

A review of isothiocyanates biofumigation activity on plant parasitic nematodes

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Abstract Natural isothiocyanates (ITCs) are toxic to a range of soil-borne pest and pathogens, including nematodes and fungi, and can thus be used as natural fumigants called biofumigants. Glucosinolates, β -thioglucoside N-hydroxysulfates, are secondary metabolites of Brassicales plants, stored in the S-cells vacuoles. Upon plant tissue damage myrosinase (thioglucoside glycohydrolase, EC 3.2.3.1), stored in contiguous cells, hydrolyses glucosinates to an unstable aglycone that eventually eliminates sulfate group producing a wide range of different volatile isothiocyanates that are extremely toxic to root-knot nematodes. In fact, among synthetic commercial nematicidal formulates we can find isothiocyanates as active ingredients. Conventional nematode control practices have included soil sterilants of great environmental impact, most of which are now banned making mandatory the development of eco-sustainable alternative tools. We reviewed the nematicidal activity of isothiocyanates as components of botanical matrixes in the frame of a holistic nematode control

approach encompassing secondary beneficial effects on soil structure and microbiology, beneficial preservation, enhanced residual life of biological activity and plant growth.

Keywords Soil amendment · Brassicaceae · Nematicidal

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are first in the ‘top 10’ list of nematodes based on scientific and economic importance followed by the cyst nematodes (*Heterodera* and *Globodera* spp.) and the root lesion nematodes (*Pratylenchus* spp) (Jones et al. 2013). The small size of plant-parasitic nematodes and the fact that many of the most important species are obligate biotrophs that cannot be cultured in large numbers, makes them extremely difficult experimental organisms. *Meloidogyne* spp. are obligate plant parasites and they are distributed worldwide. The genus consists of 98 species and their hosts encompass almost all vascular plants. The most important species (sometimes referred to as the four major species—Moens et al. 2009) are the tropical *M. arenaria*, *M. incognita* and *M. javanica*, and the temperate *M. hapla* (Jones et al. 2013).

In the past, soil fumigation with the synthetic, non-selective methyl bromide has been the preferred

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method to control plant parasitic nematodes. Methyl bromide has been banned in the developed countries from 2006 (according to the Montreal protocol) due to its negative impact on the ozone layer 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). Thus, the pesticide industry has now a bigger drive towards the development and funding of alternative management tools that are more target specific, with a lower impact on natural predators and the environment, and a favorable toxicological profile (Kruger et al. 2013).

Besides methyl bromide, and after the second world war, many other soil fumigants reached the market, including chloropicrin, 1,3-dichloropropene, ethylene-dibromide, 1,2-dibromo-3-chloropropane and methyl isothiocyanate (M-ITC) (Lembright 1990). Interestingly, M-ITC is one of the natural origin-hydrolysis product of glucosinolates (GSLs), which are compounds originating in cruciferous plants like rape, mustard, canola, cabbage, and broccoli. When the plant tissue is damaged, the GSLs encounter the endogenous enzyme myrosinase, which hydrolyses the GSLs to the biologically active ITCs (Fenwick et al. 1983). ITCs bioactivity is based on the reaction with proteins and amino acids, and the formation of stable products with sulfhydryl groups, disulfide bonds, and amino groups (Brown and Morra 1997a, b). Although natural and synthetic ITCs share the same biochemical mode of action in the target nematode, their natural or synthetic origin along with their single use or as components of complex botanical matrices affects differently the efficacy and their toxicity on non-target organisms is described.

Synthetic ITC application: a conventional practice

Control of RKN

Dazomet (3,5-dimethyl-1,3,5-thiadiazinane-2-thione) is a soil sterilant, acting on weeds as well as soil pathogens by applying to wet soil and decomposing into the active volatile M-ITC. Under the EU Regulation 1107/2009 dazomet was re-approved with

crucial restrictions of rates and years of application; and currently an EU Member State may authorize its use for 120 days/year (López-Aranda et al. 2016). The combination of dazomet with dimethyl disulfide is a potential alternative to methyl bromide for soil disinfestation, and greenhouse trials revealed that they synergically suppressed *Meloidogyne* spp. root galling (Mao et al. 2014). Dazomet has been studied as an agent to alleviate the apple replant problem and improve vigor of trees by inhibiting the growth of fungi, actinomycetes and bacteria. In specific, when used at the ratio of 0.5 g/kg, considerably higher to the registered dose for nematodes control (500–600 gr/ha for 15 cm soil depth), it decreased the soil number of fungi, bacteria and actinomycetes by 58.8% ($P < 0.01$), 15.3 and 8.5% ($P < 0.05$), and promoted root development (Liu et al. 2014a, b).

Soil degradation and adverse effects on non-target organisms

Although the efficacy provided by the plant protection products is substantial, the usefulness of any pest control measure is hampered if the soil quality and the beneficial non-target species are severely and adversely affected. In fact, the use of soil fumigation in sustained monoculture often leads to a decline in soil quality (Zhao et al. 2016) by reducing the total bacterial numbers dominated by Firmicutes and Proteobacteria (Feld et al. 2015). The bacterial and fungal biodiversity in soil was in fact decreased after treatments with Dazomet and Fosthiazate in long term glasshouse experiments (Liu et al. 2014a, b). Thus soil amending with botanical materials is seen as a means of combating declining soil fertility, also providing control over soil-borne plant pathogens (Zhao et al. 2016). Moreover, since both synthetic and natural origin ITC react in a nonspecific and irreversible mode with proteins and amino acids (Brown and Morra 1997a, b), it is likely that non-target organisms may also be affected. In fact the toxicity of the ITCs to non-target organisms in soil (Ibekwe 2004; Rumberger and Marschner 2003; Bending and Lincoln 2000) and in the aquatic environment (Schultz et al. 2005) has been demonstrated. Consequently, for non specific soil sterilants like methyl bromide, chloropicrin and combinations of chloropicrin and 1,3-dichloropropene special care was taken and soil fumigation used as a long-term approach (Louvet 1979) was applied by

trained pest control operators to lower the risks involved. Thus, for soil fumigation to be effective in the control of soil-borne pest and diseases without environmental side-effects, intensive research on the application rate and a sound knowledge of the soil and of the environmental conditions involved are required (Louvet 1979).

ITC dissipation in soil and residual biological activity

When the distribution of methyl isothiocyanate in greenhouse soils treated with the fumigant dazomet was studied, it was concluded that with independence of the soil depth, no residues of methyl isothiocyanate, above the detection limits were observed in soils covered with a polyethylene film. Additionally, the rate of liberation of methyl isothiocyanate had a half-life of 3.7 days in the surface horizon (5–10 cm) of the soil while in the subsurface horizon (15–20 cm), it was found slightly slower. With respect to the dissipation process, half-lives were lower than 1 day for both depths (0.8 and 0.9 for the surface and the subsurface horizon, respectively) (López-Fernández et al. 2016).

Brassica biofumigation practice an holistic nematode control approach

Control of RKN

In the frame of searching for natural substitutes of botanical origin the method of biofumigation has been introduced in 1870 for the control of nematodes (Van Berkum and Hoestra 1979). In detail, the ability of certain plants to suppress nematodes has been based on the nematicidal activity of the secondary metabolites among which GLS (Ntalli and Caboni 2012; Chitwood 2002; Zhao et al. 2016; Aissani et al. 2013; Akhtar and Mahmood 1996). GLSs can be found principally within Brassiceae but also in other botanical families like Poaceae, Capparaceae and Caricaceae; although *brassica* green manures are more effective in suppressing nematodes (Potter et al. 1998; Bhandari et al. 2015; Table 1). There are three chemical categories of GSLs namely aliphatic, aromatic and indole. 2-phenylethyl, benzyl, 4-methylthiobutyl, and prop-2-enyl isothiocyanate have been shown to exhibit strong activity against *M. incognita*,

with an LD₍₅₀₎ at concentrations of 11, 15, 21, and 34 μM, respectively, in vitro tests (Lazzeri et al. 2004). Recently, a review has been published describing the accumulation patterns of individual GSLs in different tissues of Poaceae and Brassicaceae species, namely *Avena sativa*, *Lolium multiflorum*, *Hordeum vulgare*, *Brassica napus*, *Brassica rapa*, *Raphanus sativa*, *Sinapis alba* and *Brassica juncea*. The nematicidal isothiocyanates, coming from aliphatic GSLs (seeds), indole GSLs (root and shoot) and aromatic GSLs (roots), are released into the soil, if their plant reservoirs are used as cover crops or in rotation programs (Bhandari et al. 2015). Kruger and co-workers have recently reported on *Eruca sativa* cv. Nemat (rocket), *Sinapis alba* cv Braco (white mustard), *Brassica juncea* cv. Caliente 199 (Indian mustard) and *Brassica napus* cv. AV Jade (canola) inclusion in crop rotation programs for soil disinfection (Kruger et al. 2013); while several studies have confirmed that the natural ITCs are toxic to a range of pathogenic soil-living organisms, including bacteria, fungi, and nematodes thus establishing biofumigation as a soil purification strategy (Laegdsmand et al. 2007; Lazzeri et al. 1993; Elbersson et al. 1996; Brown and Morra 1997a, b; Smolinska et al. 1997; Borek et al. 1997, 1998; Manici et al. 2000; Smith and Kirkegaard 2002). Rape seed, radish and buckwheat amended soil in a potato field yields more by suppressing *M. chitwoodi* (Hafez and Sundararaj 2000). Likewise, when *Meloidogyne arenaria* infested soil is amended with chrysanthemum, broccoli, tagetes, basil and marigolds at the rate of 4 kg/m² and when covered with polyethylene film, the treatment reduces nematodes in the following order of broccoli (E = 71.23%), followed by chrysanthemum (E = 67.68%) and tagetes (E = 64.79%) (Masheva et al. 2012). Wild mustard (*Brassica juncea*), successfully achieves *M. incognita* reduction in tomato plants when used as dry leaf meal, whole seed meal and hexane defatted seed meal and sinigrin is the major bioactive glucosinolate (Oliveira et al. 2011). Soil amended with four cruciferous plants viz., *Brassica rapa* (var. TL15), *Eruca sativa* (var. TMLC 2), *B. juncea* (var. PBR 97) and *B. napus* (var. GSL 1) by sowing and incorporating into beds after forty days, reduces the number of galls produced on tomato roots (Randhawa and Sharma 2008). *Eruca sativa* (rocket), *Barbarea verna* (land cress) and *Brassica nigra* (black mustard) substantially reduce *M. incognita* infestation levels

Table 1 Recent references on plant species producing glucosinolates with nematicidal activity on phytonematodes

Plant	References
<i>Armoracia rusticana</i>	Aissani et al. (2013)
<i>Avena sativa</i>	Bhandari et al. (2015)
<i>Lolium multiflorum</i>	Bhandari et al. (2015)
<i>Hordeum vulgare</i>	Bhandari et al. (2015)
<i>Brassica napus</i>	Bhandari et al. (2015), Kruger et al. (2013), Randhawa and Sharma (2008) and Hafez and Sundararaj (2000)
<i>Brassica rapa</i>	Bhandari et al. (2015) and Randhawa and Sharma (2008)
<i>Raphanus sativa</i>	Bhandari et al. (2015)
<i>Sinapis alba</i>	Bhandari et al. (2015) and Kruger et al. (2013)
<i>Brassica juncea</i>	Bhandari et al. (2015), Kruger et al. (2013), Oliveira et al. (2011), Randhawa and Sharma (2008) and Yu et al. (2007)
<i>Eruca sativa</i>	Kruger et al. (2013), Lazzeri et al. (2009), Randhawa and Sharma (2008) and Curto et al. (2016)
<i>Barbarea verna</i>	Curto et al. (2016)
<i>Brassica nigra</i>	Curto et al. (2016)
<i>Raphanus sativus</i>	Lazzeri et al. (2009)
<i>Brassica oleracea</i>	Youssef and Lashein (2013) and Masheva et al. (2012)
<i>Raphanus raphanistrum</i>	Hafez and Sundararaj (2000)
<i>Fagopyrum esculentum</i>	Hafez and Sundararaj (2000)

and enhance plant vigor when used as biofumigation agents in a tomato glasshouse (Curto et al. 2016). *Raphanus sativus* ssp *oleiformis* and *Eruca sativa* ssp *oleiformis* reduce *Heterodera schachtii* and *Meloidogyne incognita* infestation levels in zucchini roots and improve total yield (Lazzeri et al. 2009). Oriental mustard (*Brassica juncea*) bran, a by-product of mustard milling, is nematicidal against *Heterodera glycines*, followed by *Pratylenchus neglectus*, *Heterodera schachtii*, *Pratylenchus penetrans*, *Meloidogyne incognita* and *Meloidogyne hapla* (Yu et al. 2007). In extends, Brassicaceous seed meals, the residual materials of the oil extraction from seeds, contain glucosinolates that degrade to nematotoxic compounds (Zasada et al. 2009). Interestingly, *Brassica* green manures in combination with half the recommended rate of 1,3-dichloropropene (1,3-D, Telone) reduces root knot nematode, *Meloidogyne chitwoodi* to levels below detection; as well as lesion nematodes, *Pratylenchus penetrans* and stubby root nematodes, *Paratrichodorus allius*, to below economic thresholds (Riga 2011). Biofumigation with a yellow mustard green manure reduces significantly the potato cyst nematode *Globodera rostochiensis*, a quarantine species with high economic importance for potato growers worldwide (Valdesa et al. 2012). Crushed

cabbage leaves (*Brassica oleracea*) incorporated into the soil at the rates of 2.5, 5 and 10 g/pot, 10 days before transplanting tomato cv. Super Strain B reduces nematode incidence in a dose response manner promoting at the same time plant growth (Youssef and Lashein 2013). Of course, care must be taken for the *Brassica* cover crops not to serve as a nematodes host before use in rotation programs (McLeod and Warren 1993). In all cases, to achieve the best biofumigation: (1) the treatment should assure the maximum maceration time of plant tissue, by rotating the green material into the soil, (2) maximum release of GLSs avoiding degradation, and (3) reassuring enough water for GLS hydrolysis and thus ICT release (Bellostas et al. 2004). If biofumigation is done properly and according to the before-mentioned three factors, it can be as effective as using a commercial nematicide (Rahman et al. 2011).

Soil enhancement and promotion of soil microorganisms population

Soil biofumigation has beneficial impact on the soil based on (1) the reduction of water run-off and erosion, (2) the preservation of soil moisture, (3) the reduction of evaporation from soil, (4) the temperature

regulation, (5) the improvement of soil organic matter and (6) the suppression of weeds, all factors contributing to a holistic IPM approach in controlling plant parasitic nematodes (Kruger et al. 2013). Interestingly, little published data report on the beneficial impact of biofumigation on the microbial community structure. In this frame, pepper (*Capsicum annuum*) production systems under field conditions were used to study the impact of biofumigation on microbial community structure. Biofumigation with rapeseed (*Brassica napus* ‘Dwarf Essex’) meal was tested against chemical soil fumigation with dazomet and the results showed that the biofumigation increased the bacterial diversity and decreased the fungal diversity. There was a negative correlation between soil bacterial diversity and disease incidence and a positive correlation between soil fungal diversity and disease incidence. Cloning of the microbial community showed that the microbial community structures were altered by biofumigation. Soil was also evaluated for their chemical properties and it was found that the biofumigation increased soil content of total N, available P and available K (Wang et al. 2014). It has been proven that after ITC soil disinfection saprophytes such as *Penicillium* sp., *Trichoderma*, *Aspergillus* sp., *Gliocladium* sp. and several others predominate. These fungi can have an antagonistic action on soil parasitic organisms which gives rise to a synergistic action called “induced antagonism” because the fungi occurring during repopulation contribute to the inhibition of the parasite thus prolonging the disinfection effect. Thus, induced antagonism triggered by minute quantities of product may suggest the possibility of using this for (biological) integrated control (Welvaert 1974).

Lower chemical input and beneficials’ preservation

Integrating conventional management practices with novel techniques fosters sustainability of production systems and can increase economic benefit to producers while reducing chemical input. The combined treatment of *Brassica* green manures with half the recommended rate of 1,3-dichloropropene (1,3-D, Telone) had no effect on the beneficial free-living nematode populations and the non-pathogenic *Pseudomonas*. Additionally, the total cost of growing and soil-incorporating *Brassica* crops as green manures in

combination with the reduced rates of 1,3-D was approximately 35% lower than the present commercial costs for application for the full rate of this fumigant (Riga 2011). Similarly, biofumigation with a yellow mustard green manure on the potato cyst nematode *Globodera rostochiensis*, determined changes in the abundance of nematode trophic groups and the amended plots exhibited an increase of the enrichment index while the channel index decreased significantly, indicating a soil food web with a decomposition pathway dominated by bacterial-feeder nematodes (Valdesa et al. 2012). When broccoli, mustard and oilseed radish, were evaluated for their effect on earthworms (*Eisenia andrei*) and the soil microbial community, it was concluded that none of the biofumigants had a significant effect on earthworm survival or growth and that broccoli reduced earthworm reproduction while mustard induced more DNA strand breaks in earthworm cells compared to the control (Fouché et al. 2016).

Enhanced residual life and biological activity under field conditions

Biofumigation is performed by incorporating the green manure into the upper part of the soil where the ITCs are released (Matthiessen and Kirkegaard 2006; Jensen et al. 2010). These, GLS metabolic products are the factor of bioactivity and their fate in soil defines their residual life and thus efficacy. It must be noted that the ITCs originating for biofumigation are slowly released into the soil thus they might provide of a longer term biological activity compared to their directly exposed to degradation synthetic analogs (Hanschen et al. 2015). ITCs can decline due to volatility, are adsorbed to soil with high organic matter due to their lipophilicity and they decompose because of biotic and abiotic soil properties (Dungan et al. 2003; Borek et al. 1995; Gimsing et al. 2007, 2009a, b; Rumberger and Marschner 2003; Warton et al. 2003; Gimsing and Kirkegaard 2006; Poulsen et al. 2008). The ITC degradation is faster in topsoil than in sub soil (Gimsing et al. 2007). Nonetheless both chemical and natural ITC can also leach into the soil (Guo et al. 2003; Laegdsmand et al. 2007).

To study the stability of GLSs and respective nitriles as well as ITCs breakdown products, *Raphanus sativus* and *Brassica juncea* were incorporated in

soil. It was shown that the stability of GLS hydrolysis products in soil is mainly influenced by microbiota but also by their chemical structure, as methylthioalk(en)yl hydrolysis products were least stable when compared to nitriles. With regard to the degradation of pure allyl-GLS, the formation of its degradation products (nitrile or ITC) was shown to be dependent on the soil type. The iron content of the soil was assumed to be a major factor favouring nitrile formation in sandy soils with acidic pH value (Hanschen et al. 2015). Following the degradation of pure 2-propenyl glucosinolate in the soils, the nitrile as well as the isothiocyanate can be the main degradation products, depending on the soil type. The degradation was shown to be both chemically and biologically mediated since autoclaving reduced degradation thus suggesting a strong biotic degradation. The nitrile was the major product of the chemical degradation and its formation increased with iron content of the soil. Additionally, the bacterial community composition was significantly affected by adding pure 2-propenyl glucosinolate, the effect being more pronounced than in treatments with myrosinase added to the GLS (Hanschen et al. 2015). Interestingly, enhanced biodegradation of ITCs has been observed for soils frequently grown with Brassicas probably due to microorganisms favored in these conditions (Warton et al. 2003; Gimsing et al. 2009a, b, 2007; Matthiessen et al. 2004; Matthiessen and Kirkegaard 2006).

Last, a significant proportion of plant GSL can persist un-hydrolyzed in the soil for several days following *Brassica* incorporation. In specific, when the concentration of GSL and ITCs was monitored in soil following the incorporation of pulverised rape (*Brassica napus*) and mustard (*Brassica juncea*), it was found that the concentration of both GSLs and ITCs in soil was highest immediately after incorporation and they could be detected for up to 8 and 12 days, respectively (Gimsing and Kirkegaard 2006). On the other hand, the rate of decomposition of dazomet into methyl isothiocyanate was shorter and the half-life was calculated 3.7 days in the surface horizon as described by López-Fernández et al. 2016. The non-ITC liberating GSLs (predominately indolyl GSLs) from pulverised rape (*Brassica napus*) and mustard (*Brassica juncea*) were found at lower concentrations than ITC-liberating GSLs, but tended to persist longer in the soil (Gimsing and Kirkegaard 2006).

Conclusions

In the search for alternative practices to control phytonematodes, many disciplines are evaluated but none is enough on its own to achieve efficacy levels similar to the ones obtained by the traditional nematicides used in the past. Multidisciplinary strategies are needed to fill the gaps of the single plant protection methods and the farmers need to be educated to holistic approaches of pest control that see beyond efficacy. The chemical agents are still predominant to exhibit significant activity but they cannot be incorporated in plant protection schemes if not evaluated as environmentally safe and cost effective. On the other hand, non-chemical practices, such as crop rotation, are to be evaluated case by case as they may be non-applicable because of the extensive host range of *Meloidogyne* sp. Soil amending by incorporating plant tissue, before the establishment of the nematodes susceptible culture, is an effective practice to control nematodes and although considered non chemical practice, it is actually based on the nematicidal activity of the plant secondary metabolites released in the soil. The time employed for the natural decomposition process and the chemical transformation to the reactive nematicidal molecules acts as a controlled release translated to a longer residual nematicidal activity under field conditions. The cost effectiveness is guaranteed since the raw material is freely available together with the eco-sustainability being the soil biofumigant of natural origin. Additionally the development of nematode resistance is highly improbable because of the complex cluster of chemically different nematicidal components all ingredients in the raw material used for soil amendment.

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