

Journal of Advanced Zoology

ISSN: 0253-7214 Volume **44** Issue **05 Year 2023** Page **116:127**

The Biofumigation Potential of Wild Radish (*Raphanus Raphanustrum*) and Field Mustard (*Sinapis Arvensis*) in Controlling Some Phytoparisitic Nematodes

Sihem Ziouche¹, Haizia Kelaleche², Amel Bourahla³, Zahr Eddine Djazouli⁴

¹Mohammed El Bachir El Ibrahimi University, Department of Agronomic Sciences, Faculty of Natural and Life Sciences and Earth and Universe Sciences, Laboratory of valorisation of natural biological ressources (LVRBN), Bordj Bou Arreridj 34000, Algeria.

²Blida 1 University, Faculty of Natural and Life Sciences, Department of Biotechnology, Laboratory of Biotechnology Plants produtions, Blida 09000, Algeria.

³Higher School of Food Sciences and Agro-Food Industries. Algiers 16000, Algeria. Email: sihem.ziouche@univ-bba.dz

*Corresponding author's E-mail: sihem.ziouche@univ-bba.dz

Article History	Abstract		
Received: 08 June 2023 Revised: 21 Sept 2023 Accepted: 08 Dec 2023	Biofumigation is the process of introducing plants and their residues into the soil to release volatile compounds. Among these plants, those belonging to the Brassicaceae family are the most commonly used ones for biofumigation purposes. These plants are rich in glucosinolates, which upon enzymatic hydrolysis produce compounds such as isothiocyanates, which are approved as nematicides. Raphanus, Sinapis, and Eruca species genera are the most frequently used plants in the Brassicaceae family for biofumigation. This technique is becoming increasingly popular as a sustainable alternative to restricting fumigants in agriculture production. Dry plants (at vegetative and flowering stages) of Raphanus raphanustrum and Sinapis arvensis are incorporated into the soil and evaluated for their biofumigation effects on some phytoparasitic nematodes in comparison to a synthetic nematicide (tefluthrin 1.5%) and untreated control. Thirty days post-incorporation, soil samples were collected for nematode extraction and counting, and subsequently identified at the genus level. We identified four phytoparasitic nematodes that are most harmful to cultivated plants (Tylenchorhynchus, Pratylenchus, Paratylenchus, and Tylenchus). Each treatment decreased nematode population densities from 67% to 92%. Sinapis arvensis biofumigation was the most effective, reducing nematode densities by up to 92%. Conversely, Raphanus raphanustrum and Tefluthrin 1.5% treatments generally resulted in increased nematode densities (up to 74% and 67% respectively). The results of this study have given valuable information and suggest that Sinapis arvensis could be used to manage plant-parasitic nematodes, particularly the Tylenchorhynchus population. Biofumigation is a promising strategy that can be used especially by subsistence farmers for the sustainable production of crops in the presence of lower nematode pest densities.		
CC-BY-NC-SA 4.0	Keywords: Alternative Control, Brassicaceae, Biofumigation, Nematodes Dentities, Nematicide		

1. Introduction

Defending crops against telluric organisms is more difficult than foliar pests (Matthiessen and Kirkegaard, 2006). PPNs (plant parasitic nematodes) wreak havoc on a wide range of crops (Hansen and Keinath, 2013; Handiseni et al., 2017). They are a significant pest of agricultural and horticultural crops and are a major constraint on intensive agricultural production systems around the world (Nyczepir and Thomas, 2009; Singh et al., 2013; Stirling, 2014). They can also promote secondary infections and diseases due to root damage, which facilitates fungal and bacterial diseases. In furthermore, PPNs can act as vectors for various plant viruses (Jones et al., 2013). When considering both direct and indirect crop damage caused by PPNs, the estimated annual yield loss ranges from US\$80 to US\$173 billion (Elling, 2013; Youssef et al., 2013; Singh et al., 2015). Jones et al. (2013)

identified the top ten nematode pests, with root-knot nematodes (Meloidogyne), cyst nematodes (Heterodera and Globodera), and root lesion nematodes (Pratylenchus) taking the top three positions. Ten of the 98 Meloidogyne species have been identified as agricultural pests, while five cyst and six lesion nematode species are of global economic concern. Effective nematode management is therefore critical to ensuring sustainable crop production and food security in both developed and developing countries. During the last 20th century, under intensive conventional farming systems, control of economically important PPNs was generally based on the application of volatile chemical compounds with non-specific action, frequently used in a practice known as soil fumigation nematicides (Vervoort et al., 2014, Ladha Lakshmi et al., 2015, Leite et Lopes, 2018). Historically, chemical fumigation has relied on methyl bromide (Epstein, 2014, Rudolph et al. 2015, Brennan et al., 2020). However, methyl bromide was phased out under the Montreal Protocol in 2005 due to its depleting effects on the ozone layer (Laegdsmand et al., 2007, Gimsing and Kirkegaard, 2009), and thereafter suitable substitutes are on focus. To date, the global focus on sustainability in the agricultural environment is increasing with the main aim to produce healthy, safe, and good-quality crops and food. This focus includes the implementation of 'integrated pest management' (IPM), 'sustainable farming', and 'farming for the future' (Woolworths) (Kruger et al., 2013). The continuous use of chemical nematicides to control plant parasitic nematodes had a considerable environmental impact and has resulted in the onset of resistance phenomena within some populations of nematode pests and soil sterilants, the majority of which are now prohibited, necessitating the development of cheaper, safer and eco-sustainable alternative tools, i.e. biological and cultural methods to control plantparasitic nematodes to reduce the effects of widespread nematicides utilization in crop protection (Salem and Mahdy, 2015). However, a combination of increasing interest in natural soil fertility and sanctions against the use of chemical nematicides has directed researchers towards biofumigation (Matthiessen and Kirkegaard 2006; Zasada et al. 2010; Kruger et al. 2013; Ntalli et al. 2017; Daneel et al. 2018). Biofumigation, the use of naturally produced plant chemicals to control plant-parasitic nematodes (Motisi et al. 2010), offers a creative solution to an old problem. Brassicaceae plants have been widely used and studied for biofumigation because they produce a group of secondary anionic metabolites called glucosinolates (GSLs), which release bioactive gases such as isothiocyanates after enzymatic hydrolysis (Gimsing and Kirkegaard, 2009; Ntalli and Carboni, 2017; Maina et al., 2020). So far, over 130 GSL structures have been discovered and validated in the entire Brassicaceae order (Blaževi' and others, 2020). The family Brassicaceae (Cruciferae) consists of 350 genera and approximately 3.500 species, including the genera Arabidopsis, Brassica, Camelina, Crambe, Raphnus, Sinapis and Thlaspi (Abu Ghannam and Jaiswal, 2014; Fratianni et al., 2014). Research on the use of Brassicaceae crops, their residues, and their breakdown products, acting as bionematicides, started in the 1930s (Smedley, 1939). Today, Brassicaceae plants are still attracting renewed interest, mainly for their pest control properties and especially as alternatives to toxic nematicides, which are being phased out due to animal, human and environmental concerns (Cardoza and Stewart, 2004). Brassicaceae crops are particularly known for their biofumigation effects as a result of mowing and incorporation of aerial plant parts into the upper soil layers as soil amendments (Cardoza and Stewart, 2004), as well as the poor host status of several species used as cover and/or rotation crops to reduce populations of various nematode pests (Hendrika et al., 2016). The aim of this study was to gain knowledge that would allow the development of alternative approaches to chemical nematicides, by evaluating the biofumigation effects of wild radish (Raphanus raphanustrum) and field mustard (Sinapis arvensis) against a chemical nematicide (Tefluthrin 1.5%) on some plant-parasitic nematodes under field conditions.

2. Materials And Methods

Two Brassicaceae, wild radish (Raphanus raphanustrum), and mustard (Sinapis arvensis) were randomly collected in the morning during the flowering phase in April (2019). The collections were made at different farm farm-level locations in Boumerdes, Algeria (36°78′71″N, 3°30′90″E). The day after harvesting, the samples, which are complete plants, are placed in paper bags. The samples were then taken to the laboratory. The plants were then divided into their aerial parts (leaves, stems, and flowers) and their underground parts (roots). The samples were spread out on white paper and left to air dry in the laboratory at room temperature, protected from light and moisture. After drying, the samples are crushed in a porcelain mortar and then finely ground using an electric mixer. The resulting powder is collected and stored in sterile, hermetically sealed bottles at room temperature and protected from light until use.

Field Experiments

An in situ experiment was initiated on uncultivated soil, our study was carried out on a private farm in Boumerdes (36°69′63″N, 3°.31′51″E). The field experiment was set up to investigate the nematicide

effect of all treatments applied (biofumigation of wild radish (Raphanus raphanustrum); biofumigation of field mustard (Sinapis arvensis); nematicide treatment (Tefluthrin 1.5%); control treatment (no treatment)) on a loamy soil with 39% clay, 43% loam and 18% sand, the organic carbon content of 1.3% and pH-KCl of 7.4. Four replications were factorially combined in arandomized complete block design with a split-plot arrangement for a total of 16 (1.5 x 3 m) subplots. There was a 1 m space between each crossed plot. The plant powder of two crucifer species was applied at a rate of 400 g per square meter and incorporated at a depth of 10 cm by mechanical means using randomized a rotary cultivator. After incorporation, the subplots were moistened with 18 mm of water to facilitate hydrolysis. The soil was then compacted with a roller to seal the soil surface. The subplots were covered with black polyethylene plastic film, which remained in place during the 14-day incubation period (De Cauwer et al., 2019) to minimize fumigant emissions (Wang et al., 2014). Following the burial, the soil was covered for four weeks (Yim et al., 2016). Tefluthrin 1.5% was used as a nematicide at a recommended concentration of 10 g/m2, combined with 500 g of sand to aid dispersion, without covering. The control plots were not treated. The 4 plots studied included 32 samples taken before and after incubation. A soil sample of 20 cm2 and 15 cm depth was collected from each. The samples were collected with a hoe. The soil samples were placed in sealed plastic bags with an information sheet and delivered to a laboratory on the same day as they were collected.

Extracting Nematodes from Soil Samples

The extraction of the nematodes was carried out according to the technique of Dalmasso (1966). After several soi+ washes and siftings, the nematodes were removed. The nematodes separated from the sample carried out in duplicate in a completely randomized design with four replicates in each of the treatments. XLSTAT 2021 version 2.10 was used for statistical analysis of the data using one-way ANOVA. Means were compared by Duncan's multiple range test at P=0.05 (Duncan, 1955).

3. Results and Discussion Climatic conditions

Average growing season (February to June) air temperature was 99 and 108% of the long-term (1991-2020) average of 16.33°C in 2019 to 2020, respectively, and total growing season precipitation was 54.4 and 77.5% of the long-term average of 235.07 mm in 2019 to 2020 (Table 1). As a result, relatively dry soil conditions were present in February and May in both 2019 and 2020 when field mustard and wild radish powders were incorporated.

Table 1. Average daily mean air temperature and total precipitation during the 2019-2020 growing season in Boumerdes, Algeria between 2019 and 2020 in comparison to the long-term average (1990-2020). Climatic data were obtained from the Algerian National Office of Meteorology (ANOM, 2021).

	Average air temperature (C°)		perature (C°)	Total precipiation (mm)		
Month	2019	2020	1990-2020	2019	2020	1990-2020
Feb.	10,80	13,00	11,19	18,29	0,00	60,75
Mar.	13,20	15,00	13,42	37,84	55,11	58,87
Apr.	15,40	16,70	15,52	42,41	118,63	53,90
May	18,50	20,50	18,81	21,08	5,09	46,36
June	23,10	23,20	22,72	8,37	3,30	15,19
Average or total	16,20	17,68	16,33	127,99	182,13	235,07

Nematode population density

In this study, we found 8 nematode genera throughout all samples, including 7 taxa classified as phytoparasitic nematodes (PN), fungivorous nematodes (FN), omnivorous predator nematodes (OPN), represented by *Dorylaimidae*, and other nematodes (ON), mainly composed of bacterivorous nematodes (Fig. 1). However, we focused only on the phytoparasitic nematodes that are the most harmful to cultivated plants (*Tylenchorhynchus*, *Pratylenchus*, *Paratylenchus*, *Tylenchus*). In this study, when the abundance of nematodes was examined for each trophic category, significant differences between phytoparasitic nematodes and other trophic groups were found, and the higher overall nematode abundance was mainly caused by the high abundance of plant-parasitic nematodes (3215).

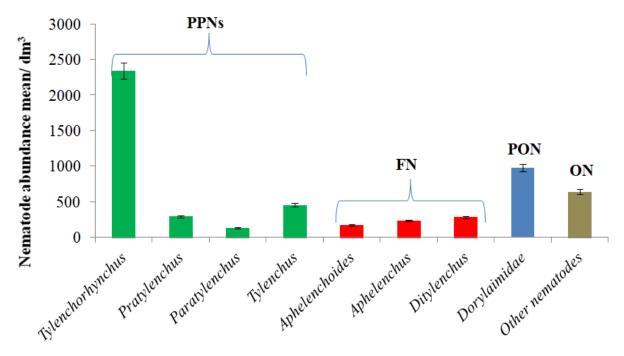


Fig. 1: The abundance mean of identified species of filiform nematodes.

FN: Fungivores nematodes, PPN: Phytoparasitic Nematodes, PON: Predator-Omnivore nematodes, ON: Other nematodes.

Among the four trophic groups, the fungivore groups *Aphelenchus*, *Aphelenchoides*, and *Ditylenchus* had the lowest mean relative abundances. They are 4.26%, 5.08%, and 3.08% respectively. The genus *Tylenchorhynchus* is the most represented (42.43%) of the four taxa found, followed by *Tylenchus* (8.25%), *Pratylenchus* (5.26%), and *Paratylenchus* with (2.36%). The relative abundance of the phytoparasitic group was much higher (58.30%). There are 17.68% of the predatory nematodes *Dorylaimidae* (Table 2).

Table 2. Mean absolute abundance and relative / group abundance (%) of the identified taxa

T	Absolute	Relative/group		
Trophic group	abundance mean	abundance (%)		
FN	685	12,42		
Aphelenchoides	170	3,08		
Aphelenchus	235	4,26		
Ditylenchus	280	5,08		
PN	3215	58,30		
Tylenchorhynchus	2340	42,43		
Pratylenchus	290	5,26		
Paratylenchus	130	2,36		
Tylenchus	455	8,25		
PON	975	17,68		
Dorylaimidae	975	17,68		
ON	640	11,60		
Total	5515	100		

FN: Fungivorous nematodes, PN: Phytoparasitic Nematodes, PON: Predator-Omnivore nematodes, ON: Other nematodes.

Phytoparasitic nematode population abundance under different treatments

The abundance of each nematode group encountered in the different plots varied considerably between treatments (Fig. 2). Species of *Tylenchorhynchus*, *Tylenchus*, *Pratylenchus*, and

Paratylenchus were detected in all plots studied ubiquitously before treatment relatively identical with a strong dominance of *Tylenchorhynchus*, in a lesser way (*Tylenchus*, *Pratylenchus*, *Paratylenchus*). After treatment, the abundance of the various nematode groups changes. There is a strong decrease in the numbers of *Tylenchorhynchus* and a slight decrease in the abundance of other nematodes in the treated plots.

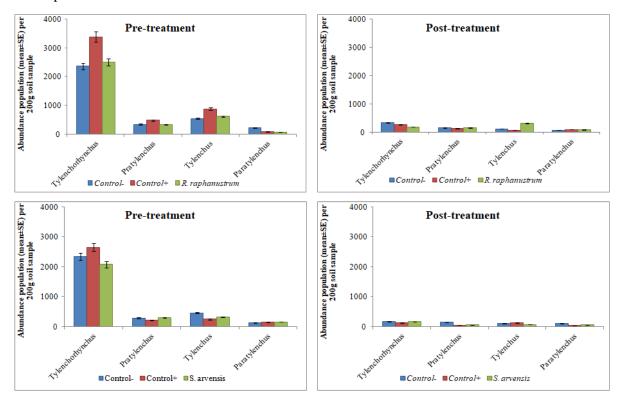


Fig 2: Effect of different treatments on phytoparasitic nematode abundance under field conditions, 2019-2020 season.

Tylenchorhynchus were significantly more abundant in treated and control plots (p<0.001). Where no significant differences in abundance were found between other nematode species, the control plots were negative (Table 3).

Table 3: Abundance (mean± SE) of phytoparasitic nematodes under diffrent treatments

	Abundance	%		
Pytoparasitic nematodes	Before treatment	After treatment	of reductions	
Tylenchorhynchus	2340 ±137a	195,625± 18a	92	
Paratylenchus	398,813±115 b	$103,125\pm14b$	74	
Pratylenchus	296,250±38b	$99,375 \pm 8b$	67	
Tylenchus	242,750±32b	81,250 ±5b	67	
P-value	0,0001	0,001		

Means followed by different superscript letters within the same column indicate significant differences calculated using a one-way ANOVA with post-hoc Tukey HSD test (n = 5), p < 0.05.

As part of our study, we adopted the General Linear Model (GLM) to evaluate the temporal variation of the structure of the abundance of the different nematode species according to the treatments: vegetable powder (*R. raphanustrum* and *S. arvensis*) and positive control (Tefluthrin 1.5%) and negative control (no treatment). This model makes it possible to study the strict and individual effects of the various factors, without considering the interactions between them. Based on the results obtained, we find that trophic group factors (F-ratio=261.291, p=0.000, p<1‰), treatments (F-ratio=9.546, p=0.000, p<1‰), follow-up periods (F-ratio=687.592, p=0.000, p<1‰) and even their interaction (F-ratio=2.407, p=0.000, p<1‰) show a very highly significant difference in nematode abundance (Table 4).

Table 4: The abundance of phytoparsitic nematodes under different treatments using G.L.M. and ANOVA models.

Source	sum of squares	df	Mean Square	F-ratio	P
Nematodes	2,65393E+07	8	3317417,667	261,291	0,000***
Treatments	242405,333	2	121202,667	9,546	0,000***
Times	8729832,296	1	8729832,296	687,592	0,000***
Trophic group x Treatments	921742,667	16	57608,917	4,537	0.000***
Trophic group x Time	2,21664E+07	8	2770802,296	218,238	0,000***
Treatments x Time	62393,481	2	31196,741	2,457	0.089*
Trophic group x Treatmentsx Time	489007,852	16	30562,991	2,407	0,000***
Error	2056792,000	162	12696,247	-	-

^{*:} Significant probability at 5%, **: Significant probability at 1%, ***: Significant probability at 1 %.

No significant differences were observed in the abundance of phytoparasitic nematodes before treatment. Regarding the temporal effect (pre- and post-treatment) of the different treatments applied, nematode abundance decreased significantly after each treatment application. As shown in Figure 3, plots treated with *S. arvensis* and control + (tefluthrin 1.5%) had the lowest abundances, showing the effectiveness of these treatments. In terms of their ability to reduce the nematode population, there were no significant differences between *R. raphanustrum* and the negative control (Fig. 3).

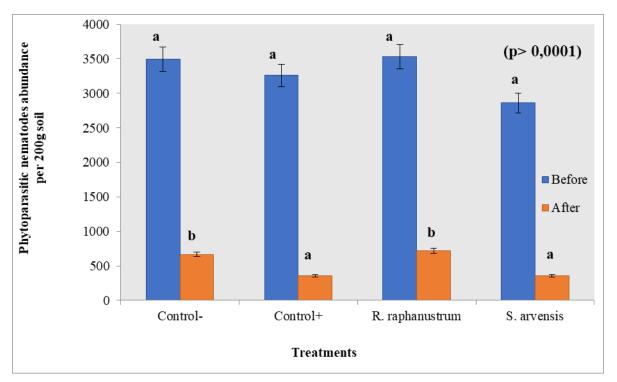


Fig 3 : The abundance of different phytoparasitic nematode populations (per 200 g soil sample) before (Bt) and after (At) treatment according to treatments.

It is expected that dry soil conditions at the time of field mustard and wild radish incorporation in each of the experiments in this study will reduce the effectiveness of the biofumigation process (Mattner et *al.*, 2008, Omirou et *al.*, 2013). More favorable soil characteristics (temperature, pH, water content, organic matter, and nutrient availability) (Westphal et *al.*, 2017) and weather conditions or areas where irrigation can be used to moisten the soil before mustard incorporation (Chen et *al.*, 2022) may have a greater positive effect on biofumigation effectiveness. Nematodes are

ubiquitous and functionally diverse (Bongers and Ferris 1999), and their communities are composed of different species that can be classified into five trophic groups based on their feeding habits: bacterivorous, fungivorous, herbivorous, omnivorous, and predatory (Yeates et al., 1993; Wasilewska, 1997). As a result of changes in ecological and edaphic conditions, the diversity of living organisms, including nematodes, varies with geographical location (Hanel, 1993). The structure of nematode communities, e.g. abundance and diversity, species distribution, and related ecosystem functions, are mainly influenced by vegetation, edaphic conditions (soil moisture, chemical, and physical characteristics), and climate elements like temperature and precipitation (Neher et al, 2003; Nielsen et al., 2014; Song et al., 2017; Thakur et al., 2019), which are important environmental factors for the survival and reproduction of nematodes (Song et al., 2017; Thakur et al., 2019). Climate change can have significant impacts on the abundance and community composition of soil nematodes (Thakur et al., 2017; Siebert et al., 2019). This is because they are essentially aquatic animals that depend on the water film around soil particles for their development and movement through their environment (Griffiths and Caul, 1993). Although there has been considerable research on the effects of temperature and rainfall on nematode diversity (Nielsen et al., 2014; Song et al., 2017; Thakur et al., 2019; Da Silva et al., 2020; Nisa, 2021; Kiptoo et al., 2022; Kitagmi, 2022), the effects of temperature and rainfall on nematode diversity are not well understood. The relative abundances of phytoparasitic nematodes were negatively associated and the shift in relative abundance was best explained by mean annual temperature, with plant parasites dominating warm sites (Procter, 1984). In addition, plant-parasitic nematodes require free moisture to develop and live in hygroscopic water around soil particles and surrounding plant tissues (Bridge and Starr, 2007). An increase in nematode mobility with water flow in the soil pore space facilitates the potential for nematodes to reach plant roots (Fujimoto et al., 2010). Considering that we used uncultivated soil for our test, the predatoromnivore nematodes (975) were the second most abundant trophic group after plant parasitic nematodes. This is because predatory and omnivorous nematodes are more sensitive to environmental perturbations (Bongers, 1990; Bongers and Bongers, 1998; Georgieva et al., 2002; Sánchez-Moreno et al., 2006; Zhao and Neher, 2013; Li et al., 2016). A large number of biotic and abiotic elements have an influence on nematode populations in the soil. Plant parasitic nematodes require significant moisture and aeration from absorbed soil water films (Dropkin, 1980; Kim, 2015). According to Mateille et al. (2014) and Palomares-Rius et al. (2015), soil texture and particle size are important in determining PPN abundance and richness (Norton, 1989; Mateille et al., 2014). The proportion of sand, clays, and silts can affect water retention and also the number of minerals and organic matter present (Saxton and Rawls, 2006; Mateille et al., 2014). This has implications for plant growth and thus food quality for PPNs, but also for movement through the water film around soil particles, stimulated by the retention of root exudates that enable nematodes to locate roots (Prot and Van Gundy, 1981). Our results showed that the species Tylenchorhynchus, Tylenchus, Pratylenchus, and Paratylenchus were abundant in sandy clay loam at pH 7.4, which is consistent with previous studies. The abundance of Tylenchorhynchus had a positive correlation with pH (Liang, 2000). Pratylenchus sp. abundance was reported to be negatively correlated with pH, suggesting that Pratylenchus may be more abundant at lower than higher pH (Spaull et al., 2001; Kawanobe et al., 2020). Previous studies have shown that *Pratylenchus* abundance is significantly influenced by soil texture (Griffin, 1996). Pratylenchus penetrans was found in loamy sands, and these species were reportedly less abundant in heavy soils (Szczygieł et al., 1983; Szczygieł and Zepp, 2004; Chen et al., 2012). According to Van Gundy et al. (1964), Tylenchulus semipenetrans were able to reproduce successfully on soils with a clay content of between 10% and 15%. This may explain the low abundance of Tylenchulus in the studied plots with their high clay content (38%). However, the relationship between pH, clay content, and the abundance of plant-parasitic nematodes was not demonstrated in our study. Several biotic and abiotic elements have an influence on nematode populations in the soil. Plant-parasitic nematodes require significant moisture and aeration from absorbed soil water films (Dropkin, 1980; Kim, 2015). The response to biofumigation found in this study is similar to that found in previous studies. A significant reduction in Pratylenchus sp. of 48-72% has been reported with the use of Raphanus sativus (Al-Rehiayani et al., 1999; Hartsema et al., 2005). Mazzola et al., (2007, 2009); Zasada et al., (2009) using Sinapis alba showed that mustard crops were effective in suppressing Pratylenchus sp. 28-90%. Worldwide, it has been shown that various activities of biofumigants have demonstrated nematicidal efficiency against different types of plant parasitic nematode populations to acceptable levels (Lazzeri, et al., 2004, Ramirez et al., 2009; Ngala et al., 2015; Chowa'nski et al., 2016; Fourie et al., 2016; Dutta and al., 2019; Maina et al., 2020; Morris et al., 2020). Brassica plants have a highly effective and specialized chemical defense mechanism which relies on the development of a family of secondary metabolic products known as glucosinolates (GSL) (Ntalli and Caboni, 2017; Maina et al., 2020). GSLs have nematicidal activity (Lazzeri et al., 1993; Riga, 2011; Ntalli and Caboni, 2017). The use of Brassicaceae cultivars and species with different GSL content in their tissues affects the efficiency of biofumigation in reducing nematode pest populations (Craig et al., 2005) and the soil characteristics at the time of incorporation of plant residues into the soil (e.g. soil moisture, temperature and pH) (Kirkegaard et al., 1998; Bending and Lincoln, 2000; Wood et al., 2017). This variability is due to the many biological and physical factors that have an impact on the effectiveness of biofumigation (Motisi et al., 2010). Furthermore, research has found a relationship between the reduction of nematode pest populations and the amount of Brassicaceae green manure incorporated into soils (Lazzeri et al., 1993; Motjahedi et al., 1993; Kwerepe and Labuschagne, 2003; Lopez-Perez et al., 2005; Matthiessen and Kirkegaard, 2006; Youssef and Lashein, 2013). In addition, for biofumigation to be effective, the biofumigant must be evenly distributed throughout the soil profile in which the target nematode pest(s) are present (Morra and Kirkegaard, 2002; Roubtsova et al., 2007). Nematicidal activity via Brassicaceae biofumigation techniques is considered a biological alternative to conventional soil fumigation (Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006; Laegdsmand et al., 2007; Clarkson et al., 2015). The suppressive effect of Tefluthrin on PPNs population densities matched what has been achieved using other fumigants or nematicides (Rowe and Powelson, 2002; Hassan, 2020; Chen, 2022). As the current study did not include the phytochemical characterization of the two plants studied (glycosinolate content), the exact reason for this was not clear.

4. Conclusion

The present study is the first field-scale demonstration that biofumigation is as effective as tefluthrin fumigation in the suppression of PPNs. Biofumigation with S. arvensis mustard may be a potential alternative to fumigation due to its favorable effect in reducing various PPNs populations, particularly in fields with high *Tylenchorhynchus* populations and improving crop health. However, biofumigation with *R. raphanustrum* did not respond well to the plant parasitic nematode populations studied. The study recommends that in both conventional and organic farming systems, biofumigation with mustard (*S. arvensis*) green manure can be used as part of an integrated pest management (IPM) programme. Therefore, knowledge of the glucosinolate content of the plants that are to be used for biofumigation is crucial. This requires knowledge of GSL and ITC production and systematic field research through analytical studies. In addition, the appropriate source and quality of organic matter to improve soil quality and suppress the growth of parasitic nematodes should be investigated in soil management research.

Abbreviations

ANOVA: Analysis of variance; FN: Fungivores nematodes; GSL: Glycosinolate; IPM: pest management program; ITC: Isocyanate; ON: Other nematodes; PN: Phytoparasitic Nematodes; PON: Predator-Omnivore nematodes; PPNs: plant parsite nematodes; *R. raphanustrum: Raphanus raphanustrum*; *S. arvensis: Sinapis arvensis*.

Acknowledgements

Not applicable

Conflict of Interest

The Authors declare that there is no conflict of interest

Authors' Contribution Statements

SZ has onceived and designed the experiment, collected the data, analyzed the data and wrote the paper, HK and AB carried out the biocontrol experiment. ZED corrected the paper. All authors have read and approved the final manuscript.

References:

- Abad P, Gouzy J, Aury J M, Castagnone-Sereno Pm:, Danchin E G J, Deleury E, Perfus-Barbeoc L, Anthouard V, Artiguenave F, Blok V C, Caillaud M C, Coutinho P M, Dasilva C, De Luca F, Deau F, Esquibet M, Flutre T, Goldstone J V, Hamamouch N, Hewezi T, (2008). Genome sequence of the metazoan plant-parasitic nematode Meloidogyne incognita. Nature Biotechnology, 26: 909–915.
- Abu-Ghannam, N. and Jaiswal, A.K., (2014). Enzymatic extraction of high-value ingredients from food waste. Presented at "Waste not Want not Recovering Value from Food Waste". Conference paper.Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland.
- Al-Rehiayani, S., Hafez, S.L., Thornton, M., Sundararaj, P., (1999). Effects of Pratylenchus neglectus, Bacillus megaterium radish or rapeseed and oil green manure on reproductive potential of Meloidogyne chitwoodi on potato. Nematropica 29: 37-49.
- Bending, G.D. and Lincoln, S.D. (2000). Inhibition of soil nitrifying bacteria communities and their activities by glucosinolate hydrolysis products. Soil Biol. Biochem. 32: 1261–1269. https://doi.org/10.1016/s0038-0717-(00)00043-2.

- Bongers, T., Bongers, M., (1998). Functional diversity of nematodes. Appl. Soil Ecol. 10: 239–251. https://doi.org/10.1016/S0929-1393(98)00123-1.
- Bongers, T. and Ferris H. (1999). Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution 14:224-228. https://doi.org/10.1016/s0169-5347(98)01583-3.
- Bongers, T., (1990). The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14–19. https://doi.org/10.1007/BF00324627.
- Bridge, J., Starr, J.L., (2007). Plant nematodes of agricultural importance: A colour handbook. Manson Publishing, London, UK.
- Brown, P.D., Morra, M.J., (1997). Control of soil-borne plant pests using glucosinolate containing plants. Adv. Agron. 61: 167-231.
- Cardoza, V., Stewart Jr., N.C., (2004). Brassica biotechnology: progress in cellular and molecular biology. In Vitro Cell. Dev. Biol. 40: 542-551.
- Chen, S.Y., Sheaffer, C.C., Wyse, D.L., Nickel, P., Kandel, H. (2012). Plant-parasitic nematode communities and their associations with soil factors in organically farmed fields in Minenesota. J. Nematol. 44: 361–369.
- Chen D., Zebarth B.J., Goyer C., Comeau L.P., ahar K., Dixon T. (2022) Effect of Biofumigation on Population Densities of Pratylenchus spp. and Verticillium spp. and Potato Yield in Eastern Canada. American Journal of Potato Research. 99(3):229-242. https://doi.org/10.1007/s12230-022-09875-2
- Chowa'nski, S.; Adamski, Z.; Marciniak, P.; Rosia'nski, G.; Büyükgüzel, E.; Büyükgüzel, K.; Falabella, P.; Scrano, L.; Ventrella, E.; Lelario, F. (2016). Review of Bioinsecticidal Activity of Solanaceae Alkaloids. Toxin 8: 1–28.
- Clarkson J., Michel V., Neilson R., (2015). Biofumigation for the control of soil-borne diseases. Mini-paper. EPI-AGRI. Soil-borne disease. ec.europa.eu .1-7.
- Craig, S.C., Saxton, A.M., Sams, C.E., (2005). Relationship of climate and genotype to seasonal variation in the glucosinoate myrosimaze system. I. Glucosinolate content in ten cultivars of Brassica oleracea grown in fall and spring seasons. J. Sci. Food Agric. 85: 671-681.
- Da Silva J.V.C.L., Hirschfeld M.N.C., Cares J.E., Esteves A.M., (2020). Land use, soil properties and climate variables influence the nematode communities in the Caatinga dry forest, Appl. Soil Ecol. 150 :103747. https://doi.org/10.1016/j. apsoil.2019.103474. pp:
- Dalmasso A., (1966). Méthode simple d'extraction des nématodes du sol. Rev. Écol. Biol. Sol, 3:473-478.
- Daneel M., Engelbrecht E., Fourie H. et Ahuja P., (2018). The host status of Brassicaceae to Meloidogyne and their effects as cover and biofumigant crops on root-knot nematode populations associated with potato and tomato under South African field conditions. Crop protecton .110: 198-206. https://doi.org/10.1016/j.cropro.2017.09.001.
- De Cauwer B, Vanbesien J, De Ryck S, Reheul D (2019). Impact of Brassica juncea biofumigation on viability of propagules of pernicious weed species. Weed Research. https://doi.org/10.1111/wre.12358.
- Dropkin V.H., (1980). Introduction to Plant Nematology. John Wiley & Sons, New York. 293.
- Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11:1–41.
- Dutta, T.K.; Khan, M.R.; Phani, V. (2019). Plant-parasitic nematode management via biofumigation using brassica and non-brassica plants: Current status and future prospects. Curr. Plant Biol., 17: 17–32. https://doi.org/10.1016/j.cpb.2009.02.001
- Epstein, S. (2014). Cognitive-experiential theory: An integrative theory of personality. Oxford University Press. Ferris, H., (2010). Contribution of nematodes to the structure and function of the soil food web. J. Nematol. 42: 63-67. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3380510
- Fourie H., Ahuja P., Lammers J., Mieke Daneel M., (2016).Brassicacea-based management strategies as an alternative to combat nematode pests: A synopsis. Crop Protection 80 :21-41. http://dx.doi.org/10.1016/j.cropro.2015.10.026
- Fratianni, F., Cardinale, F., Cozzolino, A., Granese, T., Pepe, S., Riccardi, R., Spigno, P., Coppola, R., Nazzaro, F., (2014). Polyphenol composition and antioxidant activity of two autochthonous Brassicaceae of the Campania region, Southern Italy. Food Nutr. Sci. 5:, 66-70.
- Fujimoto, Taketo, Hasegawa, Shuichi, Otobe, Kazunori, Mizukubo, Takayuki, (2010). The effect of soil water flow and soil properties on the motility of second-stagejuveniles of the root-knot nematode (Meloidogyne incognita). Soil Biol.Biochem. 42 (7): 1065–1072.
- Georgieva SS, McGrath SP, Hooper DJ, Chambers BS. (2002). Nematode communities under stress: The long-term effects of heavy metals in soil treated with sewage sludge. Applied Soil Ecology.20: 27–42. https://doi.org/10.1016/S0929-1393(02)00005-7

- Gimsing, A.; Kirkegaard, J., (2009). Glucosinolates and biofumigation: Fate of glucosinolates and their hydrolysis products in soil. Phytochem. Rev., 8: 299–310. https://doi.org/10.1007s11101-008-9105-5
- Griffiths, B.S., Caul, S., (1993). Migration of bacterial-feeding nematodes, but not protozoa, to decomposing grass residues. Biol. Fert. Soils 15: 201–207. https://doi.org/10.1007/BF00361612.
- Handiseni M, Cromwell W, Zidek M, Zhou G, Jo Y K. (2017). Use of brassicaceous seed meal extracts for managing root-knot nematode in bermudagrass. Nematropica, 47, 55–62.
- Hanel L., (1993). Diversity of soil nematode (Nematoda) in various types of ecosystems. Ekologia (Bratislava). 259 272.
- Hansen Z R, and Keinath A P., (2013). Increased pepper yields following incorporation of biofumigation cover crops and the effects on soilborne pathogen populations and pepper diseases. Applied Soil Ecology, 63, 67–77.
- Hartsema, O.H., Koot P Molendijk, L.P.G., Berg, W van den, Plentinger, M.C., Hoek, J., (2005). Rotatieonderzoek Paratrichodorus teres (1991-2000) PPO, p. 85.
- Hassan S, Al-Gendy AA, Abdel-Baset SH, Abd El-Kareem SM, Massoud IS, Abdallah MY (2020).

 Biofumigation Potentials of Some Brassica Crops to Control Root-knot Nematodes Meloidogyne spp., on Tomato under Field Conditions. J Plant Pathol Microbiol. 11:527. https://doi.org/10.35248/2157-7471.20.12.227.
- Ibrahim S K, Traboulsi A F, El-Haj S. (2006). Effect of essential oils and plant extracts on hatching, migration and mortality of Meloidogyne incognita. Phytopathologia Mediterranea, 45: 238–246.
- Jones J. T., Haegeman A., Danchin E. G. J., Gaur H. S., Helder J., Jones M. G. K., Perry R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology, 14: 946–961.
- Kawanobe M., Sugihara S., Miyamaru N., Yoshida K., Nonomura E., Oshiro H., Toyota K., (2020). Distribution of root-lesion and stunt nematodes, and their relationship with soil properties and nematode fauna in sugarcane fields in Okinawa, Japan. Agronomy, 10 (6), 762. https://doi.org/10.3390/agronomy10060762
- Kirkegaard, J.A.; Sarwar, M. (1998). Biofumigation potential of brassicas I. Plant Soil, 201: 71–89. https://doi.org/10.1023/A: 1004364713152
- Kruger, D.H.M., Fourie, J.C., Malan, A.P., (2013). Cover crops with biofumigation properties for the suppression of PPN e a review. S. Afr. J. Enol. Vitic. 34 (2): 287-295.
- Kwerepe, B.C., Labuschagne, N., (2003). Biofumigation and soil solarization as integrated pest management (IPM) components for the control of root-knot nematode, Meloidogyne incognita (Kofoid and White Chitwood) on Bambara groundnut (Vigna subterranean (L.) Verdc.). J. Agric. 11: 56-63.
- Ladhalakshmi D., Madhubala R., Sundravadana S., Laha G. S., Krishnaveni D., Sangeetha G., Ragothuman G., (2015). Biofumigation in crop disease management. Sustainable crop disease management using natural products, 389-402. https://doi.org/10.1079/9781780643236.0389
- Lazzeri, L.; Curto, G.; Leoni, O.; Dallavalle, E. (2004) Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode Meloidogyne incognita (Kofoid et White) Chitw. J. Agric. Food Chem., 52: 6703–6707. https://doi.org/10.1021/jf030776u
- Leite, I. C. H.; Lopes, U. P. (2018). Controle químico de patógenos radiculares. In: LOPES, U. P.; MICHEREFF, S. J. (eds). Desafios do Manejo de Doenças Radiculares Causadas por Fungos. Recife: EDUFRPE. Cap. 11, p. 179-192. https://repository.ufrpe.br/handle/123456789/2341
- Li, N., Pan, F.J., Han, X.Z., Zhang, B., (2016). Development of soil food web of microbes and nematodes under different agricultural practices during the early stage of pedogenesis of a Mollisol. Soil Biol. Biochem. 98: 208–216. https://doi.org/10.1016/j.soilbio.2016.04.011
- Matthiessen, J.N.; Kirkegaard, J.A. (2006). Biofumigation and enhanced biodegradation: Opportunity and challenge in soilborne pest and disease management. Crit. Rev. Plant Sci., 25: 235–265. https://doi.org/10.1080/07352680600611543
- Mazzola M.; Brown J.; Izzo, A.D.; Cohen M.F., (2007). Mechanismof action and efficacy of seedmeal-induced pathogen suppression differ in a Brassicaceae species and time-dependentmanner. Phytopathology, 97:454–460. https://doi.org/10.1094/PHYTO-97-4-0454
- Mazzola, M.; Brown, J.; Zhao, X.; Izzo, A.D.; Fazio, G. (2009). Interaction of brassicaceous seed meal and apple rootstock on recovery of Pythium spp. and Pratylenchus penetrans from roots grown in replant soils. Plant Dis., 93:51–57. https://doi.org/10.1094/PDIS-93-1-0051
- Morra, M.J.; Kirkegaard, J.A. (2002). Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biol. Biochem., 34: 1683–1690. https://doi.org/10.1016/S0038-0717(02)001513-0
- Morris E.K., Fletcher R. Stavros D. Veresoglou, (2020). Effective methods of biofumigation: a meta-analysis. Plant Soil https://doi.org/10.1007/s11104-019-04352-y

- Ngala B M, Haydock P P, Woods S, Back M A. (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: 759–769.
- Nielsen, U.N., Ayres, E., Wall, D.H., Li, G., Bardgett, R.D., Wu, T., Garey, J.R., (2014). Global-scale patterns of assemblage structure of soil nematodes in relation to climate and ecosystem properties. Glob. Ecol. Biogeogr. 23: 968–978. https://doi.org/10.1111/geb.12177.
- Ntalli N.G., Caboni P. (2012). Botanical nematicides: A review. J Agri Food Chem. 2012;60(40):9929-9940.
- Ntalli, N.G.; Caboni, P. A (2017). review of isothiocyanates biofumigation activity on plant parasitic nematodes. Phytochem. Rev., 16: 827–834. https://doi.org/10.1007/s11101-017-9491-7
- Ntalli N, Monokrousos N, Rumbos C, Kontea D, Zioga D, Argyropoulou M D, Menkissoglu-Spiroudi U, Tsiropolos N G. (2017). Greenhouse biofumigation with Melia azedarch controls Meloidogyne spp. and enhances soil biological activity. Journal of Pest Science, (91): 29–40.
- Nyczepir, A.P., Thomas, S.H., (2009). Current and future management strategies in intensive crop production systems. In: Perry, R.N., Moens, M., Starr, J. (Eds.), Root-knot Nematodes. Common wealth Agricultural Bureau International: 412-433.
- Omirou, M., Karpouzas D.G., Papadopoulou K.K., Ehaliotis C. (2013). Dissipation of pure and broccolireleased glucosinolates in soil under high and low moisture content. European Journal of Soil Biology 56: 49–55. https://doi.org/10.1016/j.scitotenv.2015.05.086
- Ramirez R A, Henderson D R, Riga E, Lacey L A, Snyder W E. (2009). Harmful effects of mustard biofumigants on entomopathogenic nematodes. Biological Control, (48): 147–154.
- Riga E. (2011). The effects of Brassica green manures on plantparasitic and free-living nematodes used in combination with reduced rates of synthetic nematicides. Journal of Nematology, 43: 119–121.
- Roubtsova, T., Lopez-Perez, J.-A., Edwards, S., Ploeg, A., (2007). Effect of Broccoli (Brassica oleraceae) tissue, incorporated at different depths in a soil column, on Meloidogyne incognita. J. Nematol. 39 (2): 111-117
- Rowe, R.C., and M.L. Powelson. (2002). Potato early dying: Management challenges in a changing production environment. Plant Disease 86: 1184–1193.
- Rudolph R E, Sams C, Steiner R, Thomas S H, Walker S, Uchanski M E. (2015). Biofumigation performance of four Brassica crops in a green chile pepper (Capsicum annuum) rotation system in southern New Mexico. Hort Science, 50: 247–253.
- Sánchez-Moreno, S., Minoshima, H., Ferris, H., Jackson, L.E., (2006). Linking soil properties and nematode community composition: effects of soil management on soil food webs. Nematology 8: 703–715. https://doi.org/10.1163/156854106778877857
- Siebert, J., Sünnemann, M., Auge, H., Berger, S., Cesarz, S., Ciobanu, M., Guerrero-Ramírez, N.R., Eisenhauer, N., (2019). The effects of drought and nutrient addition on soil organisms vary across taxonomic groups, but are constant across seasons. Sci. Rep. 9: 639. https://doi.org/10.1038/s41598-018-36777-3.
- Singh, S.K., Hodda, M., Ash, G.J., (2013). Plant-parasitic nematodes of potential phytosanitary importance, their main hosts and reported yield losses. EPPO Bull. 43:334-374.
- Smedley, E.M., (1939). Experiments on the use of isothiocyanates in the control of the potato strain of Heterodera schachtii (Schmidt). J. Helminthol. 17: 31-38.
- Song, D., Pan, K., Tariq, A., Sun, F., Li, Z., Sun, X., Zhang, L., Olusanya, O.A., Wu, X., (2017). Large-scale patterns of distribution and diversity of terrestrial nematodes. J. Appl. Ecol. 114: 161–169. https://doi.org/10.1016/j.apsoil.2017.02.013.
- Spaull, V.W.; Cadet, P., (2001). Nematodes and nutrients: Association between plant-parasitic nematodes and soil chemicals. In Proceedings of the Annual Congress-South African Sugar Technologists' Association, South African Sugar Technologists' Association, Durban, South Africa, 31 July–3 August 2001: 116–117.
- Stirling, G.R., (2014). Biological control as a component of integrated nematode management: the way forward. In: Stirling, G.R. (Ed.), Biological Control of PPN: Soil Ecosystem Management in Sustainable Agriculture, second revised edition. CABI Publishers: 393-407.
- Thakur, M.P., Tilman, D., Purschke, O., Ciobanu, M., Cowles, J., Isbell, F., Wragg, P.D., Eisenhauer, N., (2017). Climate warming promotes species diversity, but with greater taxonomic redundancy, in complex environments. Sci. Adv. 3:1700866. https://doi.org/10.1126/sciadv.1700866.
- Thakur, M.P., Real, I.M.D., Cesarz, S., Steinauer, K., Reich, P.B., Hobbie, S., Ciobanu, M., Rich, R., Worm, K., Eisenhauer, N., (2019). Soil microbial, nematode, and enzymatic responses to elevated CO2, N fertilization, warming, and reduced precipitation. Soil Biol. Biochem. 135: 184–193. https://doi.org/10.1016/j.soilbio.2019.04.020.

- Vervoort, M.T.W.; Vonk, J.A.; Brolsma, K.M.; Schütze, W.; Quist, C.W.; de Goede, R.G.M.; Ho_and, E.; Bakker, J.; Mulder, C.; Hallmann, J., (2014). Release of isothiocyanates does not explain the e_ects of biofumigation with Indian mustard cultivars on nematode assemblages. Soil Biol. Biochem., 68: 200–207. https://doi.org/10.1016/j.soilbio.2013.10.008
- Wang, Q., Ma, Y., Wang, G., Gu, Z., Sun, D., An, X., Chang, Z. (2014). Integration of biofumigation with antagonistic microorganism can control Phytophthora blight of pepper plants by regulating soil bacterial community structure. Eur. J. Soil Biol. 61:58–67. https://doi.org/10.1016/j.ejsobi.2013.12.004
- Wasilewska L., (1997). Soil invertebrates as bioindicators, with special reference to soil-inhabiting nematodes. Russian Journal of Nematology 5 (2), 113 126.
- Westphal A, Reidl K M, Cooperstone J L, Kamat S, Balasubramaniam V M, Schwartz S J, Bohm V. (2017). High-pressure processing of broccoli sprouts: Influence on bioactivation of glucosinolates to isothiocyanates. Journal of Agricultural and Food Chemistry, 65: 8578–8585
- Wood, C., Kenyon D.M., and Cooper J.M., (2017). Allyl isothiocyanate shows promise as a naturally produced suppressant of the potato cyst nematode, Globodera pallida, in biofumigation systems. Nematol. 19: 389–402.
- Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S., (1993). Feeding habits in soil nematode families and genera an outline for soil ecologists. J. Nematol. 25, 315–331.
- Yim, B., Hanschen, F.S., Wrede, A., Nitt, H., Schreiner, M., Smalla K., Winkelmann T. E. (2016) Effects of biofumigation using Brassica juncea and Raphanus sativus in comparison to disinfection using Basamid on apple plant growth and soil microbial communities at three field sites with replant disease. Plant Soil 406: 389-408. https://doi.org/10.1007/s11104-016-2876-3
- Youssef, M. M. A. and Lashein, A. M. S. (2013). Efficacy of different medicinal plants as green and dry leaves and extracts of leaves on root knot nematode, Meloidogyne incognita infecting eggplant.. Eurasian J. of Agri. and Environ. Medic., 2 (1): 10-14.
- Zasada I A, Halbrendt J M, Kokalis-Burelle N, LaMondia J, McKenry M V, Noling J W. (2010). Managing nematodes without methyl bromide. Annual Review of Phytopathology, 48: 311–328.
- Zasada, I.A., Meyer, S.L.F., Morra, M.J., (2009). Brassicaceous seed meals as soil amendments to suppress the plant-parasitic nematodes Pratylenchus penetrans and Meloidogyne incognita. J. Nematol. 41 (3): 221.
- Zhao, J., Neher, D.A., (2013). Soil nematode genera that predict specific types of disturbance. Appl. Soil Ecol. 64: 135–141. https://doi.org/10.1016/j.apsoil.2012.11. 008.