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Revista Facultad Nacional de Agronomía

In vitro study on the nematicidal effect of different plant extracts on *Pratylenchus penetrans* and *Meloidogyne chitwoodi*

UNIVERSIDAD NACIONAL DE COLOMBIA

Estudio *in vitro* sobre el efecto nematicida de diferentes extractos de plantas en *Pratylenchus penetrans* y *Meloidogyne chitwoodi*

doi: 10.15446/rfnam.v72n3.76070

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ABSTRACT

Keywords:
Brassica spp.
Glucosinolates
Lolium spp.
Lupinus sp.

The purpose of this *in vitro* study was to evaluate the nematicidal effect of different glucosinolates and other secondary metabolites extracted from several plant species on the plant-parasitic nematodes *Pratylenchus penetrans* and *Meloidogyne chitwoodi*. Glucosinolate extracts from 16 species of genera *Brassica*, seven *Lolium* species and one species of *Lupinus* were used to investigate their nematicidal effect *in vitro*. From the tested extracts, the one obtained from *Brassica juncea* (oriental) showed the most promising results, controlling both nematode species. *Lupinus* sp. also showed positive results when tested against *P. penetrans*.

RESUMEN

Palabras clave:
Brassica spp.
Glucosinolatos
Lolium spp.
Lupinus sp.

El propósito de este estudio *in vitro* fue evaluar el efecto nematicida de diferentes glucosinolatos y otros metabolitos secundarios extraídos de varias especies de plantas, sobre los nemátodos *Pratylenchus penetrans y Meloidogyne chitwoodi* que afectan negativamente diversas plantas. Extractos de glucosinolatos provenientes de 16 especies del género *Brassica,* siete especies de *Lolium* y una especie de *Lupinus* fueron usados para investigar su efecto nematicida *in vitro*. De los extractos probados, el que proviene de *Brassica juncea* (oriental) mostró los resultados más promisorios para el control de las dos especies de nemátodos en estudio. *Lupinus* sp. también mostró resultados positivos para el control de *P. penetrans*.

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Received: November 9, 2018; Accepted: July 5, 2019
Rev. Fac. Nac. Agron. Medellín 72(3): 8945-8952. 2019
ISSN 0304-2847 / e-ISSN 2248-7026

The identification of phytochemical based strategies for the control of plant pathogens is important since it can be used in sustainable production systems where there are not many possibilities to manage plant-parasitic nematodes. It can also be essential for the development of new nematicides in traditional agriculture (Chitwood, 2002). Nowadays, further research on environmentally friendly biofumigants is suggested (Devi, 2018).

Meloidogyne chitwoodi Golden, O'Bannon, Santo, & Finley is an important pathogen of potato and other crops in the western part of Europe and is also a major pest of potato in the Northwestern states of the United States (Castagnone-Sereno *et al*., 1999). *Pratylenchus penetrans* is an obligate plant parasite of a wide range of hosts, mainly in temperate climates. It is one of the principal nematodes infesting ornamental plants and causes serious losses in different crops (Peng and Moens, 2002).

Plant compounds may act as a repellent, attractant, hatching stimulants or inhibitors and nematotoxicants. They can be used as fumigants or introduced in crop rotation programs for nematode control (Chitwood, 2002).

Glucosinolates are a group of allelochemicals that occur in all plants of the order Brassicales or Capparales (Cronquist, 1981), being the Brassicaceae family the most numerous and important group (Fahley *et al*., 2001). Glucosinolate (GLSs) have sulfur, and that explains its strong flavor (Avato *et al*., 2013). More than 130 glucosinolates have been identified (Fahley *et al*., 2001; Kirkegaard *et al*., 1999) and may be divided into three subclasses comprising aliphatic, phenyl, and indol-3-ylmethyl glucosinolates (Buskov *et al*., 2002). Isothiocyanates (ITC) and other plant compounds such as nitriles and thiocyanates (Buskov *et al*., 2002) are released from Brassicaceae when glucosinolates are hydrolyzed by the action of the enzyme myrosinase (Figure 1) (Kirkegaard *et al*., 1999). These hydrolysis products have shown various bioactive effects against some soilborne diseases and nematodes (Rosa *et al*., 1997).

The glucosinolate content average is higher on field-grown *B. juncea*, *B. napus*, *B. campestris*, and *Eruca sativa*, compared to the greenhouse and high tunnel cultivation (Antonious *et al*., 2009).

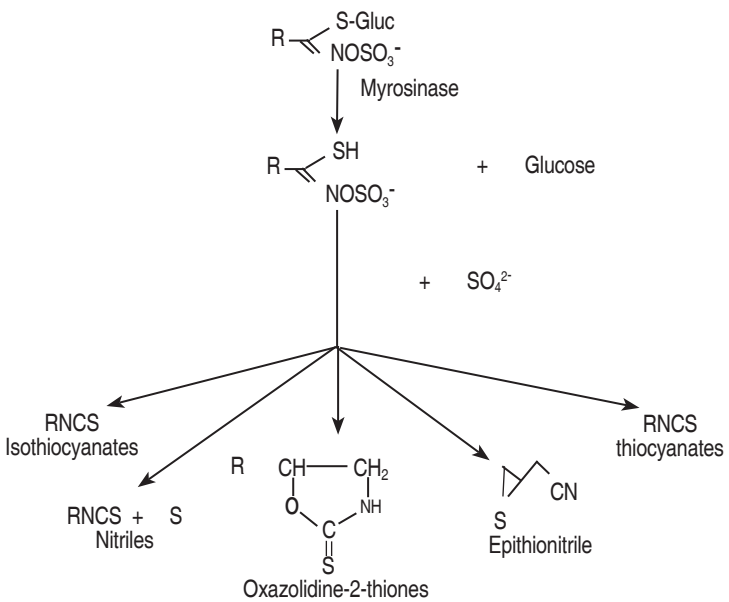

Figure 1. Enzymatic hydrolysis of glucosinolates via myrosinase activity (Kirkegaard *et al*.,1999).

Brassica species have shown a significant reduction in nematode numbers of *P. neglectus* (Potter *et al*., 1998) and *P. penetrans* (McFadden *et al*., 1992) when used as green manures. The same has been observed for *M. chitwoodi* (Mojtahedi *et al*., 1993). Other nematode species have shown mortality up to 100% when low doses of glucosinolates from Brassicaceaes have been applied, *in vitro*; such as in the case of *Globodera rostochiensis* (Serra *et al.,* 2002), *G. pallida* (Lord *et al*., 2011) and *M. incognita* (Oliveira *et al*., 2010). There is also a biocidal property with the use of GLSs on *Xiphinema index* and *Heterodera carotae* (Avato *et al*., 2013). Width control spectrum has been found by using these species (Björkman *et al*., 2011). Other plant compounds as the ones released from *Lupinus* sp. and several kinds of grass have proved to have a nematicidal effect; mulching with *Pennisetum purpureum* has been used for the control of *M. javanica* (Matsumoto *et al*., 2002) and the application of Sudan's grass extracts has reduced *M. hapla* juveniles that penetrate lettuce roots (Wildmer and Abawi, 2007). Anastasiadis and Karanastasi (2011) also presented the effectivity of brassica and ryegrass soil amendments on *M. incognit*a and *javanica*. The Fabaceae *Medicago sativa* has been found a good control against *G. rostochiensis* (D'Addabbo *et al*., 2011).

Considering the potential of these species as possible biocides, the purpose of this trial was to determine the nematicidal effects of plant extracts on plant-parasitic nematodes.

MATERIALS AND METHODS

Extracts

Brassicaceous accessions from a local field were tested: *B. fruticulosa* subsp*. mauritanica*, *B. fruticulosa*, *B. tournefortii*, *B. tournefortii*, *Sinapis arvensis* subsp. *arvensis*, *B. carinata*, *Raphanus sativus*, *R. sativus*, *B. juncea*, *B. juncea* (oriental), *B. tournefortii*, *B. oxyrrhina*, *B. napus* subsp. *oleifera*, *Sinapis alba*, *Crambe abbyssinica* and *Crambe hispanica* (identified as extract: 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 13, 15, 17, 18, 20 and 21, respectively, showed in Figure 2). The extracts of Brassicaceae and one extract of *Lupinus* sp. were obtained with a blend of 3 g of fresh shoot material in 6 mL of phosphate buffer (25 mM, pH 7, with 15 mg L^{-1} streptomycin), later sieved, centrifuged and used directly to make dilutions. Seven grasses, belonging to *Lolium* spp., were collected from dried meadows, and these samples were roasted for 3 hours from 160 to 260 °C before extracting the active components. Grasses were identified by letters (A, B, C, D, E, F, and G), as shown in Table 2. Grass A was not heat-treated while G was the most exposed to high temperatures. Grass extracts were made by blending 10 g of dried grass for one hour in 40 mL of extraction buffer.

Dilutions

Extract dilutions were made to test the dose-response of nematode migration activity. The dilutions show doses that could be obtained by practical quantities of crop residue on field. 6 mL from each extract of the stock-solution was used. This volume was diluted to 1/3, where 6 mL was used to make the dilutions 1/4, 1/16, and 1/64. The final dilutions were prepared with the same phosphate buffer (pH 7) as the original extracts. These dilutions were used for the filter plate experiments.

Filter plate experiments

The filter plate experiment was used to test the dose-response of nematode migration activity. There were two plates placed one on top of each other. The upper plate was a 96-wells high filter plate with a small tube underneath. The lower plate was a standard 96-wells plate. The upper plates were filled with cigarette filters. It was pipetted 700 µL of the plant extract solution on these filters, then 200 µL of the nematode suspension (200 *P. penetrans* and 100 J2 *M. chitwoodi* nematodes). This proportion was selected because *Meloidogyne* migrates faster, so there would be more nematodes per well. The plates were covered and stored at 20 °C for 24 hours. After 24 hours, the upper plate was transferred to a new lower plate, and 200 µL of buffer was added. The whole set was place at 20 °C for 24 hours again, so the rest of the nematodes could migrate. The plates were counted again after 48 hours. Four replications per dilution were scored, and four wells with 900 µL of buffer per plate were used as a control. For each well, the number of nematodes was scored. Juveniles and adults *P. penetrans* were counted separately.

Nematode counting for each dilution was compared and statistically analyzed with STATISTIX for Windows. All data were *log(x+1)* transformed for an appropriate analysis. Tukey tests were used to compare treatments with significant differences (P<0.05).

RESULTS AND DISCUSSION

All the Brassicaseous and grass extracts showed nematicidal or repellent effect at a high concentration (1/4 dilution), whereas at lower concentrations (dilutions 1/16 and 1/64) differences were not always observed (Figures 2, 3 and 4). This nematicidal effect was a function of the dilution and time of exposure.

Glucosinolates relative slight structural differences could confer deeply different nematicidal effects, confirming that

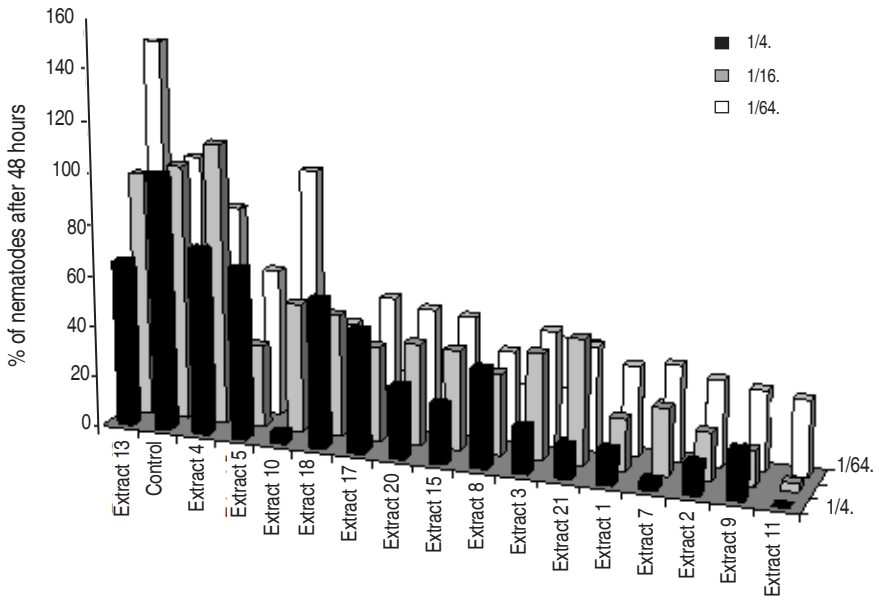

Figure 2. Percentage over the control of total *P. penetrans* found after 48 hours for different Brassicaceae extracts at different concentrations.

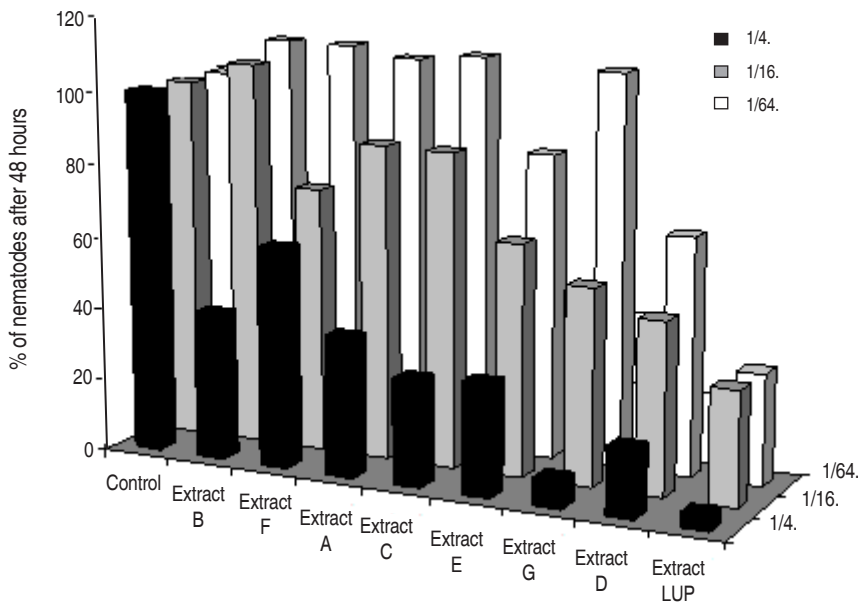

Figure 3. Percentage over the control of total *P. penetrans* found after 48 hours for the different grass extracts and *Lupinus* sp. at different concentrations.

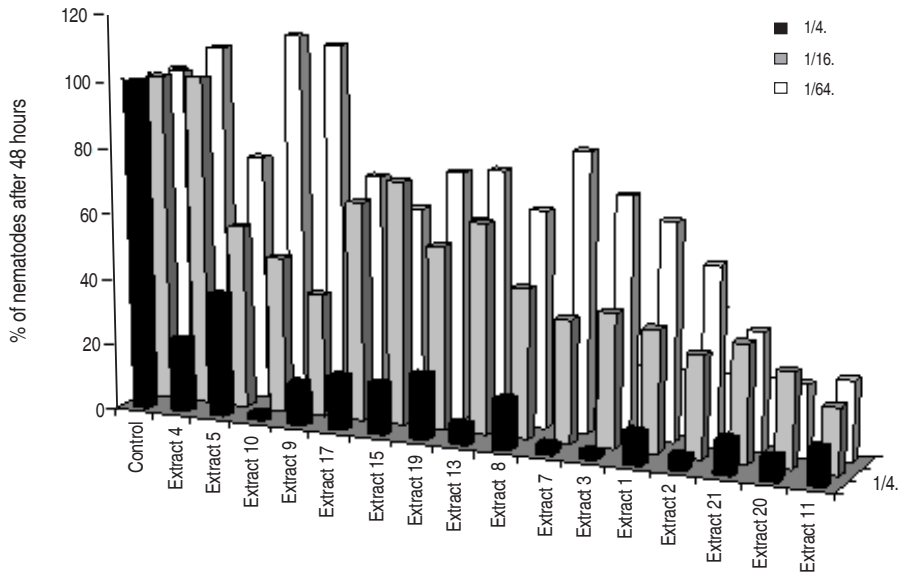

Figure 4. Percentage over the control of total *M. chitwoodi* found after 48 hours for different Brassicaceae extracts at different concentrations.

biological activity was a function of the concentration of the product and the chemical properties of the R chain (Serra *et al*., 2002; Zasada and Ferris, 2003).

Best nematicidal effects at 1/4 dilution were observed with Extracts 11, grass G and *Lupinus* for *P. penetrans* (Tables 1 and 2), and with extracts 10, 3, and 7 for *M. chitwoodi* (Table 3). At 1/16 dilution the extracts with higher effect were 11, 9, and *Lupinus* sp. for *P. penetrans* and extract 11 for *M. chitwoodi*. At the lower concentration of 1/64 most effective extracts were 2, 11, 9, and *Lupinus* for *P. penetrans*, and 20 and 11 for *M. chitwoodi*.

Table 1. Mean of total nematodes present after 48 hours for different Brassicaceae extracts at different concentrations in *P. penetrans* corrected over the control.

Extract id.	Plant extract	Dilution		
		1/4	1/16	1/64
1	*Brassica fruticulosa* subsp. *mauritanica*	12.7 cdef	19.7 de	35.0 cd
2	*B. fruticulosa*	12.1 cdef	18.5 de	30.3 d
3	*B. tournefortii*	14.8 cdef	40.5 cd	44.5 bcd
4	*B. tournefortii*	70.6 a	103.2 a	78.1 abc
5	*Sinapis arvensis* subsp. *Arvensis*	66.8 a	31.8 cd	56.7 bcd
7	*B. carinata*	3.6 fg	23.6 cde	35.4 cd
8	*Raphanus sativus*	38.4 abcd	29.8 cde	32.9 cd
9	*R. sativus*	18.3 bcde	13.5 e	27.9 d
10	*B. juncea*	4.1 efg	48.6 abc	96.9 ab
11	*B. juncea-oriental*	0.0 h	3.2 e	28.4 d
13	*B. tournefortii*	63.5 a	90.4 ab	141.7 a
15	*B. oxyrrhina*	22.7 bcde	38.6 cd	43.1 bcd
17	*B. napus* subsp. *Oleifera*	45.9 abc	36.4 cd	50.2 bcd
18	*Sinapis alba*	57.6 ab	47.1 bc	39.7 bcd
20	*Crambe abbyssinica*	276 bcde	38.1 cd	44.6 bcd
21	*Crambe hispanica*	7.7 defg	48.5 abc	40.4 bcd

Means with the same letter within dilution are not significantly different ($P>0.05$)

Table 2. Mean of total nematodes present after 48 hours for different plant grass extracts at different concentrations in *P. penetrans* corrected over the control.

Plant extract	Extract id.	Dilution		
		1/4	1/16	1/64
Lolium spp.	A	36.2 a	85.2 ab	105.9 a
	B	38.5 a	105.8 a	108.6 a
	C	27.9 a	86.1 ab	96.5 a
	D	18.3 ab	46.8 d	63.1 a
	E	30.4 a	63.6 bcd	84.1 a
	F	59.7 a	72.5 bc	110.1 a
	G	3.0 c	53.2 cd	106.4 a
Lupinus sp.	LUP	3.6 bc	30.6 e	30.6 b

Means with the same letter within dilution are not significantly different ($P>0.05$)

Table 3. Mean of total nematodes present after 48 hours for different Brassicaceae extracts at different concentrations in *M. chitwoodi* corrected over the control.

Extract id.	Plant extract	Dilution		
		1/4	1/16	1/64
1	*Brassica fruticulosa* subsp. *mauritanica*	5.1 abc	35.3 bcd	58.4 abc
2	*B. fruticulosa*	3.9 bc	27.6 cd	45.5 abcd
3	*B. tournefortii*	2.0 c	39.8 bcd	71.0 ab
4	*B. tournefortii*	21.2 ab	99.7a	107.8 a
5	*Sinapis arvensis* subsp. *arvensis*	37.1 a	54.9 abc	69.9 ab
7	*B. carinata*	2.2 c	36.5 bcd	81.5 ab
8	*Raphanus sativus*	12.8 abc	42.0 abcd	61.9 ab
9	*R. sativus*	10.1 abc	36.3 bcd	109.0 a
10	*B. juncea*	1.9 c	42.0 abcd	112.1 a
11	*B. juncea, oriental*	11.2 abc	17.7 d	23.8 cd
13	*B. tournefortii*	4.0 bc	55.6 abc	64.4 ab
15	*B. oxyrrhina*	10.5 abc	73.0 ab	59.9 ab
17	*B. napus* subsp. *oleifera*	16.0 abc	65.6 abc	70.6 ab
19	*Sinapis alba*	16.6 abc	52.1 abc	72.8 ab
20	*Crambe abbyssinica*	6.9 abc	27.0 cd	20.5 d
21	*Crambe hispanica*	10.2 abc	34.2 bcd	32.7 bcd

Means with the same letter within dilution are not significantly different ($P>0.05$)

In cases where the metabolite had a better effect when used in a lower concentration (e.g., 1/64) could be assumed that its effect did not only depend on the concentration of the plant extract, but it was also related to the optimal dilution at which the compound gave positive results for nematode management.

For *P. penetrans* control the principal compounds involved are sinigrine and gluconapin according to preliminary studies performed by the Plant Research International in Wageningen. The first one was found in high concentrations in extracts 7 and 11 (*Raphanus sativus* and *Brassica juncea* oriental, respectively), while the latter was in excess in

extracts 1 and 2 (*Brassica fructiculosa* both species) (data not shown). In the experience of Lazzeri *et al.* (1993) with a concentration of 0.05%, sinigrin and gluconapin showed nematicidal effect after 96 hours and 114 hours, respectively, for the control of *Heterodera schachtii*. *Brassica juncea* products have also shown a reduction in *Globodera pallida* (Lord *et al.*, 2011), *P. penetrans* and *M. incognita* (Zasada *et al.*, 2009). Ngala *et al.* (2014) also found nematicidal effects for the same nematode with *B. juncea* and *Raphanus sativus* deterred its multiplication.

For *M. chitwoodi* control showed a clear correlation between the type of glucosinolate so its control effectiveness could not be done easily because most of the extracts controlled the nematode effectively, at least for the lowest concentration (Table 3). However, it could be assumed that several isothiocyanates or the combination of them have detrimental effects on *M. chitwoodi* populations; therefore, its use could be highly appreciated in crops where this nematode causes economic losses. Further chemical studies of the most promissory plant species are necessary in order to determine specific compounds that have nematicidal or nematostatic effects.

CONCLUSIONS

Glucosinolates present in *Brassica juncea* and *Lupinus* sp. showed the most significant nematicidal effect with the lowest dilution (1/4). It seemed that the high concentration of these glucosinolates in the plant tissue is the direct responsible for the biocidal effect of these plants. Given the high effectiveness of most of the tested *Brassica* spp. for the control of *M. chitwoodi* and *P. penetrans*.

ACKNOWLEDGEMENTS

The author is grateful to the staff from Plant Research International - Wageningen for any input given. This study was part of a graded project presented in February 2004 for the course Ecological Aspects of Biointeractions. For the MSc in Plant Sciences, at Wageningen University in The Netherlands.

REFERENCES

Anastasiadis I and Karanastasi E. 2011. Factors affecting the efficacy of Brassica species and ryegrass (*Lolium perenne* L.) on root-knot nematode infestation of tomato. Communications in Agricultural and Applied Biological Sciences 76(3): 333-340.

Antonious GF, Bomford M and Vincelli P. 2009. Screening *Brassica* species for glucosinolate content. Journal of Environmental Science and Health 44(3): 311-316. doi: 10.1080/03601230902728476

Avato P, D'Addabbo T, Leonetti P and Argentieri M. 2013. Nematicidal potential of Brassicaceae. Phytochemistry Reviews 12(4): 791-802. doi: 10.1007/s11101-013-9303-7

Björkman M, Klingen I, Birch ANE, Bones AM, Bruce TJA, Johansen TJ, Meadow R, Mølmann J, Seljåsen R, Smart LE and Stewart D. 2011. Phytochemicals of Brassicaceae in plant protection and human health – influences of climate, environment and agronomic practice. Phytochemistry 72(7): 538-556. doi: 10.1016/j.phytochem.2011.01.014

Buskov S, Serra B, Rosa E, Sørensen H, Sørensen J. 2002. Effect of intact glucosinolates and products produced from glucosinolates in myrosinase-catalyzed hydrolysis on the potato cyst nematode (*Globodera rostochiensis* Cv. Woll). Journal of Agricultural and Food Chemistry 50(4): 690-695. doi: 10.1021/jf010470s

Castagnone-Sereno P, Leroy F, Bongiovanni M, Zijlstra C and Abad P. 1999. Specific diagnosis of two root-knot nematodes, *Meloidogyne chitwoodi* and *M. fallax*, with satellite DNA probes. Phytopathology 89(5): 380-384. doi: 10.1094/PHYTO.1999.89.5.380

Chitwood DJ. 2002. Phytochemical based strategies for nematode control. Annual Review of Phytopathology 40: 221-249. doi: 10.1146/annurev.phyto.40.032602.130045

Cronquist A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York. 1262 p.

D'Addabbo T, Carbonara T, Leonetti P, Radicci V, Tava A and Avato P. 2011. Control of plant parasitic nematodes with active saponins and biomass from *Medicago sativa*. Phytochemistry Reviews 10(4): 503-519. doi: 10.1007/s11101-010-9180-2

Devi G. 2018. Biofumigation: A potential aspect for suppression of plant-parasitic nematodes. International Journal of Environment, Agriculture and Biotechnology (3)4: 1285-1291. doi: 10.22161/ijeab/3.4.20

Fahley JW, Zalcmann AT and Talay P. 2001. The chemical diversity and distribution of glucosinolates and isothyocyanates among plants. Phytochemistry 56(1): 5-51. doi: 10.1016/S0031-9422(00)00316-2

Kirkegaard J, Matthiessen J, Wong P, Mead A, Sarwar M and Smith B. 1999. Exploiting the biofumigation potential of Brassicas in farming systems. pp 1-7. In: Proceedings of the 10th International Rapeseed Congress. The regional Institute Ltd. Canberra.

Lazzeri L, Tacconi R and Palmieri S. 1993. In vitro activity of some glucosinolates and their reaction products toward a population of the nematode *Heterodera schachtii*. Journal of Agricultural and Food Chemistry 41(5): 825-829. doi: 10.1021/jf00029a028

Lord J, Lazzeri L, Atkinson H and Urwin P. 2011. Biofumigation for control of pale potato cyst nematodes: Activity of brassica leaf extracts and green manures on *Globodera pallida* in vitro and in soil. Journal of Agricultural and Food Chemistry 59(14): 7882-7890. doi: 10.1021/jf200925k

Matsumoto MN, Goulart AMC, Homechin M, Sakamoto MMY and Santiago DC. 2002. Mulching with *Pennisetum purpureum* and other cultural practices for management of *Meloidogyne javanica* on tomato under greenhouse conditions. Nematologia Brasileira 26: 101-104.

McFadden W, Potter J and Brandle J. 1992. Use of *Brassica spp.* as green manure for biological control of *Pratylenchus penetrans*. Phytopathology 82(2): 246.

Mojtahedi H, Santo GS, Wilson JH and Hang AN. 1993. Managing *Meloidogyne chitwoodi* on potato with rapeseed as green manure. Plant Disease 77(1): 42-46. doi: 10.1094/PD-77-0042

Ngala M, Haydock P, Woods S and Back M. 2015. Biofumigation with *Brassica juncea, Raphanus sativus,* and *Eruca sativa* for the management of field populations of the potato cyst nematode *Globodera pallida*. Pest Management Science 71(5): 759-769. doi: 10.1002/ps.3849

Oliveira RDL, Dhingra OD, Lima AO, Jham GN, Berhow MA, Holloway RK and Vaughn S. 2010. Glucosinolate content and nematicidal activity of Brazilian wild mustard tissues against *Meloidogyne incognita* in tomato. Plant and Soil 341(1-2): 155-164. doi: 10.1007/s11104-010-0631-8

Peng Y and Moens M. 2002. Tolerance of Rosa rootstocks and species to *Pratylenchus penetrans*. Nematology 4(3): 395-401. doi: 10.1163/156854102760199231

Potter M, Davies K and Rathjen A. 1998. Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematode *Pratylenchus neglectus*. Journal of Chemical Ecology 24(1): 67-80. doi: 10.1023/A:1022336812240

Rosa EAS, Heaney RK, Fenwick GR and Portas CAM. 1997. Chapter 3: Glucosinolates in crop plants. pp. 99-215. In: Janick J (ed). Horticultural Review. Vol. 19. John Wiley & Sons, Inc. 441 p.

Serra B, Rosa E, Iori R, Barillari J, Cardoso A, Abreu C and Rollin, P. 2002. In vitro activity of 2-phenylethyl glucosinolate, and its hydrolysis derivatives on the root-knot nematode *Globodera rostochiensis* (Woll.). Scientia Horticulturae 92(1): 75-81. doi: 10.1016/S0304-4238(01)00277-1

Wildmer TL and Abawi GS. 2007. Mechanism of suppression of *Meloidogyne hapla* and its damage by a green manure of Sudan grass. Plant Disease 84: 562-568. doi: 10.1094/PDIS.2000.84.5.562

Zasada I and Ferris H. 2003. Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipenetrans* to isothiocyanates in laboratory assays. Nematology 93(6): 747-750. doi: 10.1094/PHYTO.2003.93.6.747

Zasada IA, Meyer SLF and Morra MJ. 2009. Brassicaceous seed meals as soil amendments to suppress the plant-parasitic nematodes *Pratylenchus penetrans* and *Meloidogyne incognita*. The Journal of Nematology 41 (3): 221-227.