

Nematicidal activity of *Solanum sisymbriifolium* and *S. nigrum* extracts against the root-lesion nematode *Pratylenchus goodeyi*

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The root-lesion nematode, *Pratylenchus goodeyi*, is a parasite of banana plants, frequently detected in Madeira Island (Portugal) affecting culture development and consequently the production, with economical damages. To identify the phytochemicals of *Solanum sisymbriifolium* and *S. nigrum* with nematicidal properties and determine the effect of those components on *P. goodeyi*, an extraction sequence of at least 10 hours each from dried plants was used. The chosen solvent sequence was: dichloromethane, acetone, ethanol and water. According to the results, both plants have in their composition chemical components mainly found in water extracts, which affects the mobility and mortality of the root-lesion nematode. *S. sisymbriifolium* and *S. nigrum* have potential to be used as a natural and environmentally friendly nematicide to control *P. goodeyi*.

Keywords Banana plant; root-lesion nematode; *Solanum*; Nematicidal activity

1. Introduction

The root-lesion nematode *Pratylenchus goodeyi* (Cobb) Sher & Allen [1] is very common in Madeira Island affecting banana culture. In order to control nematode populations, farmers use phytopharmaceutical products, which also contribute to contaminate soil, groundwater and air. It is therefore of great importance to study alternative routes to those products by seeking less harmful chemicals to the environment and humans. Thus, some plants with nematicidal potential and its application have been analyzed [2,3].

It is known that the incorporation of organic waste has a considerable impact on physical and biological properties of soil, promoting a favorable environment for the development of nematode antagonists [4-6]. In some cases, it can be also ascertain toxicity to some nematodes. Since plants are capable of producing a large variety of secondary metabolites with multiple applications, much research has been conducted to find substances in plant tissues that may have an effect on nematodes [7-12]. Several benefits can result from the identification of phytochemicals involved in these interactions, which may be used as nematicidal or can serve as a model for the development of synthetic products with positive activity on nematodes or on the environment around them [13].

Several chemical compounds present in *Solanum* species, as steroidal glycosides and alkaloids among others, have a broad spectrum of activity [15-22] and is therefore of great interest to develop studies for the application of this plant genus in different areas. Among this species *Solanum sisymbriifolium* Lam, which does not exist in Madeira Island, has been successfully used to control populations of potato-cyst nematodes, *Globodera spp.* [14] whereas *S. nigrum* L. very common in Madeira is believed to have therapeutic properties against some types of tumors since some compounds showed cytotoxic effects in tumor cells [15]. Recent studies revealed that *S. sisymbriifolium* and *S. nigrum* are not good or non-hosts of *P. goodeyi* [23]. In addition, the incorporation of these plants into soil, improved banana plant growth, directly through the release of exudates with nematicidal effect and indirectly by promoting the development of antagonists and making the rhizosphere unfavorable to the nematode.

In order to search for nematicidal substances plant extracts from *S. sisymbriifolium* and *S. nigrum* were evaluated against *P. goodeyi*.

2. Materials and Methods

2.1 Samples preparation

S. sisymbriifolium “Pion” seeds were provided by “Vandijke Semo Seed & Services”, Netherlands. *S. nigrum* plants were obtained from nature and kept in the laboratory for the production of fruits and seeds.

S. sisymbriifolium and *S. nigrum* seeds were germinated in sterile peat and the plants kept in greenhouse until they reached a height of 50 to 60 cm. After, the plants were collected and placed in a drying chamber at 30°C (named dry plant). Once dried the plants were ground in a cutting mill (Mod. 5KH35KG 254E, Arthur H. Thomas Co. Phila., PA., U.S.A.) passed through sieves of 40 and 60 mesh (type AS200, Retsch). The fraction of 40-60 mesh size (425-250 µm) was used to the extraction.

The water content was determinate by a moisture balance (Gibertini-Eurotherm).

2.2 Sequential extraction

Milled dried material was placed in cartridges and subjected to a sequential extraction of at least 10 hours each with various solvents. The chosen solvent sequence was dichloromethane (DMC), acetone (Acet), ethanol (EtOH) and water. Each extraction was followed by solvent evaporation, in a rotative evaporator (R-200 Büchi), combined with a vacuum pump (V-500 Büchi Vac®) and a bath (B-490 Büchi), at a maximum temperature of 40°C. The extractives were collected and after drying under vacuum until constant weight, the extractives percentage was gravimetrically determined.

After ethanol extraction, the plant material that remained on the cartridges was washed with ethanol and dried at 30°C. This material was refluxed for 1 hour to obtain the extracts in water. The extract solutions were filtered under vacuum (G4 porosity), lyophilized and gravimetrically quantified.

All extracts were stored in cold and dark conditions until chemical analysis or mortality assessment on *P. goodeyi*. Five determinations were performed and the results were expressed as a percentage of the extract by dry matter.

2.3 *Pratylenchus goodeyi* and mortality determination

Populations of root-lesion nematode *P. goodeyi* were reared on *in vitro* banana plants, maintained in the laboratory in pots containing sterilized soil. *P. goodeyi* was previously extracted from infected banana roots by the maceration-sieving method [24, 25]. Nematodes were transferred to 10 ml of sterile water and quantified before inoculation in the soil. Three holes with a glass stick were made around the banana plant, which were covered with soil after inoculation. These potted plants remained in the laboratory until June - October 2009, being watered when necessary.

A concentration of 25 g per 100 ml from fresh plant material was effective on nematode mortality as previously determined [23]. Extractives solutions obtained from either dichloromethane, acetone, ethanol or water were prepared using the extract quantities corresponding to the initial concentration for both plants. They were placed in syringes to where 15 *P. goodeyi* were transferred. Five replicates per treatment were made and sterile distilled water was used as control. Mortality was determined at room temperature and in the dark for 10 days.

The nematodes were considered dead, when, after being transferred to sterile water and stimulated by touch, remained inactive for 2 h. Registered mortality was converted into cumulative mortality corrected by Abbott's formula [26] and data were statistically analysed.

2.4 Statistical analysis

Data are presented as the mean value \pm standard deviation (SD) of five replicates. The data were statistically analysed using “SPSS (Statistical Package for the Social Sciences) 15.0 for Windows” program. Normal distribution was verified through Kolmogorov-Smirnov and Shapiro-Wilk normality tests. Data were subjected to an analysis of variance (ANOVA). Differences between means were reported as significant if $p < 0.05$, using Tukey's test [27].

3. Results and Discussion

3.1 Fractions of *Solanum sisymbriifolium* and *S. nigrum* dry material

Figure 1 shows the amount of extractives from sequential Soxhlet extraction of *S. sisymbriifolium* and *S. nigrum* plants that was determined after a complete extraction. The results clearly indicate a predominant amount of polar fractions. Water extracts revealed the highest amount of extractives from either *S. sisymbriifolium* or *S. nigrum*, followed by ethanol extracts. The components extracted in dichloromethane and acetone were 6 to 8-fold lower, respectively, than the components extracted in ethanol and water.

Normal distribution through Kolmogorov-Smirnov and Shapiro-Wilk normality tests were confirmed ($p>0.05$). There were significant differences by ANOVA for *S. sisymbriifolium* and Tukey test detected differences between treatments ($p<0.05$).

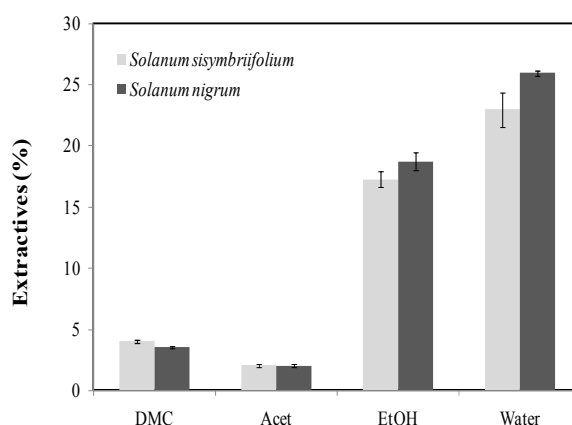


Fig. 1 Percentage of extractives from dry *Solanum sisymbriifolium* and *S. nigrum* plants obtained from solvents sequence: dichloromethane (DMC), acetone (Acet), ethanol (EtOH) and water. Results are the mean of 5 replicates \pm SD.

3.2 *Pratylenchus goodeyi* mortality and mobility in *Solanum sisymbriifolium* and *S. nigrum* extracts

Bioassays of the *S. sisymbriifolium* and *S. nigrum* extracts against the nematode *P. goodeyi* were made with the different extractives fractions in concentration corresponding to have 25 g of fresh plant per 100 ml of water. The extracts revealed differences in the toxicity level.

The mobility of *P. goodeyi* was not or little affected in dichloromethane and acetone extracts from both plants but very affected in ethanol and water extracts as shown in Figs. 2a and b. Nematodes placed in water extracts revealed lack of mobility on the second day and the recuperation of their mobility, tested on sterile water, diminished onwards. Further studies would be necessary to determine this effect through time (hours).

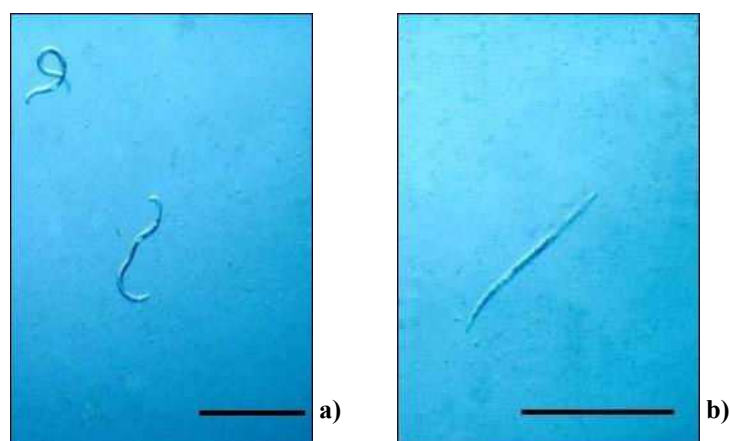


Fig. 2- Mobility of the *Pratylenchus goodeyi* in dichloromethane and acetone extracts a) and in ethanol and water extracts b) from both plants. Scale bar = 500 μ m.

The values obtained for *P. goodeyi* mortality, subjected to different extractives, showed a normal distribution by Kolmogorov-Smirnov and Shapiro-Wilk ($p > 0.05$) normality tests. Tukey test showed significant differences in *P. goodeyi* mortality within the treatments, being the water the most effective, reaching values of 99% for both plants. Dichloromethane and acetone extractives were statistically insignificant on the nematodes death as shown in Figs. 3a and b.

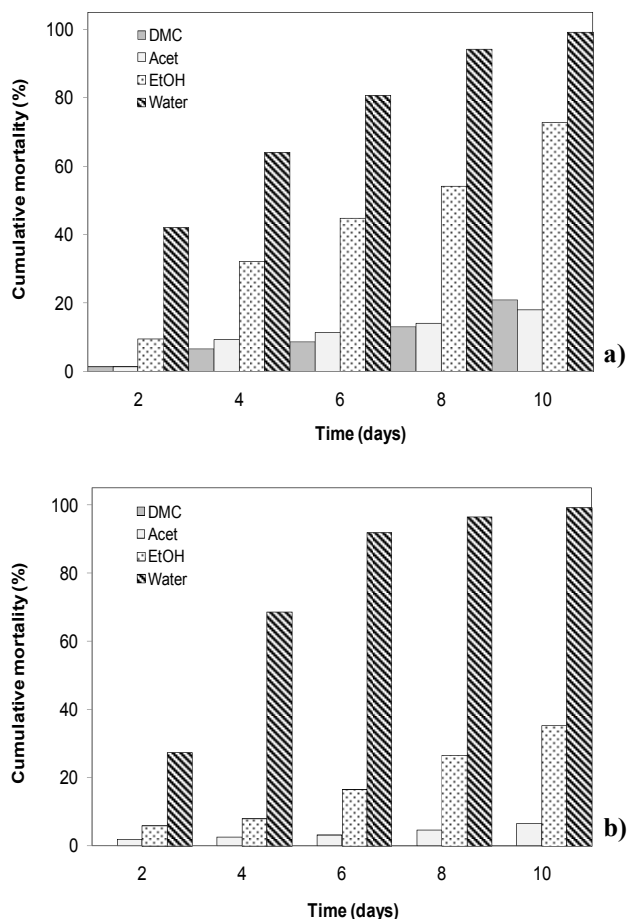


Fig. 3 *Pratylenchus goodeyi* cumulative mortality for 10 days in *Solanum sisymbriifolium* a) and *S. nigrum* b) solvents sequence extracts.

Many plant products remain undiscovered although some are known to exert nematocidal activity. Plant extracts from *S. sisymbriifolium* and *S. nigrum* affected *P. goodeyi* mobility. While motion was little or unaffected in dichloromethane and acetone extracts, ethanol extract reduced nematodes mobility, which was seriously distressed in the water extract as motionless was observed after the first day of exposure. Therefore, water extracts contain substances that had an immobilizing effect on the root-lesion nematode.

In the present study, water extracts from *S. sisymbriifolium* and *S. nigrum* plants exhibited the strongest nematocidal activity against *P. goodeyi* suggesting that some nematocidal or nematostatic properties were present. These results are very encouraging whereas they suggest that both plants can potentially be used towards *P. goodeyi*.

In order to find out phytochemicals or precursors of substances biosynthesized in response to plant/nematode interactions with potential to be explored as a natural nematocide, further analyses are needed to separate water extract into smaller fractions or into individual compounds and determine its effectiveness on *P. goodeyi*.

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