Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2023; 11(6): 60-65

Received: 20-10-2023
Accepted: 22-11-2023

Sowmya R
Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Pankaj
Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Anju Kamra
Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Corresponding Author:
Sowmya R
Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Biofumigation for nematode management: Advantages and limitations

Sowmya R, Pankaj and Anju Kamra

DOI: https://doi.org/10.22271/j.ento.2023.v11.i6a.9261

Abstract
Plant-parasitic nematodes are the foremost cause of yield loss around 12.3% ($157 billion) globally and 21.3% ($1.58 billion) nationally. The adverse effects of synthetic nematicides on the environment and public health have prompted a reassessment of non-chemical approaches for managing nematodes. One such approach is Biofumigation, wherein fresh plant biomass is incorporated into the soil and covered for two to three weeks with polythene to suppress soil-borne pests and pathogens. The mechanism of biofumigant is due to the release of volatile isothiocyanates by the hydrolysis of glucosinolates present in plants belonging to the Brassicaceae, Caricaceae, and Capparaceae. The production of volatile nematode antagonistic compounds by non-brassica plants expands the scope of Biofumigation. These compounds inhibit nematode movement, cripple the host's finding ability, and may also cause an ovicidal effect. Biofumigation is reported to effectively control fungal pathogens and weeds, improve soil properties, and enhance beneficial soil microorganisms. However, the approach has some limitations, like the unavailability of plant biomass in off-seasons and poor efficacy in dry soil and deeper layers of the soil. The beneficial entomopathogenic nematodes may also be reduced in the presence of a biofumigant. This technique can, however, be cost-effectively included in integrated nematode management for acceptable levels of nematode management.

Keywords: Brassicaceae, plant-parasitic nematodes, isothiocyanate and glucosinolates

Introduction
Plant parasitic nematodes, or PPNs, are small microscopic roundworms that primarily form an obligatory parasitic bond with their hosts. They are also known as the "unseen enemies" of plants due to the non-specific disease symptoms and frequently go unnoticed. Because PPNs are more well-adapted to a variety of agroclimatic zones, they are highly diversified and ubiquitous in all cropping systems. Annually, the percent loss of horticultural crops is around 21.3%, estimated to Rs 102,039.79 million ($1.58 billion); the losses in nineteen horticultural crops (banana, citrus, grapes, guava, papaya, pomegranate, bitter gourd, carrot, capsicum, chilli, cucumber, okra, tomato, bottle gourd, brinjal, and potato) were estimated at Rs 50,224.98 million. And in the case of eleven field crops (maize, rice, chickpea, castor, wheat, black gram, green gram, sunflower, cotton, jute, and groundnut), it was amounting at Rs. 51,814.81 million (Kumar *et al.*, 2020) [17]. Government regulations have gradually eliminated the usage of synthetic chemicals due to their detrimental impact on the environment (Warnock *et al.*, 2017) [34]. Due to tedious registration criteria at both the state and central levels in various countries, nematode management through fumigant or non-fumigant methods is constantly changing. Therefore, effective management is essential to ensure cost-effective crop production and maximize yields. Using bio-fumigants against plant parasitic nematodes is one such strategy.

History of Biofumigation
Biofumigation is the process of incorporating fresh plant biomass into the soil, which destroys soil-borne pathogens and pests by releasing several chemical substances (Kirkegaard *et al.*, 1993) [15]. At the beginning of the 17th century, the unique properties of Glucosinolates (GSLs) and isothiocyanates (ITCs) were observed. The fumigant action of volatile compounds released during organic matter biodegradation suppresses plant pathogens (Buena *et al.*, 2007) [6]. GSLs and ITCs are the key active compounds in Biofumigation.

Principles of Biofumigation

GL-MYR process is the production of glucosinolates which are sulfur-containing secondary metabolites by certain crop that are hydrolyzed by the enzyme myrosinase (MYR) to form ITCs (Fig. 1). Numerous soil-borne pathogens are toxically affected by ITCs. During the maceration of the plant biomass, damage or breaking of the plant cell walls releases the active component ITCs (Motisi *et al*., 2010) [23].

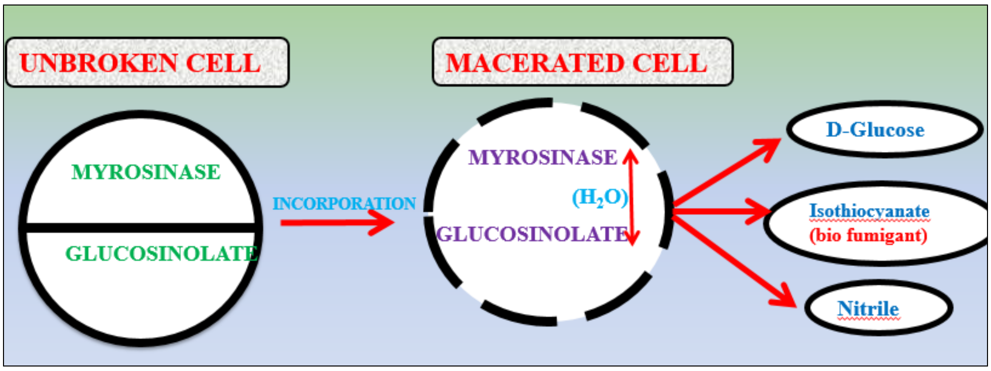

Fig 1: Process of Biofumigation

More than 350 genera and 3000 species constitute the Brassicaceae family, several of which have been experimentally shown to contain GSL. Conversely, GSLs are not exclusive to brassicas. Around 500 non-brassica species have been documented to possess one or more of the 120 well-known GSLs (Fahey *et al*., 2001) [10]. Each GSL has chemical properties and can be categorized into three types: aliphatic, aromatic and indole (Zasada & Ferris, 2004; Padilla *et al*., 2007) [36, 27]. Most of the genera belongs to the families Brassicaceae, Capparaceae, and Caricaceae contains GSL content (Dutta *et al*., 2019) [9]. Plants exhibit significant variation in the amount of GSL present in their cells. As a result, it is essential to identify species which are suppresses the soil-borne diseases and pests, including nematodes in effective manner. The majority of plant species that are typically considered for Biofumigation are found in the family Brassicaceae, which includes *Brassica oleracea* (cole crops: broccoli, cabbage, cauliflower, Brussels sprouts), *Raphanus sativus* (radish), and *B. napus* (canola, rapeseed), *Sinapis alba* (white mustard), *B. juncea* (Indian mustard), *B. rapa* (turnip), *B. campestris* (field mustard), *B. nigra* (black mustard), *B. carinata* (Ethiopian mustard) and *Eruca sativa* (salad rocket) (Sarwar *et al*., 1998; Ploeg, 2007) [31, 28] (Table 1).

Table 1: Commercially available Bio-fumigant plants

	Plants	Varieties	Country
Bio-fumigant	*Eruca sativus*	Nemat	Italy
	B. juncea	Nemfix	Australia
	B. juncea	Mustclean	Australia
	B. juncea	Fumus	Australia
	B. carinata	CT 207/ ISCI 7	Italy
	B. napus/ B.campestris	BioQure	New Zealand
	B. juncea	ISCI 99/ ISCI 61	Italy
Nematode resistant cover crop	*Raphinus sativus*	Adigo	Germany

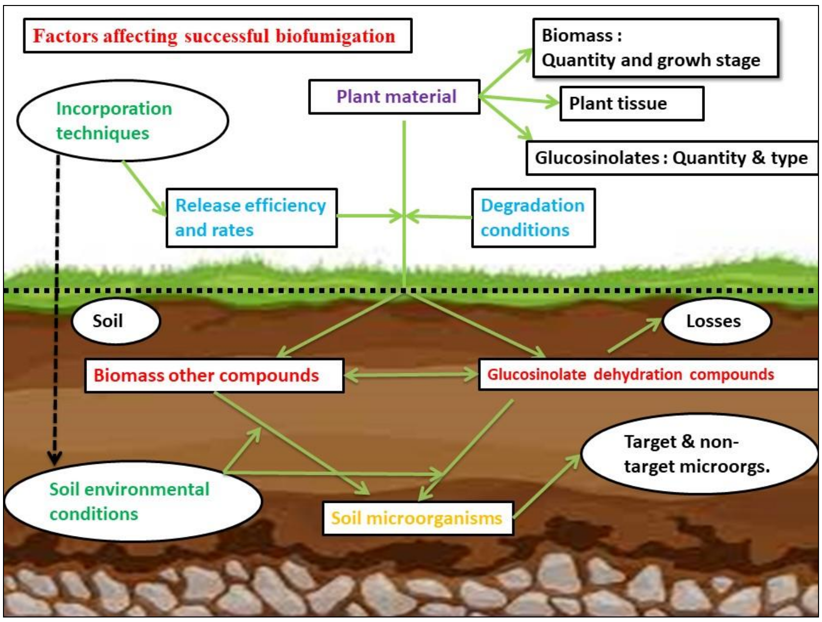

Fig 2: Interconnecting components of the Biofumigation process

While maximum dry matter production and elevated glucosinolate production occur towards the final stage of the growth phase, it is generally considered the ideal time for Biofumigation. A vital role played by the plant material is the release of an enormous amount of glucosinolate, besides various techniques for appropriate tissue breakdown have been examined (Matthiessen *et al.*, 2006) [20] (Fig. 2). It is also essential to understand the pathogen life cycle in the soil and the potential side effects on beneficial microorganisms. Finally, the amount, stage of growth, and type of plant tissue loss caused by soil microorganisms, soil environmental factors, and susceptibility of the target microorganism all influence biomass and other compounds involved in the degradation conditions. However, environmental factors such as the presence of specific ions or the pH of a solution sway myrosinase hydrolysis.

Table 2: Optimum conditions for the Biofumigation process

Factors	Optimum level	References
Temperature	20 – 25 °C	Lopez-Perez *et al.*, 2005 [19]
Moisture	50% of field capacity	Watts *et al.*, 2018 [33]
Stage of crop	Flowering stage	Fourie *et al.*, 2016 [11]
Incorporation methods	Tractor-drawn tissue pulverising instrument Cutting and Chopping method	Matthiessen *et al.*, 2006 [20]
Soil Texture	Clay loam (2.03% Organic matter and 25% clay)	Dutta *et al.*, 2019 [9]

For Biofumigation, it came to light that soil moisture of 50% field capacity and water-saturated soil performed more effectively than soil moisture of 25%, 75%, or 100% of field capacity. Low soil temperatures during Biofumigation reduce the enzymatic reaction; hence, it is not advised to incorporate green manure when the soil temperature is almost 0°C. The presence of organic matter appears to immobilize degradation products, inhibiting them from reaching the target pests. This happens due to an optimum water-to-air ratio of soil pores for volatile organic compound diffusion and retention (Watts *et al.*, 2018) [33]. The incorporation methods are a tractor-drawn tissue pulverizing instrument and the cutting-chopping method. In soil texture tests, sandy loam soil had the highest rate of volatilization compared to loamy soil. For small areas, solarization by covering the soil with transparent plastic sheet to trap sunlight and sequestering the nematotoxic chemical is an effective approach for raising the temperature in the soil (Blok *et al.*, 2000; Oka, 2010) [7, 26].

Mode of Application

Brassica plants and bio-fumigation for PPN control:

Cover cropping, utilization of whole plant or extracted plant products including the use of concentrated essential oils or distilled essences from the plants and commercially formulated defatted seed meal (Douda *et al.*, 2010; Meyer *et al.*, 2011) [8, 21]. At particular stage the plant materials should be incorporated into the soil. Then only the plant tissues decompose to release GLS, which are then hydrolyzed by enzymes to form isothiocyanates (Morra and Kirkegaard, 2002) [22]. GLS are also known as organic anions containing glucose and sulfur (Fahey *et al.*, 2001) [10]. These secondary metabolites are stored in S-cell vacuoles and are produced in large quantities by various Brassicaceae species (Westphal *et al.*, 2016) [35] (Table 3 & 4). A toxic isothiocyanate is produced during cell breakdown by the interaction between GSLs and the catalytic enzyme myrosinase. These chemicals are subsequently released into the soil (Kruger *et al.*, 2013) [18].

Table 3: Bio-fumigation using Brassica plants as slashing methods for PPN management

Biofumigant crops	Nematodes	Management status	Country
B.rapa, B. juncea, E.sativa	*M. incognita*	Increase in seedling height in tomato and reduction of galls	India
S. alba	*G. rostochiensis*	Decreased PPN and enhanced the growth of beneficial nematode	Belgium
B. juncea, S. alba, E. sativa	*M. javanica*	Suppression of *M. javanica*	South Africa
Cabbage	*Helicotylenchus Pratylenchus, Meloidogyne, Heterodera*	Reduction in infective juvenile population of PPNs	Kenya
Broccoli	*M. incognita, M. javanica*	Reduction of galls	USA

Table 4: Brassica plants as cover crops to manage PPN

Biofumigant crops	Nematodes	Management status	Country
S. alba	*G. rostochiensis, G. pallid*	Reduction in egg hatching of cysts	The Netherlands
B. juncea, B.rapa, B. napus, R.sativus	*M. incognita*	Improved yield and increase in juvenile mortality	USA
B. napus, R. sativus	*P. penetrans*	Nematode community remain unaltered until a year after cover crop growth	USA
S. alba, E. sativa	*M. incognita*	67% reduction of nematodes	Kenya

Seed meal as bio-fumigant to manage PPN

An additional approach for lowering PPN levels in the soil is to employ high-nitrogen seed meals from different brassica crops. Among many brassica crops, the other GSL compound is

- Sinalbin, or 4-hydroxybenzyl GSL, in *S. Alba*
- Sinigrin or 2-propenyl GSL in *B. carinata* and *B. juncea*
- 3-Butenyl GSL in *B. napus*

In the INM module, dazitol, a substance derived from mustard seeds and comprising up to 4.37% ITC, can be relied on as a substitute for commercial fumigants (Dutta *et al.*, 2019) [9].

Advantages of seed meal: The latter are not PPN hosts and can be readily integrated into the soil without incurring the risk of frost damage (Zasada *et al*., 2009) [37]. Higher concentration of GSL in defatted meals primarily sinigrin, speeds up the production of allyl ITC in soil.

Disadvantages of seed meal: Due to their scarcity and expensive cost, seed meals have endured as an unappealing alternative. (Zasada *et al*., 2009) [37]. In commercial crop production, the challenges of incorporating seed meal into a commercial crop's established root zone, such as raspberry, restricts the seed meal's utility (Gigot *et al*., 2013) [12].

Bio-Fumigation With Non-Brassica Plants To Manage PPN: The category of non-brassica plant material such as *Melia azedarach* (chinaberry) and *Azadirachta indica* (neem) (Barros *et al*., 2014) [2]. It is also known that nematotoxic cyanogenic glucosides are produced by other plants, including sudangrass (Sorghum sudanense), clover, and flax. Due to the production of linamarin, a cyanogenic glucoside, cassava roots have long been used by Brazilians as a nematode preventative.

Sorghum: In the vacuole of epidermal cells, dhurrin is typically present. Dhurrinase is an enzyme that hydrolyzes dhurrin to release glucose and the unstable p-hydroxymandelo nitrile, which is rapidly converted to the toxins hydrogen cyanide (HCN) and p-hydroxybenzaldehyde by the action of -hydroxynitrilelyase or at a basic $_{P}$H. As plants get older, their dhurrin concentration reduced (Bolarinwa *et al*., 2016) [3].
In peach orchards, to control the ring nematode, *Criconemoides xenoplax* (which predisposes to peach tree short-life disease), sorghum was utilized as a cover crop, rotation crop, and green manure. Because *C. xenoplaxis* may create the cyanide-degrading enzyme cyanoalanine synthase, sorghum's organic matter decomposition had a suppressive effect on nematodes in situ throughout the proliferation of hostile microorganisms. (Table 5). The effective disinfestation of *M. incognita* by soil amendment with a sorghum-sudan grass hybrid in tomato plants, was documented.

Table 5: Sorghum and sudan grass Biofumigation for PPN control

Biofumigant crops	Nematodes	Management status	Country
Sorghum	*C. xenoplax*	At the time of initial experimental stage reduction in nematode population	USA
Sudangrass	*M. chitwoodi,*	Reduced *M. chitwood,*	USA
Sorghum, Sudangrass	*M. chitwoodi, M. hapla*	Nematode populations is reduced	USA
Sorghum, Sudangrass	*M. incognita*	*in vitro* condition decreased egg hatching and reduction gall index in host plant	Italy

Marigold: Secondary metabolite in plants, α-terthienyl (thiophene-polyacetylenic sulfur compound), is abundant in the roots of marigold (Tagetes spp., Asteraceae or Compositae family). When α -terthienyl is photoactivated with near ultraviolet light (325-400 nm), reactive oxygen species are formed, which are phytotoxic to insects and nematodes (Table 6). Nevertheless, in the rhizosphere, irradiation of terthienyl (essential for nematode activity) may not take place in the absence of light. Due to the fact that Tagetes spp. development primarily causes endoparasitic nematode disruption, it was assumed that α-terthienyl was stimulated inside the living root system of Tagetes through mechanisms other than light.

Table 6: Marigold as bio-fumigant

Bio-fumigation crops	Nematodes	Host Plant	Pot study	Management status	Country
T. tenuifolia, T. lucida	*Tylenchorhynchus brassicae,*	Cabbage, cauliflower	Incorporate into the soil	Reduce the population of nematodes	India
T.patula	*M. incognita*	Tomato	soil amendment	Minimal impact on nematode population	USA

Advantages of Biofumigation

The mechanism of Biofumigation are to stimulate the antagonistic microorganisms in the soil and help in the release of nitrogenous compounds that are lethal to plant parasitic nematodes (PPNs). Biosolarization, which refers to the incorporation of soil with organic matter before the solarization, releasing organic acids helps in suppression of pest and disease, by including cytochrome P450 and soluble UDP (uridine diphosphate) glucosyl transferase. The advantage of brassica plants as cover crops is that they can significantly suppress the PPNs, other soil-borne pests (Brown, 1997) [5], pathogens, and weeds. By plummeting soil erosion, these cover crops may enhance soil fertility and structure (Nyczepir and Thomas 2009) [25]. Due to long term incorporation of organic matter, community-based soil food web analysis shows the abundance of bacterivores (enrichment index values) due to cover crops (Grabau *et al*., 2017) [13]. The Biofumigation of Brassicas leads to a proliferation in the beneficial nematode community in the soil profile (Valdes *et al*., 2012) [32]. Mustard seed meal amendments modify the soil bacterial community, produce nitric oxide by bacteria, and induce plant systemic resistance (Meyer *et al*., 2011) [21]. The proliferation of certain bacterial community, such as *Burkholderia* spp., *Defluvibacter* spp., *Rhodanobacter* spp., etc., are able to degrade recalcitrant chemicals, including herbicides, pesticides, and industrial wastes by the incorporation of Brassica seed meal. Biofumigation is considered an eco-friendly approach to control PPNs than chemical nematicides (Kruger *et al*., 2013) [18]. The amine and thiol groups of numerous enzymes, become irreversibly alkylated due to reactions in active site of ITC and nucleophiles of nematodes (Avato *et al*., 2013) [1]. ITCs show equal or greater nematicidal effects compared to synthetic ones.

Disadvantages of Biofumigation

Although seed meals are readily available, they are rather costly. The non-availability of plant biomass in the off-season. Application of mustard biofumigants exerted a destructive effect on entomopathogenic nematodes such as *Steinernema* and *Heterorhabditis* spp. (Henderson *et al*., 2009) [14]. Certain isolates of fungi, like *Aspergillus flavus*, compete with plant myrosinase during Biofumigation to release desulphoglucosinolates and nitriles that are less toxic than isothiocyanates. Comparatively ineffective to chemical products. Biofumigation with plants releases low-toxic gas so that the thick-walled pathogen escapes. Some of the Brassicaceae crops act as reservoir to PPNs, even though shows an allelopathic effect against them. The numerous cruciferous plants have been known to become infested by the majority of the commercially important PPNs. (Fourie *et al*., 2016) [11]. Brassicas' effectiveness in the olericulture sector is limited by their susceptibility to PPNs and the requirement of cultivating them during the winter. Biofumigation may have no effect on nematode populations that are found in deeper soil layers. (Rahman and Somers, 2013) [29], and these act as reservoirs for the succeeding season. The concentration of ITC varies with the cultivar, soil type, and temperature. In soil, isothiocyanates usually remain for 10 hr. ITCs negatively impact a variety of soil biota, and their unregulated emission can cause soil food webs to become unstable. (Grabau *et al*., 2017) [13].

Summary and future prospects

Regarding the increasing demand on chemical control alternatives for managing nematodes in a wide range of crops and the limited fumigation options accessible for use prior in crop planting, Biofumigation can be adapted. For a long period of time, it has been believed to possess untapped potential and has demonstrated positive outcomes when used correctly to manage nematodes. Understanding the intricate relationships and variables involved in Biofumigation is challenging. First and foremost, it suppresses the nematode and secondarily enhances the soil microflora. To achieve acceptable levels of nematode management, this technology can be simply and affordably integrated with other strategies, such as INM, bio-control agents, etc. More field research is needed, even though greenhouse experiments are essential for obtaining an improved evaluation of the suppression of nematode diseases by eliminating external factors. For the biofumigant crops to properly exhibit their nematotoxic qualities, the greenhouse temperatures may be too high. In field conditions, the precise positions and function of different nematicidal compounds in Tagetes spp. are poorly understood. Future research ought to look into the possibility of using multiple cultural strategies simultaneously in the same field. The factors including the existence of susceptible nematode stages. Further development and enhancement of the conventional knowledge about the utilization of Brassica intercropped with other vegetable crops that farmers are exposed to. The ideal planting period to maximize biomass output at the intended time is determined by the variables controlling the biomass used as a biofumigant of Indian cultivars with high GSL concentrations are identified and bred. Long-term research is desirable to understand the vulnerability of soil microbes towards Biofumigation strategy.

References

1. Avato P, D'Addabbo T, Leonetti P, Argentieri MP. Nematicidal potential of Brassicaceae. Phytochemistry Reviews. 2013;12(4):791-802.
2. Barros AF, Estupiñan-López L, Campos VP, Silva AP, Pedroso MP, Silva JC, *et al*. Volatile organic compounds from seed meal are toxic to *Meloidogyne incognita*. Tropical Plant Pathology. 2017;42(6):443-450.
3. Bolarinwa IF, Oke MO, Olaniyan SA, Ajala AS. A review of cyanogenic glycosides in edible plants. *Toxicology* - New Aspects to This Scientific Conundrum; c2016.
4. Brennan RJB, Samantha Glaze-Corcoran, Robert Wick, Masoud Hashemi. Biofumigation: An alternative strategy for the control of plant parasitic nematodes. Journal of Integrative Agriculture. 2020;19(7):1680-1690
5. Brown PD. Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy*. 1997;61:168-231.
6. Buena AP, García-Álvarez A, Díez-Rojo MA, Ros C, Fernández P, Lacasa A, *et al*. Use of pepper crop residues for the control of root-knot nematodes. Bioresource Technology. 2007;98(15):2846-2851.
7. Blok WJ, Lamers JG, Termorshuizen AJ, Bollen GJ. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology. 2000;90(3):253-259.
8. Douda O, Zouhar M, Mazáková J, Nováková E, Pavela R. Using plant essences as alternative mean for northern root-knot nematode (*Meloidogyne hapla*) management. Journal of pest science. 2010;83(3):217-221.
9. Dutta TK, Khan MR, Phani V. Plant-parasitic nematode management via Biofumigation using brassica and non-brassica plants: Current status and future prospects. Current Plant Biology. 2019;17:17-32.
10. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 2001;56(1):5-51.
11. Fourie H, Ahuja P, Lammers J, Daneel M. Brassicacea-based management strategies as an alternative to combat nematode pests: A synopsis. Crop Protection. 2016;80:21-41.
12. Gigot JA, Zasada IA, Walters TW. Integration of brassicaceous seed meals into red raspberry production systems. Applied soil ecology. 2013;64:23-31.
13. Grabau ZJ, Maung ZTZ, Noyes DC, Baas DG, Werling BP, Brainard DC, *et al*. Effects of cover crops on *Pratylenchus penetrans* and the nematode community in carrot production. Journal of Nematology. 2017;49(1):114.
14. Henderson DR, Riga E, Ramirez RA, Wilson J, Snyder WE. Mustard Biofumigation disrupts biological control by *Steinernema* spp. nematodes in the soil. Biological Control. 2009;48(3):316-322.
15. Kirkegaard J. Biofumigation-using Brassica species to control pests and diseases in horticulture and agriculture. Proceedings of the 9th Australian Research Assembly on Brassica. Wagga. NSW Agriculture; c1993.
16. Kirkegaard JA, Sarwar M, Matthiessen JN. Assessing the Biofumigation potential of crucifers. In International Symposium Brassica 97, Xth Crucifer Genetics Workshop. 1998;459:105-112.
17. Kumar V, Khan MR, Walia RK. Crop Loss Estimations due to Plant-Parasitic Nematodes in Major Crops in India. National Academy Science Letters, 2020, 1-4.
18. Kruger DHM, Fourie JC, Malan AP. Cover crops with Biofumigation properties for the suppression of plant-parasitic nematodes: A review. South African Journal of Enology and Viticulture. 2013;34(2):287-295.
19. López-Pérez JA, Roubtsova T, Ploeg A. Effect of three plant residues and chicken manure used as biofumigants at three temperatures on Meloidogyne incognita infestation of tomato in greenhouse experiments. Journal

of nematology. 2005;37(4):489.
20. Matthiessen JN, Kirkegaard JA. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Critical reviews in plant sciences. 2006;25(3):235-265.
21. Meyer SL, Zasada IA, Orisajo SB, Morra MJ. Mustard seed meal mixtures: management of *Meloidogyne incognita* on pepper and potential phytotoxicity. Journal of Nematology. 2011;43(1):7.
22. Morra MJ, Kirkegaard JA. Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biology and Biochemistry. 2002;34(11):1683-1690.
23. Motisi N, Doré T, Lucas P, Montfort F. Dealing with the variability in Biofumigation efficacy through an epidemiological framework. Soil Biology and Biochemistry. 2010;42(12):2044-2057.
24. Munnecke DE, Martin JP. Release of methylisothiocyanate from soils treated with Mylone (3, 5-dimethyl-tetrahydro-1, 3, 5, 2H-thiadiazine-2-thione). Phytopathology. 1964;54(8):941.
25. Nyczepir AP, Thomas SH. 18 Current and Future Management Strategies in Intensive Crop Production Systems. Root-knot nematodes, 2009, 412.
26. Oka Y. Mechanisms of nematode suppression by organic soil amendments: A review. Applied Soil Ecology. 2010;44(2):101-115.
27. Padilla G, Cartea ME, Velasco P, de Haro A, Ordás A. Variation of glucosinolates in vegetable crops of *Brassica rapa*. Phytochemistry. 2007;68(4):536-545.
28. Ploeg AT. Biofumigation to manage plant-parasitic nematodes. In: Ciancio, A. And Mukerji, K.G. (eds). Integrated management and biocontrol of vegetable and grain crops nematodes. Springer-Verlag, Berlin, 2007, 239–248.
29. Rahman L, Somers T. Suppression of root knot nematode (*Meloidogyne javanica*) after incorporation of Indian mustard cv. Nemfix as green manure and seed meal in vineyards. Australasian Plant Pathology. 2013;34(1):77-83.
30. Rodman JE. Divergence, convergence, and parallelism in phytochemical characters: the glucosinolate-myrosinase system. Phytochemistry and angiosperm phylogeny, 1981, 43-79.
31. Sarwar M, Kirkegaard JA, Wong PTW, Desmarchelier JM. Biofumigation potential of brassicas. III. *In vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. Plant Soil. 1998;201:103-112.
32. Valdes Y, Viaene N, Moens M. Effects of yellow mustard amendments on the soil nematode community in a potato field with focus on *Globodera rostochiensis*. Applied soil ecology. 2012;59:39-47.
33. Watts WD. Factors affecting Biofumigation success against potato cyst nematodes (Doctoral dissertation, Harper Adams University); c2018.
34. Warnock ND, Wilson L, Patten C, Fleming CC, Maule AG, Dalzell JJ. Nematode neuropeptides as transgenic nematicides. *PLoS pathogens*. 2017;13(2):237.
35. Westphal A, Kücke M, Heuer H. Soil amendment with digestate from bio-energy fermenters for mitigating damage to *Beta vulgaris* subspp. by *Heterodera schachtii*. Applied soil ecology. 2016;99:129-136.
36. Zasada IA, Ferris H. Nematode suppression with brassicaceous amendments: application based upon glucosinolate profiles. Soil Biology and Biochemistry. 2004;36(7):1017-1024.
37. Zasada IA, Meyer SLF, Morra MJ. Brassicaceous seed meals as soil amendments to suppress the plant-parasitic nematodes *Pratylenchus penetrans* and *Meloidogyne incognita*. Journal of Nematology. 2009;41(3):221.