SCAP
SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

Control of the root lesion *Pratylenchus penetrans* - the effect of nematicidal activity of plant-derived compounds

O controlo do nemátode das lesões radiculares *Pratylenchus penetrans* - actividade nematicida de compostos derivados de plantas

Pedro Barbosa[1], Jorge M.S. Faria[2], Ana Cristina Figueiredo[3], Manuel Mota[4] & Cláudia S.L. Vicente[1,2,*]

[1] *MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal*
[2] *Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, Oeiras, Portugal*
[3] *Centro de Estudos do Ambiente e do Mar (CESAM Lisboa), Faculdade de Ciências da Universidade de Lisboa, Biotecnologia Vegetal, DBV, C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal*
[4] *MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Department of Biology, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal*
*(*E-mail: cvicente@uevora.pt)*

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ABSTRACT

The root lesion nematode, *Pratylenchus penetrans*, is one of the most harmful plant parasites, responsible for worldwide productivity losses in a significant number of plant hosts. Generally, chemical control relies on synthetic compounds used through fumigation or direct contact, which offers a systemic protection. These control methods are costly and hazardous to the environment and to humans. Phytochemicals may play an important role in nematode control. The nematicidal activity of eight compounds that occur naturaly in plants, from two classes of compounds, was assessed at 2 mg/mL, for 24 h. Bioassays were performed following the standard direct contact methodology. *P. penetrans* was remarkably tolerant to the tested compounds, with mortality range between 1.0 and 5.8 %. To the best of our knowledge, the nematicidal activity of 4 phenolic compounds (catechin, caffeic acid, gallic acid and gentisic acid) was evaluated for the first time for *P. penetrans*.

Keywords: biopesticides, biocontrol, plant-parasitic nematodes, soil health

RESUMO

O nemátode das lesões radiculares, *Pratylenchus penetrans*, é um dos fitoparasitas mais destrutivo, responsável por perdas a nível mundial num significativo número de plantas hospedeiras. O controlo químico destes organismos baseia-se em fumigantes e não fumigantes, que providenciam uma protecção sistémica. Tais métodos de controlo são dispendiosos e prejudiciais quer para o ambiente quer para os humanos. Os compostos produzidos naturalmente pelas plantas poderão desempenhar um papel importante no controlo de nemátodes. A actividade nematicida de 8 compostos que ocorrem naturalmente em plantas, de duas classes de compostos, foi avaliada a 2 mg/mL durante 24 h. Os ensaios foram efectuados segundo a metodologia de contacto directo usualmente empregue. *P. penetrans* revelou-se extremamente insensível aos compostos testados com valores de mortalidade compreendidos entre 1.0 e 5.8%. No nosso conhecimento, a actividade nematicida de 4 compostos fenólicos (catequina, ácido cafeico, ácido gálico e ácido gentísico) foi testada pela primeira vez em *P. penetrans*.

Palavras-chave: Biocontrolo, compostos bioactivos, nemátodes fitoparasitas, saúde do solo

INTRODUCTION

Soil health is the capacity of soil to maintain important ecosystem functions, such as sustaining living organisms (*i.e.*, plants, animals, and humans). In general, free-living nematodes contribute for soil health as important key species in nutrient cycles and in the multitrophic interactions with other soil microbes. However, the presence of plant-parasitic nematodes (PPN), may cause detrimental effects on plant hosts and other soil microorganisms, disrupting the equilibrium of soil ecosystem services (Brussaard, 1997).

The root-lesion nematodes (RLN) from genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae) are migratory PPN that attack a wide variety of important food and fed crops (*e.g.*, potato, carrot, soybean, maize, alfafa) causing severe economic constraints in the affected cultures (Jones *et al*., 2013). Plants infected by the RLNs present a stunted and necrotic root system (as result of RLN feeding habits) with poor development in aboveground plant parts. *Pratylenchus penetrans* is one of the most devastating RLN being reported in more than 400 plant hosts worldwide (Vicente *et al*., 2021). In Europe, *P. penetrans* has been detected in potato cultures in Portugal (Esteves *et al*., 2015) and in association with olive trees in Spain, Italy, and Turkey (Ali *et al*., 2014).

With the continuous withdrawal of chemical nematicides, such as Aldicarb, Carbofuran and 1,3-Dichloropropene, the current PPN control measures are far from being effective and sustainable. Plant-derived compounds, mostly plant secondary metabolites (*e.g.*, alkaloids, essential oils components, phenolic compounds) are safer to humans and environment and have already been applied against important PPN pests (Barbosa *et al*., 2012; Faria *et al*., 2021). Within the framework of the national project PratyOmics - Plant metabolomics for the control of the root-lesion nematode *Pratylenchus penetrans* (PTDC/ASP-PLA/0197/2020), the present work aims to evaluate the nematicidal activity of seven plant-derived compounds in direct contact bioassays against *P. penetrans.*

MATERIAL AND METHODS

Nematode Culture

Pratylenchus penetrans A44L4 was obtained in portuguese potato fields in 2010 by NematoLab team (Centre for Functional Ecology, University of Coimbra) (Esteves, et al., 2015). Nematodes are routinely multiplied in carrot disks according to Boisseau & Sarah (2008) and, when needed, extracted for 24 h in distilled water containing 50 μg/mL carbenecillin and kanamycin. Following, a nematode suspension was prepared with approximately 50-75 mixed-stage RLN per 100 μL distilled water.

Nematicidal Activity

Tested compounds (Table 1) were diluted in acetone (purity 99.8%, Carl Roth GmbH + Co. KG) to 200 mg/mL and maintained at -20ºC until use (2 mg/mL final concentration). Bioassays were conduted in a 96-well microtiter plate. For each compound, 1 μL of the solution was added to a mixed-stage nematode suspension (99 μL). Plates were covered with their lid and maintained at 25°C in the dark. After 24 h, dead and live nematodes were counted under a binocular microscope Olympus SZX-12 (Olympus Corporation, Tokyo, Japan). Nematodes were considered dead if they failed to respond to the gentle physical prodding with a needle. Two independent biological trials were performed with five replicates at similar conditions. Negative control was performed with acetone (purity 99.8%, Carl Roth GmbH + Co. KG.).

Data Analysis

Corrected mortality values (M_c) were obtained following the Schneider-Orelli formula (Puntener, 1981): $M_C = M_T - M_0 / 100 - M_0$, in which M_0 is the mortality in the control, and M_T is the mortality in treatments.

RESULTS AND DISCUSSION

Mean corrected mortality values obtained for control treatments, using acetone, was 2.2%. For the seven phenolic compounds tested((+)-catechin,

caffeic acid, coumaric acid, ferulic acid, gallic acid, gentisic acid and quercetin), *P. penetrans* mortality was relatively low, after 24 h of direct contact (Table 1). The highest M_c value was 5.8% recorded for gentisic acid, while the lowest Mc (1.0%) was obtained for gallic acid. The monoterpene carvacrol, reached full mortality (M_c=100%).

Tabla 1 - Nematicidal activity of commercial pure compounds (2 mg/mL in acetone) against *Pratylenchus penetrans* exposed for 24 h

Compound	Grade Supplier	Class	Corrected mortality (M_c)
(+)-Catechin	98% - Aldrich	I	1.98 ± 1.31
Caffeic acid	98% HPLC - Fluka	I	4.64 ± 1.54
Coumaric acid	98% HPLC - Fluka	I	1.88 ± 0.81
Ferulic acid	for research only - Extrasynthese	I	1.99 ± 0.84
Gallic acid	for research only - Extrasynthese	I	1.00 ± 1.02
Gentisic acid	for research only - Extrasynthese	I	5.83 ± 2.53
Quercetin	95% - Sigma-Aldrich	I	1.04 ± 0.87
Carvacrol	98% - Sigma-Aldrich	II	100.00 ± 0.00

Class: I, Phenolic compounds; II, Monoterpene

The phenolic compounds showed little or no nematicidal effect in *P. penetrans*, and only caffeic acid and gentisic acid could induce a higher M_c than the negative control (acetone). Only the monoterpene cravacol showed a high activity against this RLN.

Previous studies (Nguyen *et al*., 2013, d'Errico *et al*., 2018) showed that the efficiency of gallic acid, extracted from *Terminalia nigrovenulosa*, and SaviotaN (extracted from chestnut) against *Meloidogyne incognita*. However, in *P. penetrans*, its activity is reduced. Wuyts *et al*. (2006) showed that coumaric acid, ferulic acid, caffeic acid and quercetin displayed no activity (*i.e*., motility) in this nematode. According to our results, the seven phenolic compounds had little or no effect to *P. penetrans*, when tested individually, supporting the results observed by Wuyts *et al*. (2006). So far, this is the first work where the nematicidal activity of catechin, caffeic acid, gallic acid and gentisic acid is assessed against *P. penetrans*. Several monoterpenes have been already tested in *P. penetrans*. Rong and Qing (2000) showed that citronellol, carvacrol and (-)-perillaldehyde are potential nematicides against this RLN.

Plant secondary metabolites such as terpenoids and phenolics, have different mode of actions in nematodes (e.g., attract or repel, stimulate or inhibit egg-hatching, or exhibit nematicidal properties) (Ntalli *et al*., 2020). Beside these direct effects, and in the context of soil health and ecological equilibrium, these compounds can interact with multiple target sites, and therefore reduce the likehood of developing pest resistance (Chitwood *et al*., 2002).

CONCLUSIONS

Plant-based compounds with nematicidal effect against PPN might be good alternatives to synthetic nematicides and are less harmful for soil health. However, it is important to conduct previous rigorous tests, since not all compounds are effective. In PratyOmics, we will employ plant metabolomics on resistant/susceptible potato cultivars of P. penetrans to discover new bioactive compounds with antagonistic effect against this RLN.

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