 20 (15 min), 30 mL of 10% aqueous NaOCl and 120 mL of distilled water, with agitation. Subsequently, the seeds were rinsed in distilled water during 10 min (×3) and dried on filter paper in the dark at 25 °C for 24 h. Then, these seeds were placed in Petri dishes on filter paper moistened with distilled water and kept at 25–27 °C in the dark. Following germination,

individual seeds were transplanted into plastic pots with a diameter of 5 cm. These pots were filled with a steam-sterilized mixture of loam soil and sand in a 1:2 ratio (v/v), totaling 60 cm^3. For *S. sisymbriifolium* seeds, germination took place in polystyrene plates filled with sterile peat within a controlled glasshouse environment. Fifteen days after germination the seedlings of both species were transplanted individually into pots filled with 500 g of steam-sterilized soil that contained sand, soil and peat (2:1:1 v/v) and maintained in the same conditions during one or two months. To obtain fruits, plants were transplanted to the field when they had two pairs of true leaves and fruits were collected 3–4 weeks after flower pollination. Immature fruits and fully ripe fruits were collected separately. Potato plants, *S. tuberosum* ssp. *tuberosum*, of the cultivar Désirée were propagated from sprouted potato tuber pieces. These sprouts were carefully placed into plastic pots with 5 cm diameter, containing a steam-sterilized mixture of sand and loam soil 2:1 ratio (v/v) for infectivity assays. For reproduction assays and to obtain root exudates, the sprouts were planted in pots containing 500 g of the same soil mixture. All plants, regardless of the assay, were maintained under consistent conditions in a glasshouse, as previously described (2.1).

2.3. Root exudates

To obtain the roots, one-month-old plants of *S. linnaeanum*, *S. lycopersicum* cv. Coração de boi, *S. sisymbriifolium* cv. Sis 6001 and *S. tuberosum* ssp. *tuberosum* cv. Désirée were grown as described above (2.2). Root exudates were obtained adding 1.5 L of distilled water, through successive leaching of soil, from three pots having plants grown in 500 g of soil. The distilled water was gradually added to the first pot and the water collected was passed through the second and then finally passed through the third pot. The exudates were collected in a glass container, filtered using Whatman filter paper and stored at 4 °C.

2.4. Plant extracts

One or two months after transplantation of *S. linnaeanum* and *S. sisymbriifolium*, the plants were uprooted and the roots washed thoroughly in running tap water, cut (separated from the aerial part), dried in an oven at 30 °C with air circulation, for two days, and frozen at −20 °C until required for the preparation of the extracts. An extraction method was devised that is simple and does not require added chemicals. The root extracts were obtained from dried and pulverized roots, using a coffee mill. A decoction was prepared by treatment of the powdered roots with distilled water (1:10), in an Erlenmeyer flask, with heating under reflux in a boiling water bath for 60 min. Immature fruits of the *S. linnaeanum* and *S. sisymbriifolium* plants were collected and immediately frozen at −20 °C until needed for extraction. Decoctions from the fruits were obtained by extraction with distilled water (1:20), using an Ultra-Turrax homogenizer and subsequent heating under the same conditions as described previously for the roots. The extracts of roots and immature fruits were filtered under vacuum, concentrated in a rotary evaporator at 45 °C, frozen, lyophilized and stored at −20 °C until used. Aqueous extracts of *S. linnaeanum* (Sl) and *S. sisymbriifolium* (Ss) one-month-old (R1M) and two-months-old (R2M) roots and immature fruits (F) were obtained in a total of six extracts: Sl R1M; Sl R2M; Sl F; Ss R1M; Ss R2M; and Ss F. Extractive yields, expressed in dry weight of extracted material, were calculated for each extract: Sl R1M = 28%; Sl R2M = 20%; Sl F = 42%; Ss R1M = 9%; Ss R2M = 13%; and Ss F = 46%. The extracts were prepared from a very representative number of plants (average of 120 plants/extract) and the lyophilized extracts obtained were properly homogenized and stored. For all the bioassays, the extracts were dissolved in distilled water, using a vortex stirrer and ultrasound to help the homogenization. An extract concentration of 0.8 mg/mL was used for all the bioassays described and distilled water was used as a control. Two independent assays, with five replicates in each, were performed for each extract.

2.5. Hatching bioassay

Each treatment consisted of five replicates of 30 eggs placed in 2 mL of each extract and distilled water was used as control. The experiments were conducted in glass-staining blocks maintained in a moist chamber, in the dark, at 22 ± 2 °C. Two independent bioassays were performed and the hatched J2 were counted every 24 h (except at weekends), for 20 days (i.e. until the average hatching percentage in the controls exceeded 80%). The hatched J2 were removed from the glass blocks at the successive counts. After the last count, the cumulative hatching percentage (CH%) was determined.

2.6. Mortality bioassay

Egg masses of *M. chitwoodi* were picked from infected tomato roots and placed in a muslin sieve of 300 μm pore in a glass bowl (9 cm diameter), containing distilled water, and kept at 22 ± 2 °C. Twenty-four hours later, the resulting J2 suspension was discarded and those that hatched in the subsequent 24 h were used for the bioassay. The freshly hatched J2 were hand-picked with an eyelash and transferred into 100 μL of distilled water placed in glass-staining blocks. Each treatment consisted of five replicates of 15 J2 each, which were placed in 1 mL of each treatment (extract or control) and held in a moist chamber, in the dark, at 22 ± 2 °C. Observations were made at 3, 24, 72, 168 and 240 h after exposure. The J2 that did not move when touched with a bristle were transferred to distilled water and were considered dead if they still failed to react to probing with a bristle 1 h later, after addition of sodium hydroxide (NaOH, 40 mg/mL). Two independent bioassays were done, distilled water being used as control. After the last count, mortality data was converted to cumulative mortality percentage (CM%).

2.7. Infectivity bioassay

For the infectivity bioassay, only the most active extract of each plant species, according to the results of the hatching and mortality bioassays, was used, that is: R1M for *S. linnaeanum* and F for *S. sisymbriifolium*. Potato plants (cv. Désirée), were obtained from pieces of potato tubers with sprouts, grown in 60 cm^3 plastic pots filled with a mixture of autoclaved loam soil and sand (1:2 v/v). A J2 suspension, obtained from egg masses as described for the mortality tests (2.6), was divided into three centrifuge tubes, centrifuged during 5 min at 4 °C and 1500 g, and as much supernatant as possible was removed. Either distilled water or plant extracts were added to each tube, vortexed and the volume divided into 5 tubes/treatment. Around 300 J2 per tube were incubated in the dark, at room temperature, for 4 or 7 days. The J2 exposed in this way were centrifuged, as mentioned above, 1 mL of distilled water was added and the tubes vortexed immediately before the J2 were inoculated near the roots (in three holes made in soil around the plants) of one-week-old potato plants. Thus, plants were never in contact with the extracts. Each treatment consisted of five replicates. The pots were placed at randomly assigned positions in the same glasshouse and conditions used were as described for obtaining the nematode isolates (2.1). The plants were watered when needed. Seven DAI the plants were uprooted, and their roots were washed free of soil and stained with acid fuchsin according to Byrd et al. (1983) [39]. Nematodes in the entire root system of each plant were counted using a stereomicroscope, and the infectivity was expressed as a percentage (I% = (number of nematodes inside the roots/number of nematodes inoculated) x 100). Two independent bioassays were performed, and distilled water was used as control.

2.8. Reproduction bioassay

As for the infectivity assays (2.7), only the most active extract of each plant species was used (Sl R1M and Ss F). Potato plants (cv. Désirée) were obtained from pieces of potato tubers with sprouts, grown in 600 cm^3 pots filled with a mixture of autoclaved loam soil and sand (1:2 v/v). An egg suspension, obtained according to Hussey and Barker (1973) [40], was divided into three centrifuge tubes, centrifuged for 5 min at 4 °C and 1500 g and as much supernatant as possible was discarded. Distilled water or plant extract was then added to each tube, vortexed and the volume divided into 5 tubes/treatment. Around 1800 eggs per tube (initial population, Pi) were incubated in the dark at room temperature for 4 or 7 days. Eggs exposed in this way went through the same processes as the J2 in the infectivity bioassays before inoculation into pots that each contained a one-week-old potato plant. Each treatment consisted of five replicates. The inoculated pots were placed at randomly assigned positions in the same glasshouse under the conditions described for obtaining the nematodes isolates (2.1). The plants were regularly watered as needed. Sixty DAI the plants were uprooted, and their roots were washed free of soil and weighed. The root systems were stained with phloxine B (0.0015% solution) for 15 min, as described by Eisenback et al. (1981), and galls and eggs masses counted [41]. Eggs were extracted using the previously mentioned procedure (2.1) and counted to determine the final population (Pf). Two independent bioassays were done, distilled water was used as control, and the reproduction factor (RF = Pf/Pi) was calculated.

2.9. HPLC-ESI/MS^n

A Liquid Chromatograph of High Performance (Finnigan Surveyor, THERMO) coupled to a linear ion trap mass spectrometer (LIT-MS) (LTQ XL, Thermo Scientific) was used to screen phytochemicals of the extracts. For chromatographic separation, a column Waters Spherisorb ODS2 (3 μm, 150 × 2.1 mm) was preceded by a guard cartridge Waters Spherisorb ODS2 (5 μm, 10 × 4.6 mm) and 0.1% aqueous formic acid (solvent A) and acetonitrile with 0.1% of formic acid (solvent B) were used as mobile phase, according to the following elution profile: 0–5 min, 5–30% B; 5–40 min, 30–65% B; 40–50 min, 65–100% B, at a flow rate of 200 μL/min. The mass spectrometer was operated in the positive electrospray ionization (ESI) mode and programmed to perform three scans: a full mass (MS) and a MS2 and MS3 of the most abundant ion. Helium was the collision gas with a normalized energy of 35%. Nitrogen was used as nebulizing gas, with a sheath and auxiliary gas flow of 40.00 and 5.00 arbitrary units, respectively. Capillary temperature and voltage were set at 275 °C and 40.00 V, respectively and the source voltage was set at 5.00 kV. Data treatment was performed using XCALIBUR software (Thermo Scientific).

2.10. Statistical analysis

Data for CH%, CM%, I%, and RF (averages from the two independent bioassays) were assessed to confirm compliance with the statistical assumptions of normality and homogeneity of variances using a one-way ANOVA. Subsequently, an analysis of variance was performed, and the means were compared using the least significant difference (LSD) test with a significance level set at $P < 0.05$. The statistical analysis was conducted using Statistic 10 software (Statsoft Inc.). In order to satisfy the assumptions of ANOVA, a square root ($\sqrt{x}$) transformation was applied to ensure normal distribution and homogeneity of variance for the data that would otherwise not meet the assumptions.

3. Results

3.1. Hatching

The root exudates of *S. linnaeanum* and *S. sisymbriifolium* cv. Sis 6001, compared to the tomato root exudates (cumulative hatching percentage (CH%) of 90.6%), had no inhibitory effect on the hatching of *M. chitwoodi*, and had similar percentages of hatching

stimulation (92.8–97.3%) after 20 days. The control with potato root exudates had slightly lower hatching (88.1%) than the control with tomato exudates (90.55%) (Fig. 1). The results of the two independent assays performed were similar.

All *Solanum* extracts, except Sl F, with a CH% of 76.33%, reduced the hatching of J2 after 20 days of continuous exposure, compared to the control (85.15%). For the *S. linnaeanum* root extracts, the hatching rate of the R1M extract (40.16%) was not significantly different from that of the R2M extract (33.86%) (Fig. 2A). In *S. sisymbriifolium*, the greatest hatching decrease occurred with the F extract, with a hatching rate of only 23.81% (Fig. 2B). The results of the two independent assays performed were similar.

3.2. Mortality

The *Solanum* extracts had no effect on J2 mortality. After 10 days of exposure, the cumulative mortality percentage (CM%) in *S. linnaeanum* extracts (2.7–12%) was not significantly different from the mortality in the control (10.7%), although slightly lower for the extract of R2M (2.7%) (Fig. 3A). The CM% in *S. sisymbriifolium* extracts (8–10.7%) was not different from the control (10.7%) (Fig. 3B).

3.3. Infectivity

The selected *S. linnaeanum* (Sl R1M) and *S. sisymbriifolium* (Ss F) aqueous extracts were effective at reducing *M. chitwoodi* infection of potato roots, after exposure to the extracts for periods of 4 or 7 days. For the two times of exposure to the *Solanum* extracts, the infectivity percentage (I%) was significantly lower than in the control (Fig. 4). After 7 days of exposure to the *S. sisymbriifolium* extract, the infectivity percentage was zero, with no nematodes detected inside the roots but, despite the infectivity also being low in the control after 7 days of exposure, there were significant differences between the two extracts and the control (Fig. 4B).

3.4. Reproduction

The selected *Solanum* extracts, Sl R1M and Ss F, were effective at reducing *M. chitwoodi* reproduction on potato roots after 7 days of exposure. A reduction in the numbers of galls, egg masses and eggs/gram of root was already noticed after 4 days of exposure to the extracts (Tables A1 and A2). After 4 days of exposure, the reproduction factor (RF) was 8 and 4, for Sl R1M and Ss F, respectively, not significantly different from the RF in the control (RF = 10) (Fig. 5A). After 7 days of exposure, the RF values were 7 and 3, for Sl R1M and Ss F, respectively, significantly lower than in the control (RF = 11), which was more evident for the *S. sisymbriifolium* extract (Fig. 5B). The results of the two independent assays performed were similar.

The results of this study reveal that the selected extracts (Sl R1M and Ss F) reduced nematode hatching, diminished the ability to infect a susceptible host after 4 days of exposure, and diminished the reproduction of *M. chitwoodi* on a susceptible host after 7 days of exposure.

3.5. HPLC-ESI/MS^n

Solasodine and its glycosides were detected in the selected aqueous extracts, *S. linnaeanum* root extract (Sl R) and Ss F, which have been shown to have nematicidal activity. The identification of these spirostane-type alkaloids was based on ions at *m/z* 415 (protonated molecule) and at *m/z* 253, 271 and 157 in MS2 and/or MS3 according to Yuan et al. (2019) [42]. Therefore, in Ss F extract the solasodine and its glycoside solamargine were detected and their identification was confirmed by authentic standards. For the Sl R

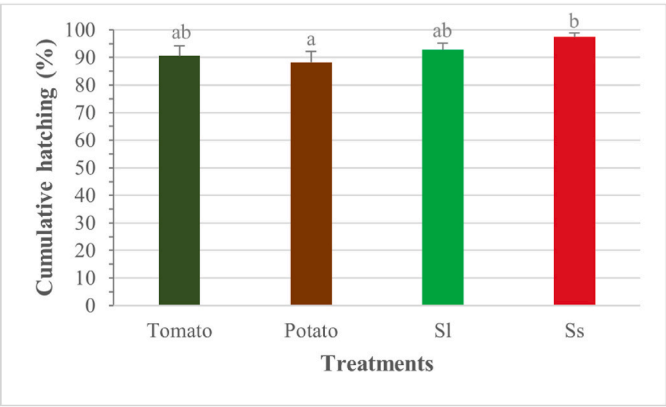

Fig. 1. Cumulative hatching percentage of *Meloidogyne chitwoodi* second-stage juveniles (J2) exposed to root exudates of *Solanum linnaeanum* (Sl) and *Solanum sisymbriifolium* cv. Sis 6001 (Ss), compared with tomato and potato root exudates, after 20 days of exposure. Percentages are averages of the two independent bioassays (30 eggs/replicate and 5 replicates/bioassay), error bars show standard errors and letters indicate significant differences among means (LSD test, $P < 0.05$).

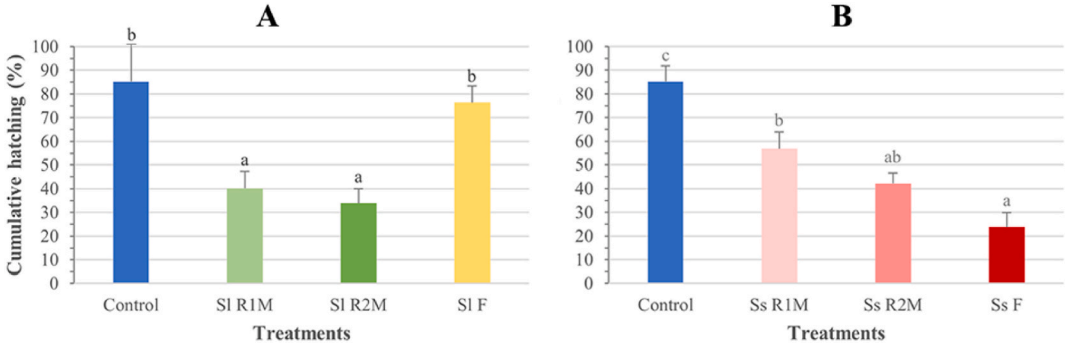

Fig. 2. Cumulative hatching percentage of *Meloidogyne chitwoodi* second-stage juveniles (J2) exposed to different *Solanum linnaeanum* (Sl) (**A**) and *Solanum sisymbriifolium* cv. Sis 6001 (Ss) (**B**) extracts, compared with the distilled water control, after 20 days of exposure. R1M – aqueous extract of one-month-old roots; R2M – aqueous extract of two-months-old roots; F - aqueous extract of immature fruits. Percentages are averages of two independent bioassays (30 eggs/replicate and 5 replicates/treatment); error bars show standard errors and letters indicate significant differences among means (LSD test, $P < 0.05$).

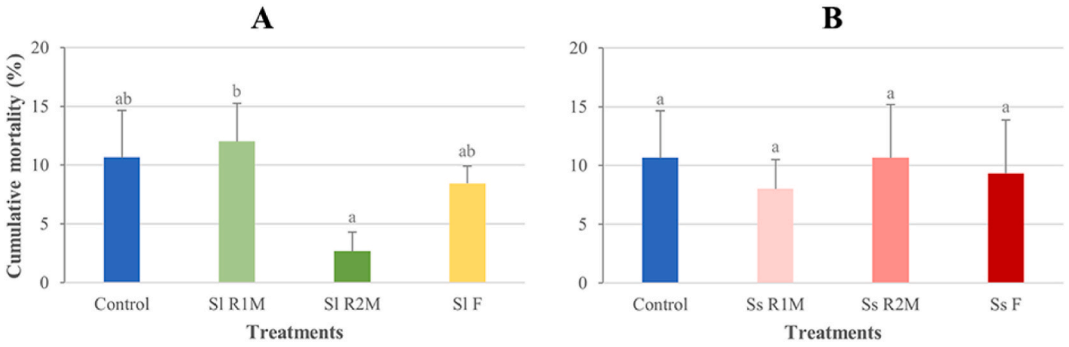

Fig. 3. Cumulative mortality percentage of *Meloidogyne chitwoodi* second-stage juveniles (J2) exposed to different *Solanum linnaeanum* (Sl) (**A**) and *Solanum sisymbriifolium* cv. Sis 6001 (Ss) (**B**) extracts, compared with the distilled water control, after 10 days of exposure. R1M – aqueous extract of one-month-old roots; R2M – aqueous extract of two-months-old roots; F - aqueous extract of immature fruits. Percentages are averages of two independent bioassays (15 J^2/replicate and 5 replicates/treatment); error bars show standard errors and letters indicate significant differences among means (LSD test, $P < 0.05$).

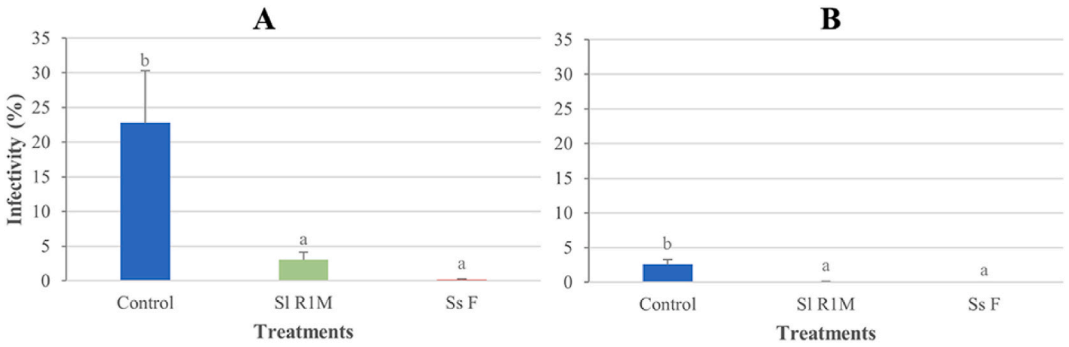

Fig. 4. Infectivity percentage of *Meloidogyne chitwoodi* on potato cv. Désirée after exposure to aqueous extracts of *Solanum linnaeanum* one-month old roots (Sl R1M) and *Solanum sisymbriifolium* cv. Sis 6001 immature fruits (Ss F) at 0.8 mg/mL, compared with the distilled water control, after 4 (**A**) or 7 (**B**) days of exposure and allowing 7 days from inoculation of 300 s-stage juveniles before counting. Percentages are averages of two independent bioassays (5 replicates/treatment); error bars show standard errors and letters indicate significant differences among means (LSD test, $P < 0.05$).

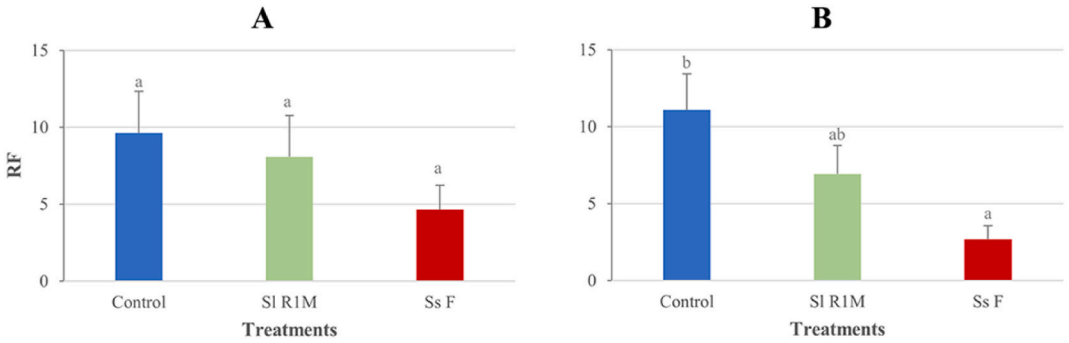

Fig. 5. Reproduction factor of *Meloidogyne chitwoodi* on potato cv. Désirée after exposure to aqueous extracts of *Solanum linnaeanum* one-month old roots (Sl R1M) and *Solanum sisymbriifolium* cv. Sis 6001 immature fruits (Ss F) at 0.8 mg/mL, compared with the distilled water control after 4 (**A**) or 7 (**B**) days of exposure and allowing 60 days from inoculation of 1800 eggs before counting. Values are averages of two independent bioassays (5 replicates/treatment); error bars show standard errors and letters indicate significant differences among means (LSD test, $P < 0.05$).

extract, the solasodine has also been identified, as well as solasodine-derived glycosides.

4. Discussion

Some natural phytochemicals released by several plant species have been shown to have nematicidal properties. The plant root exudates may act as stimulants or inhibitors of J2 hatching. In this study, *S. linnaeanum* and *S. sisymbriifolium* cv. Sis 6001 root exudates did not affect the hatching of *M. chitwoodi* J2. These results agree with the study carried out by Dias et al. (2012) with root exudates of *S. sisymbriifolium* from cultivars Domino, Sis 4004 and Pion [26]. Results of our study show, for the first time, some nematicidal activity of *S. linnaeanum* and *S. sisymbriifolium* cv. Sis 6001 aqueous extracts against *M. chitwoodi.* The population of *M. chitwoodi* is difficult to multiply at some periods of the year, which may explain the low level of infectivity in the control of the infectivity bioassays. The plant extracts from both *Solanum* species affected *M. chitwoodi* hatching and its capacity to infect and reproduce in a susceptible host, *S. tuberosum* spp. *tuberosum* cv. Désirée, suggesting that compounds with nematicidal properties are present. In fact, it is well known that the plants of *Solanum* species are sources of secondary metabolites that act as bioactive compounds such as alkaloids, namely steroidal glycoalkaloids, triterpenoids and saponins, that have a broad spectrum of pharmacological and toxicological activity [15].

Solanum sisymbriifolium is already used as a trap crop in some countries, reducing *G. pallida* populations (50–80%) by stimulating hatching of their eggs but not allowing the nematodes to complete their life cycle [26,30,36,43,44]. Although the nematicidal properties of the biologically active metabolites of *S. sisymbriifolium* are poorly understood, it contains several of them, such as flavonoids, glycoalkaloids and steroids [45–47]. Recently, the presence of some SGAs, such as α-solamargine and other solasodine-type glycoalkaloids, were detected in *S. sisymbriifolium*. These phytochemicals demonstrated nematicidal properties against *G. pallida* and may contribute to plant defenses [25]. In this study, the screening for phytochemicals in the Ss F extract prove the existence of solasodine and its glycoside solamargine in *S. sisymbriifolium* fruit. Solasodine and its glycosides, which occurs in other *Solanum* species were also identified in the root extract of *S. linnaeanum* [48].

Many wild *Solanum* spp. demonstrate resistance to *Meloidogyne* spp. [4]. So, before the evaluation of the nematicidal activity of *S. linnaeanum* and *S. sisymbriifolium* cv. Sis 6001, a first study was done on the resistance of these plant species to *M. chitwoodi*, and both were resistant [29]. Dias et al. (2012) evaluated other *S. sisymbriifolium* cultivars (Domino, Pion, Sis 4004 and Sharp) and all were resistant to *M. chitwoodi* [26]. These previous studies are important because a plant's resistant to a nematode, suggests an incompatibility in the plant-nematode interaction. The reduced number of nematodes observed inside the roots may be related to the existence of phytochemicals in exudates produced by the roots that prevent J2 penetration [49].

The *Solanum* extracts studied had no effect on *M. chitwoodi* mortality, although studies done with other PPN species and other plant extracts demonstrate effects on nematode mortality. Such is the case for *S. nigrum* and *S. sisymbriifolium* aqueous extracts against *Pratylenchus goodeyi*, in which nematode mobility and mortality were both affected [15]. Extracts from other plants, outside the Solanaceae family, have also shown nematicidal effects against several *Meloidogyne* species, mainly affecting nematodes mobility [24,49]. Control measures currently used against other *Meloidogyne* species, including the use of some nematicides, have proved to be less effective against *M. chitwoodi* [9]. This may be reflected in the fact that some plant extracts affect the mortality of other *Meloidogyne* species but not the mortality of *M. chitwoodi*.

As demonstrated in this study, the plant extracts prevent nematode eggs from hatching and thus suppress the PPN population densities, as verified for other plants [18,22,23,51–54]. In the case of *M. chitwoodi*, aqueous extracts from *S. linnaeanum* and *S. sisymbriifolium* showed *in vitro* nematicidal activity at 0.8 mg/mL. The most active extracts (Sl R1M and Ss F), based on the results of hatching and mortality assays, reduced the hatching of the nematodes and thereby diminished the number able to infect a susceptible host after 4 days of exposure to the extracts and reduced the reproduction of *M. chitwoodi* on a susceptible host after 7 days of exposure to the extracts.

Most studies done with plant extracts have only been against *M. incognita* or *M. javanica* [18,22–24,51,55]. The lower efficacy of chemical nematicides against *M. chitwoodi*, compared to other RKN species, make the results of this research with *M. chitwoodi*

promising. The ability of *M. chitwoodi* to develop at lower temperatures than other species of RKN means that information from tests at different temperatures and in field conditions will also be helpful.

For easer production, a simple preparation method can be used with water as the extraction solvent, such as was done by Pestana et al. (2008, 2014) and Ntalli et al. (2020a, 2020b) [15,31,50,56]. The utilization of water rather than other solvents reduces the risk of secondary effects on the nematodes. Such effects could mask the impact of the extract and influence the outcome of the assays.

Biopesticides could be a good alternative to synthetic pesticides. The use of natural products, with low impact on the environment and non-target organisms, represents a vital option for PPN control. The use of *S. linnaeanum* and *S. sisymbriifolium* may be an appropriate nematicidal approach to include in IPM. The great intra- and inter-cultivar variation of *S. sisymbriifolium*, observed in some research, makes it crucial to know the reactions of the crops that will be used and the RKN species present in each field. Furthermore, extracts of other *S. sisymbriifolium* cultivars should be evaluated. In Portugal, it may be an advantage to use *S. linnaeanum*, already found to occur in certain places, instead of *S. sisymbriifolium*, which is considered invasive in some countries and whose use may be a risk.

When *M. chitwoodi* is detected in seed potato lots, zero tolerance is generally applied, and the tolerance by the market for the tuber damage caused by it is very low. However, for industrial processing, there are acceptable limits even though they vary from country to country [57]. Despite the level of *M. chitwoodi* reproduction being reduced by exposure to the extracts at a concentration of 0.8 mg/mL, relative to the control, the RF it is still higher than 2. It will be important, in a next step, to test the effectiveness of the extracts in field conditions and to test higher concentrations of the extracts.

5. Conclusion

Aqueous extracts of *S. linnaeanum* and *S. sisymbriifolium* were shown to contain phytochemicals that possess nematicidal activity against *M. chitwoodi.* As synthetic nematicides are now being taken off the market, the results of this study have significant implications for RKN management but perhaps also for other PPN. For this reason, further studies are required to identify and characterize the active phytochemical(s) present in these *Solanum* species that may be possible to use as environmentally friendly nematicides for PPN, and to elucidate the underlying modes of action of the extracts. Further studies of these extracts should also be carried out in different crops to evaluate their nematicidal properties against other PPN under commercial field conditions. To obtain successful and maximum control efficacy in the use of plants with nematicidal activity, several factors should be investigated, such as: soil type, timing of incorporation into the soil, stability of nematicidal compounds in the soil, nematode-host status of the candidate plants, phytotoxicity to crops and toxicity to non-target organisms.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this paper.

Data availability statement

This work belongs to a PHD thesis.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendices.

Table A1
Numbers of galls, egg masses and eggs per gram of root of *Meloidogyne chitwoodi* on potato cv. Désirée after exposure to aqueous extracts of *Solanum linnaeanum* one-month-old roots (Sl R1M) and *Solanum sisymbriifolium* cv. Sis 6001 immature fruits (Ss F) at 0.8 mg/mL, compared with the distilled water control, after 4 days of exposure and 60 days after inoculation of 1800 eggs.

Treatments	Galls*	Egg masses*	Eggs g^{-1} root*
Control	89 ± 17.66 b	63 ± 14.80 b	33,664 ± 14619.96 b
Sl R1M	35 ± 10.24 a	29 ± 8.63 a	18,726 ± 6437.69 ab
Ss F	23 ± 7.81 a	18 ± 6.43 a	7153 ± 1866.46 a

* Values are averages of the two bioassays (5 replicates/treatment) with standard errors and letters indicating significant differences among means (LSD test, $P < 0.05$).

Table A2
Numbers of galls, egg masses and eggs per gram of root of *Meloidogyne chitwoodi* on potato cv. Désirée after exposure to aqueous extracts of *Solanum linnaeanum* one-month-old roots (Sl R1M) and *Solanum sisymbriifolium* cv. Sis 6001 immature fruits (Ss F) at 0.8 mg/mL, compared with the distilled water control, after 7 days of exposure and 60 days after inoculation of 1800 eggs.

Treatments	Galls*	Egg masses*	Eggs g^{-1} root*
Control	108 ± 22.60 b	78 ± 17.62 b	15,109 ± 3526.85 b
Sl R1M	38 ± 9.28 ab	28 ± 7.88 ab	7562 ± 1071.74 ab
Ss F	14 ± 3.03 a	10 ± 2.51 a	2622 ± 1042.70 a

* Values are averages of the two bioassays (5 replicates/treatment) with standard errors and letters indicating significant differences among means (LSD test, $P < 0.05$).

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