

19 Biofumigation in Crop Disease Management

**D. Ladhalakshmi,^{1*} R. Madhubala,² S. Sundravadana,³ G.S. Laha,¹
D. Krishnaveni,¹ G. Sangeetha⁴ and G. Ragothuman⁵**

¹Indian Institute of Rice Research, Hyderabad, India; ²National Institute of Plant Health Management, Hyderabad, India; ³Coconut Research Station, Tamil Nadu Agricultural University, Coimbatore, India; ⁴Central Horticulture Experimental Station, Bhubaneswar (IIHR, Bengaluru), India; ⁵Coconut Development Board, Assam, India

19.1 Introduction

Plant diseases have been associated with crop plants since agriculture began and, until recently, were routinely managed through application of synthetic fungicides. Among the plant diseases, soil-borne diseases are the important factor limiting the yield of crops resulting in serious economic losses in many countries. The major soil-borne pathogens include fungi (*Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium* spp., *Pythium* spp. and *Phytophthora* spp.), bacteria (*Erwinia* spp., *Ralstonia* spp., *Rhizomonas* spp., *Agrobacterium* spp. and *Streptomyces* spp.) and nematodes belonging to the genera *Meloidogyne*, *Heterodera*, *Longidorus* and *Paratrichodorus*. In general, soil-borne diseases are difficult to control because the causal agents can survive in the soil for long periods in the absence of host. Effective management of soil-borne diseases is possible only through detailed study of their ways of survival and dissemination, effect of environmental conditions, role of cultural practices and host plant resistance. Fumigation and

drenching of soil with synthetic chemicals has been practised in agriculture for many years to manage soil-borne diseases and pests of economically important crops. Some commonly used fumigants are methyl bromide, methomex and 1,3-dichloropropene-chloropicrin. Dichloro-diphenyltrichloroethane (DDT), which was used as a chemical fumigant for the control of soil-borne pathogens, was later withdrawn from the market due to its adverse effect on the environment (Gamliel *et al.*, 2000). Apart from DDT, methyl bromide and chloropicrin are also used to control fungal pathogens (Lazzeri *et al.*, 2004), whereas metham sodium is specific against the fungi, *Verticillium* (Larkin and Griffin, 2007). Methyl bromide has been banned in developed countries since 2005 and will be banned in developing countries by 2015 because of its ozone depleting nature. These fumigants are highly volatile and non-specific, and their use leads to environmental pollution, ecological problems and destruction of beneficial microbial communities in the soil. Similarly, nematicides are highly toxic to humans, contaminate groundwater

* E-mail: ladhasavitha@gmail.com

and can be absorbed by plants (Oka, 2010). Synthetic pesticides and fumigation chemicals like methyl bromide are able to cause damage to the ozone layer, are harmful to our environment and to humans as well, hence many developed and developing countries have banned the usage of these chemicals to reduce the risk.

Therefore, an alternative method is needed that can still control crop diseases without affecting human health and the environment. Attempts have been made to use non-chemical alternatives such as biofumigation, biocontrol and soil solarization in the place of pesticides. Brassicaceae plants contain glucosinolates and the products of these glucosinolates upon enzymatic hydrolysis exhibit fungicidal activity. Hence these plants may be used as an alternative approach for the management of soil-borne diseases (Walker *et al.*, 1937). Utilizing crop residues to reduce soil-borne pathogen populations has been examined many times over the years as a method to control these pathogens (Patrick *et al.*, 1964; Lewis and Papavizas, 1975). The value of this process is being re-examined mainly because of the deleterious environmental effects and costs of fumigants. Further, the rotation of crops with green manure crops as potential biofumigants has been widely explored in recent decades by many researchers.

19.2 Biofumigation

Biofumigation is a process, whereby volatile chemicals released from decomposing plant material are utilized for suppressing the growth of soil pathogens, nematodes, insects and germinating weed seeds. The term biofumigation was first coined by Kirkegaard *et al.* (1993), who specifically described using glucosinolate hydrolysis products, notably isothiocyanates. During decomposition, in addition to isothiocyanates, plant tissues release nitriles and oxazolidinethiones. Incorporation of glucosinolate-containing plants reduced the initial inoculum of certain soil-borne diseases according to Kirkegaard *et al.* (1993, 1998). Scientific studies have proved that volatiles released during the degradation of organic matter are responsible for the suppression of plant pathogens (Piedra Buena *et al.*, 2007; Clarke, 2010; Lord *et al.*, 2011).

19.3 Plant Species with Glucosinolates (GSLs)

Different plant species that contain glucosinolates and sulfur compounds have been reported to have biocidal activity. Glucosinolate-containing plants belong to the Brassicaceae, Capparidaceae, Tropaeolaceae, Moringaceae and Amaryllidaceae families. The family Brassicaceae (brassicacae) contains more than 350 genera with 3000 species, of which many are known to contain GSLs. Other than brassica plants, about 500 species of non-brassica dicotyledonous plants also contain GSLs (Fahey *et al.*, 2001; Larkin and Griffin, 2007; Wang *et al.*, 2009) and these glucosinolates can be grouped into different classes, namely, aliphatic, aromatic and indolyl forms (Zasada and Ferris, 2004; Padilla *et al.*, 2007). Kruger *et al.* (2013) studied the biofumigation properties of *Eruca sativa* cv. Nemat, *Sinapis alba* cv. Braco, *Brassica juncea* cv. Caliente 199, and *Brassica napus* cv. AV Jade (canola) and suggested these species for suppression of soil-borne plant pathogens in South Africa.

In Brassicas, the most extensively produced GSLs are aliphatic (e.g. glucoraphenin, glucoerucin, glucocheirolin, glucosinigrin), ω -methylthioalkyl (e.g. glucobenzosissymbrin, glucomalcomiin), aromatic (e.g. glucotropaeolin, glucobarbarin, glucosinalbin) and heterocyclic or indole (e.g. glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin) containing either straight or branched chain carbons (Fahey *et al.*, 2001). Allium species contain sulfur compounds known as disulfides (DS) and thiosulfonates (Ti), which have shown antimicrobial activity against plant pathogens (Auger and Thibout, 2004).

Plants contain GSLs that are hydrolysed by an enzyme, myrosinase, in the presence of water into various products upon tissue degradation. Generally GSLs are polar and highly water soluble (Gimsing *et al.*, 2005). The plant species used for soil fumigation are listed in Table 19.1.

19.4 Glucosinolates

Glucosinolates are stored in cell vacuoles (Rausch and Wachter, 2005), whereas myrosinase is accumulated in myrosin cells (Hoagland *et al.*, 1991). Glucosinolates consist of sulfur

Table 19.1. Plant species used for soil fumigation, as an alternative to chemical fumigation. (From: Lazzeri and Manici, 2001; Keusgen et al., 2002; Karavina and Mandumbu, 2012.)

Plant species	Common name	Family
<i>Alliaria petiolata</i>	Garlic mustard	Brassicaceae
<i>Allium ursinum</i>	Bear's garlic	Amaryllidaceae
<i>Allium vineale</i>	Wild onion	Amaryllidaceae
<i>Arabidopsis thaliana</i>	Thale cress	Brassicaceae
<i>Azima tetracantha</i>	Needle bush	Salvadoraceae
<i>Brassica campestris rapa</i>	Turnip	Brassicaceae
<i>Brassica carinata</i>	Ethiopian mustard	Brassicaceae
<i>Brassica fruticulosa</i>	Mediterranean cabbage	Brassicaceae
<i>Brassica juncea</i>	Indian mustard	Brassicaceae
<i>Brassica napus</i>	Rape/canola	Brassicaceae
<i>Brassica nigra</i>	Black mustard	Brassicaceae
<i>Brassica oleraceae acephala</i>	Kale	Brassicaceae
<i>Brassica oleraceae</i>	Cabbage	Brassicaceae
<i>Cardamine cordifolia</i>	Heartleaf bittercress	Brassicaceae
<i>Cardamine diphylla</i>	Pepper root	Brassicaceae
<i>Carica papaya</i>	Pawpaw	Caricaceae
<i>Cleome hassleriana</i>	Spider flower	Capparidaceae
<i>Diploaxis tenuifolia</i>	Perennial wall-rocket	Brassicaceae
<i>Eruca sativa</i>	Salad rocket	Brassicaceae
<i>Iberis amara</i>	Rocket candytuft	Brassicaceae
<i>Lepidium sativa</i>	Garden cress	Brassicaceae
<i>Moringa oleifera</i>	Moringa	Moringaceae
<i>Moringa stenopetala</i>	Cabbage tree	Moringaceae
<i>Rapistrum rugosum All.</i>	Common giant mustard	Brassicaceae
<i>Rhaphanus sativus</i>	Radish	Brassicaceae
<i>Sinapis alba</i>	White mustard	Brassicaceae
<i>Thlaspi arvense</i>	Field pennycress	Brassicaceae
<i>Tropaeolum maju</i>	Indian cress	Tropaeolaceae

and nitrogen compounds and their quantity varies with plant species; for example, species such as Indian/brown mustard (*Brassica juncea*), black mustard (*Brassica nigra*) and white mustard (*Sinapis alba*) contain higher amounts of glucosinolates. The aliphatic group of glucosinolates is found in foliage of rapeseed (*Brassica napus*), and consists of 3-butenyl (gluconapin), R-2-hydroxy-3-butenyl (progoitrin/glucorapiferin) and 4-pentenyl (glucobrassicinapin). Similarly, an aromatic glucosinolate found in the roots of rapeseed (*Brassica napus*) (Fig. 19.1) consists of 2-phenylethyl (gluconasturtiin). Earlier studies inferred that aliphatic GSLs degrade much more easily than aromatic GSLs.

19.4.1 Mode of action

Glucosinolates and the enzyme myrosinase are separated in living cells, and tissue degradation due to insect feeding, mechanical damage or infection helps to bring them together. At this point, glucosinolates are degraded by the myrosinase enzyme through the process of hydrolysis, thereby volatile products including isothiocyanates (ITCs), organic cyanides, ionic thiocyanates and oxazolidinethiones are released. These volatile products are likely to have biological activity against plant pathogens. The enzymatic mechanism of myrosinase involves two steps: the glycosylation step, in which the glycosyl enzyme

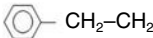
Aliphatic glucosinolates	Aromatic glucosinolates
$\text{CH}_2 = \text{CH}-\text{CH}_2-\text{CH}_2$ [$\text{CH}_2\text{CHCH}_2\text{CH}_2$]	2-phenylethyl-(Gluconasturtiin)
3-butenyl (Gluconapin)	 CH_2-CH_2
$\text{CH}_2 = \text{CH}-\text{CH}-\text{CH}_2$ -[$\text{CH}_2\text{CH}(\text{OH})\text{CHCH}_2$]	$[\text{C}_6\text{H}_5(\text{CH}_2)_2]$
<i>R</i> -2-hydroxy-3-butenyl (Progoitrin)	
$\text{CH}_2 = \text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2$ [$\text{CH}_2\text{CH}(\text{CH}_2)_3$]	
4-pentenyl (Glucobrassicinapin)	

Fig. 19.1. Aliphatic and aromatic glucosinolates. (Courtesy of Matthew Back and Melvyn, Harper Adams University, Newport.)

is formed and subsequently the aglycone is released; followed by the deglycolation step in which the glycosyl enzyme is hydrolysed by a water molecule (Burmeister *et al.*, 1997).

These hydrolysis products, in particular the ITCs, are known to have broad biocidal activity including insecticidal, nematocidal, fungicidal, antibiotic and phytotoxic effects (Fenwick and Heaney, 1983; Chew, 1988; Brown and Morra, 1997; Rosa, 1997).

19.5 *Allium* Sp. for Biofumigation

Like *Brassica* spp., *Allium* spp. also have biofumigation properties because of their sulfur components, mainly three disulfides: dimethyl disulfide (DMDS), dipropyl disulfide (DPDS) and diallyl disulfide (DADS), with an efficacy superior to that of DMDS. Similar to enzyme myrosinase, alliinase is also stored in the vacuoles. Upon mechanical disruption or insect feeding, alliinase is released, which reacts with S-alk(en)yl-L-cysteine sulfoxides (RCSOs) (Lancaster *et al.*, 1988) and releases sulfenic acids (Ferary and Auger, 1996). Many RCSOs (R= methyl, propyl, 1-propenyl) are present in onions, giving rise to DPDS (Arnault *et al.*, 2004) as the end product of biosynthesis. In bear's garlic (*A. ursinum*), the major RCSO is methoinin (S-methyl-L-cysteine

sulfoxide); it gives rise to dimethyl thiosulfinate (DMTi), which gets rearranged into DMDS. The biocidal activity of *Allium* has been proved and therefore it was suggested for soil fumigation (Auger and Thibout, 2004). GSLs are present in 16 families of dicotyledonous angiosperms including a large number of edible species, and at least 120 types of ITCs have been identified in these plants (Fahey *et al.*, 2001).

19.6 Management of Soil-borne Diseases

Brassica napus contains several types of GSLs including but-3-enyl, benzyl, phenethyl and 2-hydroxy-but-3-enyl (Bjerg and Sorensen, 1987; Gardiner *et al.*, 1999). Different stages of plant growth contain various glucosinolates, namely, glucoerucin, glucotropaeolin, glucoraphenin, glucobrassicin, and gluconasturtin. The roots contain mostly the GSL gluconasturtin, whereas the shoots have more aliphatic GSLs (Sarwar *et al.*, 1998). It was confirmed that the root tissue of the *Brassicas*, canola and Indian mustard released volatile compounds, namely, methyl ITC and phenyl ethyl ITC, which inhibited the growth of pure cultures of the fungal pathogen that causes take-all of wheat, *Gaeumannomyces graminis* var. *tritici*, at low concentration

(Angus *et al.*, 1994). Higher fungitoxic activity of plants, namely, *Iberis amara* L. (selection ISC14), *Rapistrum rugosum* All. (selection ISC14) and *Cleome hassleriana* L. (selection ISC12) was observed against *Pythium* sp. under *in vitro* conditions, and the activity was mainly due to the degradation products of glucosinolates (Lazzeri and Manici, 2001).

The effect of biofumigation on soil-borne fungal pathogens has been studied by many researchers, e.g. *Rhizoctonia* sp., *Verticillium* sp., *Sclerotinia* sp., *Colletotrichum* sp., *Fusarium* sp., *Pythium* sp., *Phytophthora* spp. (Steffek *et al.*, 2006; Zurera *et al.*, 2007; Mattner *et al.*, 2008; Friberg *et al.*, 2009; Omirou *et al.*, 2011). 2-propenyl ITC has been proved to be toxic to *Verticillium dahliae*, *Helminthosporium solani*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Phytophthora capsici* (Chung *et al.*, 2002). A higher percentage of fungal suppression was observed when both mustard roots and shoots were used for biofumigation (Snapp *et al.*, 2007). A reduced number of microsclerotia of *V. dahliae* in strawberry fields were observed as a result of biofumigation with different glucosinolate-containing *Brassica* spp. (Steffek *et al.*, 2006). The number of microsclerotia varied between 0% and 30% depending on the field characteristics and the biofumigant plant species used.

Incorporation of mustard (*Brassica juncea*) as a green manure decreased the inoculum density of *Rhizoctonia solani* (Friberg *et al.*, 2009). However, thick-walled hyphae (pseudosclerotia) of *R. solani* were less susceptible to GSL hydrolysis products than young hyphae (Yulianti *et al.*, 2006). Canola green manures are effective biofumigants against black scurf caused by *R. solani* (Larkin and Honeycutt, 2006; Larkin and Griffin, 2007). Wang *et al.* (2009) reported that production of methyl sulfide and dimethyl disulfide gases from white mustard (*Sinapis alba*) under natural field environments reduced soil-borne pathogens, namely, *V. dahliae*, *F. oxysporum* and *T. semipenetrans*. Taylor (2013) reported that benzyl ITC inhibited the growth of *R. solani* and *Helminthosporium solani* under *in vitro* conditions. Gas chromatography–mass spectrometry (GC-MS) studies confirmed that concentrations of specific ITCs produced during glucosinolate hydrolysis altered throughout the growth period. Hence, the efficacy of the method may depend on the specific biofumigant cultivar being grown

and the time of incorporation of the plant material into the soil. Kirkegaard and Sarwar (1998) reported that aliphatic glucosinolates content was greater in shoots, while aromatic glucosinolates, particularly 2-phenylethyl glucosinolates, were dominant in the roots. The concentration of individual and total GSLs in both root and shoot tissues varied within the species. These findings help us to select or develop brassicas with enhanced biofumigation potential. The most significant pathogen suppression was observed with *R. solani* when it was exposed to benzyl or methyl ITC, and *H. solani* was sensitive to 2-phenylethyl ITC (Taylor, 2013).

Larkin and Griffin (2007) tested various *Brassica* crops, namely, canola, rapeseed, radish, turnip, yellow mustard and Indian mustard for the management of soil-borne potato pathogens. Volatiles released from leaf of *Brassica* species and barley were harmful to *R. solani*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum* and *Fusarium sambucinum* under *in vitro* conditions, whereas Indian mustard inhibited the growth completely up to 80–100%. These *Brassica* crops and barley under greenhouse conditions reduced the inoculum levels of *R. solani* (20–56% reduction); radish, rapeseed and Indian mustard reduced potato seedling disease by 40–83%. Canola and rapeseed grown as green manure rotation crops reduced black scurf by 70–80% in potato. Satisfactory results were obtained with Indian mustard against powdery scab and common scab diseases of potato, whereas *Rhizoctonia* diseases were reduced with rapeseed and canola treatment. The combination of mustard blend (mustards and rapeseed) grown as green manure was found to be the most effective in reducing potato scurf disease by up to 54% and increasing the yield by 25% compared with soybean cover crop (Larkin and Halloran, 2014).

The biofumigation process has been modified a little to increase the efficiency of the process. Blok *et al.* (2000) reported that *Brassica juncea* was decomposed anaerobically in field soil with temporary irrigation and covered with polythene sheets, and the technique, developed in the Netherlands and Japan, was named biological soil disinfestation (BSD). Model experiments of BSD with wheat bran or *Brassica juncea* and *Avena strigosa* plants as biomass sources successfully controlled the wilt pathogen populations of

tomato (*Fusarium oxysporum* f. sp. *lycopersici*) and spinach (*F. oxysporum* f. sp. *spinacea*) when incorporated into soil (Mowlick *et al.*, 2013).

19.7 Seed Meal as a Source of Biofumigation

Seed meal, a by-product derivative of oil extraction of the seeds from the glucosinolate-containing plant species can also be used for soil-borne pathogen suppression, wherein it forms a source of nitrogen and other nutrients. Several reports have shown that amending the soil with *Brassica* sp. as seed meal suppressed many plant fungal pathogens (Lodha and Sharma, 2002; Kirkegaard and Matthiessen, 2004; Matthiessen and Kirkegaard, 2006). Mustard seed meal application significantly reduced the stem infection in lily caused by *Rhizoctonia solani* (Van Os *et al.*, 2004; Van Os and Lazzeri, 2006). Apple replant disease is commonly characterized as a pathogen complex involving the genera *Rhizoctonia*, *Cylindrocarpon*, *Pythium* and *Phytophthora* (*P. cactorum*) and lesion nematode *Pratylenchus penetrans* (Mazzola and Mullinix, 2005). The pathogen complex varies from site to site, even among orchards within close proximity (Traquair, 1984). The efficacy of brassicaceous seed meals (*Brassica juncea*, *Brassica napus* and *Sinapis alba*) for the management of apple replant disease was studied by Mazzola and Brown (2010). The study inferred that *B. juncea* and *S. alba* seed meal soil amendments were effective when it was combined with mefenoxam – a post plant fumigant – in terms of disease control, tree growth and overall fruit yields of Gala/M26 apple under a conventional production system. Similarly, a seed meal blend of *B. juncea*:*B. napus* (1:1 ratio) performed well in terms of disease control and vegetative growth of Gala/M26 under organic systems. Hence, the study results concluded that these amendments act as an alternative to soil fumigation for the control of apple replant disease in both conventional and organic systems.

Apple root infection by *R. solani* AG-5 was suppressed by allyl isothiocyanate (AITC), which was released from *B. juncea* seed meal amendments (Mazzola and Zhao, 2010). Seed meals of *B. juncea* and *B. napus* at a concentration of 0.5% significantly reduced the infection

of *R. solani* AG 8 in wheat (*Triticum aestivum* L.) compared to the unamended control (Handiseni *et al.*, 2013). Radish (*Raphanus sativus* L.), mustard (*B. juncea* (L.) Czern) and winter rapeseed (*B. napus* L.) were evaluated for their biofumigant activity against *R. solani* in bell pepper. The crops were disked into the soil and immediately covered with virtually impermeable film (VIF) to reduce the escape of volatile pesticidal compounds. It was revealed that mustard followed by rapeseed and radish reduced populations of *R. solani*, and the concentration of ITCs was high in mustard followed by the other crops (Hansen and Keinath, 2013). *Fusarium oxysporum*, *R. solani*, *Macrophomina phaseolina* and *S. rolfsii* are the common fungal pathogens infecting soybean causing damping-off, root rot and wilt diseases resulting in serious economic losses. Management of these pathogens is difficult due to their broader host range and nature of survival mechanisms in the soil. Payzalla *et al.* (2009) evaluated the effect of mustard seed meal as a biofumigant in lab, greenhouse and field conditions against the root rot and wilt pathogens infecting soybean. Mustard seed meal resulted in decreased linear growth of *R. solani* as compared with the control. In pot culture experiments, suppression of disease as well as increased plant growth were observed in mustard seed meal-treated pots compared to the untreated control. The sensitivity of the pathogen to seed meal differed at all levels and, among the pathogens, *R. solani* was the most sensitive. Under field conditions also, mustard seed meal was compared with Rhizolex® fungicide and both mustard seed meal and Rhizolex® reduced the disease incidence by 69.5% and 74.4%, respectively, 4 months after planting. Studies conducted by Handiseni *et al.* (2013) revealed that soils amended with *Sinapis alba* seed meal had the lowest severity of root rot caused by *R. solani* Kuhn anastomosis group (AG) 8 in wheat.

White lupine (*Lupinus albus*) mainly used as green manure, is infected by a wilt pathogen *F. oxysporum* f. sp. *lupine*. Shaban *et al.* (2011) studied the effect of mustard and canola seed meal against *F. oxysporum* f. sp. *lupine* as a biofumigant and compared it with Topsin M-70® fungicide under lab, greenhouse and field conditions. Under lab conditions, mustard seed meal decreased the growth of wilt pathogen of lupine and the growth decreased further with increasing concentrations

of seed meal, whereas canola seed meal reduced the growth of the pathogen only at high concentrations. In the pot culture studies, mustard seed meal treatment reduced the percentage of disease reduction up to 85.7%, followed by canola seed meal treatment (71.4%) and the fungicide Topsin M-70® treatment (64.3%), and the reduction in disease was reflected in increased growth parameters of lupine such as plant height, number of pods, weight of seeds and root length of plants grown. Field experiments with mustard seed meal treatment reduced the disease incidence by 83.6% at 30 and 90 days after planting for the first season (2008/2009) and 87.5% and 87.8% for the second season (2009/2010).

Fan *et al.* (2008) evaluated powdered tissues of *Brassica oleracea* var. *caulorapa* against 28 fungal isolates from 16 hosts under *in vitro* conditions. One gramme of powder of *B. oleracea* var. *caulorapa* could suppress the growth of *Ceratobasidium fimbriata* up to 68.6% and *V. dahliae* up to 68.7%. The findings also suggested that the amount of plant tissue should be standardized depending on target pathogen species for better results.

Other than *Brassica* plants, the alliaceous crops, namely, onion (*Allium cepa* L.) and garlic (*A. sativum* L.) also exhibited multiple bioactive properties against variety of soil microorganisms including fungi, bacteria and nematodes (Timonin and Thexton, 1950; Bianchi *et al.*, 1997). In *Allium* spp., suppression was due to the production of volatile sulfur compounds released via cleavage of certain S-alk(en)yl cysteine sulfoxides. The quality and quantity of volatile sulfur compounds varied among members of the Alliaceae (Jones *et al.*, 2004). In addition these bioactive compounds inhibited the germination of weed species like *Echinochloa crusgalli*, *Sisymbrium irio* and *Solanum oleraceus* in soil at ambient temperature (23°C) (Mallek *et al.*, 2007).

19.8 Management of Nematode Infection

Biofumigation has also been reported to reduce nematode populations (Henderson *et al.*, 2009; Zasada *et al.*, 2009). Green manures like *Brassica* sp. were more effective in suppressing nematodes under controlled conditions (Mojtahedi

et al., 1991; Mojtahedi *et al.*, 1993; Potter *et al.*, 1998). Rahman and Somers (2005) reported that when *B. juncea* cv. Nemfix (Indian mustard) was incorporated into the soil as a green manure, a suppressed population of *M. javanica* was observed. In addition to glucosinolate content of the brassica plants, secondary metabolites that are released during the biofumigation process might also play a role in the process of suppressing the nematode population (Piedra Buena *et al.*, 2006). Piedra Buena *et al.* (2006) reported that other than glucosinolates, secondary metabolites that are released during the biofumigation process also suppressed the nematode population. It was reported that root-knot nematode species can complete their life cycle on several *Brassica* spp., but their susceptibility varies with species (McLeod and Steel, 1999). The efficacy of biofumigation depends upon the selection of cover crop, because the selected crop should either be resistant or have a poor host status for the target pest (Vianene and Abawi, 1998). Melakeberhan *et al.* (2006) reported that *Eruca sativa* cv. Nemat was suitable trap crop for *Meloidogyne hapla* root-knot nematode wherein no eggs were produced in 80% of the plants. In addition to the selection of *Brassica* plants, soil temperature and duration of exposure of treatments played an important role in the efficacy of the biofumigation process. Ploeg and Stapleton (2001) tested the effect of time and temperature in combination with brassica soil residues on the suppression of *M. incognita* and *M. javanica*. It was found that in a temperature range of 30–35°C for 10 days, the treatment almost eliminated the galls on the roots. Methanol extracts of *Terminalia arjuna* (Combretaceae) bark, particularly 3,4-dihydroxybenzoic acid (3,4-DHBA) exhibited nematicidal activity against juveniles of *Meloidogyne incognita* collected from roots of infected cucumber plants (Nguyen *et al.*, 2013). Biofumigation with broccoli (*B. oleracea* L. var. *italica* L.) plant parts efficiently controlled *M. incognita* and produced significantly higher yields in the organic tomato fields in Turkey to those found with treatment consisting of grafting the susceptible cultivars with resistant root stock (Kaskavalci *et al.*, 2009). Henderson *et al.* (2009) reported that in potato crop, both *Brassica carinata* seed meal and *Steinernema* spp. reduced root-knot nematode damage to potato tubers and increased marketable tuber yields.

19.9 Influence of Biofumigation on Soil

Application of crops as biofumigants also improves soil structure and physical properties, soil infiltration and nutrient values (Cherr *et al.*, 2006). Among the different crops tested, soil treated with canola had the highest dissolved carbon and cation concentration (K + sodium – Na). Solubility of Fe/Al phosphate increased the soil pH from 4.8 to 5.3–6.2 in the rhizosphere region (Wang *et al.*, 2007). In addition to improving soil structure, the added plant materials can also change the native microbial community with respect to competition, parasitism, antagonism and predation against the soil-borne pathogens (Raaijmakers *et al.*, 2009) due to the changes in plant root secretions (Xu *et al.*, 2009). *Brassica* sp. as plant material or its seed meal was found to influence microbial community structures (Vera *et al.*, 1987; Williams-Woodward *et al.*, 1997; Mazzola *et al.*, 2001; Cohen and Mazzola, 2006; Hoagland *et al.*, 2008; Friberg *et al.*, 2009; Omirou *et al.*, 2011). Incorporation of *Brassica* plant material for biofumigation altered the microbial community in the soil.

19.10 Compatibility with Biocontrol Agents

The population of the rhizosphere microorganisms, namely, *Trichoderma* spp., *Pythium* spp., fluorescent *Pseudomonads*, *Streptomyces* spp., actinomycetes and other antagonists of soil-borne pathogens was either increased or decreased due to the effect of *Brassica napus* seed meal depending on the plant species and soil type (Mazzola *et al.*, 2001; Cohen and Mazzola, 2006; Mazzola and Zhao, 2010; Mazzola *et al.*, 2012). Wang *et al.* (2014) tested the compatibility of antagonistic *Bacillus amyloliquefaciens* strain BS211 along with biofumigation to control the pepper disease caused by *Phytophthora capsici* under controlled conditions. Application of the biofumigant along with the antagonistic bacteria reduced the disease incidence and increased soil bacterial diversity. Stefania Galletti *et al.* (2008) studied the compatibility of beneficial fungus *Trichoderma* with *Brassica carinata*

seed meal (BCSM). Forty isolates of *Trichoderma* spp. were tested against seed meal and volatiles released by BCSM. *Trichoderma* spp. were found to be generally less sensitive than the tested fungal pathogens (*P. ultimum*, *R. solani* and *F. oxysporum*). In addition, the author also pointed out that there was a reduction in allyl-isothiocyanate concentration in the soil. This may be due to the activity of *Trichoderma* isolates which protected against the biocidal compounds.

19.11 Compatibility with Other Techniques of Disease Management

The biofumigation process may not kill the pathogen completely, but the target pathogen group may be weakened so that it cannot survive in those environmental conditions. Hence, biofumigation can be combined with other techniques like soil solarization. Solarization, alone or combined with biofumigation or low doses of fumigants, has gained wider adoption as a methyl bromide alternative in areas with sunny climates and where it suits the cropping season and the pest and disease complex, especially countries like Morocco, Israel, Jordan and Brazil. Biofumigation is widely used at a commercial level in many developing and developed countries to control soil-borne pathogens (Zurera *et al.*, 2007; Fan *et al.*, 2008; Mattner *et al.*, 2008; Njoroge *et al.*, 2008; Bensen *et al.*, 2009). There are several patents for commercial manufacturing of biofumigants for pest control using *Brassica* seed products. Bello *et al.* (2003) reviewed the switching over of Spain to biofumigation and biosolarization as the main non-chemical alternatives, followed by soil-less cultivation, crop rotation, resistant varieties and grafting. These alternatives are more effective when combined in integrated crop management (ICM) systems.

The combination of biofumigation and soil solarization has been found to be synergistic in improving the efficacy of both procedures and thereby reducing the time required for solarization and the rates of amendment needed for biofumigation (Ndiaye *et al.*, 2007; Medina *et al.*, 2009; Porras *et al.*, 2009). Adoption of non-chemical alternatives such as substrates, grafting, resistant varieties, steam, solarization, biofumigation and biodisinfection has been

Table 19.2. Alternative soil technologies adopted in different countries instead of methyl bromide. (From: MBTOC 2010 Assessment Report.)

Country	Soil technologies selected
Peru	Steam, floating trays, solarization, biocontrol agents and biofumigation
Uruguay	Solarization + chemicals (1,3-D/Pic, MI, MS, DMDS), biofumigation and steam
Egypt	Substrates, steam, biofumigation, grafting
Lebanon	1,3-D, 1,3-D/Pic, metham sodium, solarization, solarization + reduced doses of chemicals, grafting, crop rotation, biofumigation and floating trays
Bosnia and Herzegovina	Floating trays, solarization and biofumigation
Macedonia	Floating trays and solarization + biofumigation

increasing tremendously in recent years. In Europe, the non-chemical alternatives applied in commercial strawberry fruit production are crop rotation, which is widely used in Denmark, Germany, the Netherlands and Poland; steam, which is used to protect strawberry in Belgium, France and Germany; solarization, which is used in Cyprus; and mulches, which are used against weeds in countries like Estonia, Germany and Slovenia. These alternatives are used extensively as methyl bromide alternatives in *Solanaceous* crops in Mediterranean countries and other areas of the world (Besri, 2002; Fennimore *et al.*, 2006). Solarization combined with biofumigation resulted in significant increase in tomato yields and decreased densities of certain pathogens and nematodes according to Iapichino *et al.* (2008). In Spain, biofumigation and biosolarization (biodisinfestation) are the main non-chemical alternatives that are increasingly used in pepper and tomato production.

19.12 Conclusion

Farmers have been accustomed to using very high levels of fungicide or fumigant for the

management of soil-borne diseases. In recent years, public concern about the environment has increased the need to develop and implement effective non-chemical alternatives instead of chemical fumigants. The results of biofumigation studies have already shown its definite potential and good results for the management of nematodes, soil-borne diseases and weeds whenever its methodologies are applied correctly. Plants containing glucosinolates, especially *Brassica* spp., exhibit biocidal activity against soil-borne pathogens of various crops and can be exclusively used for the biofumigation process. Despite its limitations, biofumigation has many potential benefits that could be exploited in disease management very well in the future. Furthermore, it could act as a very good alternative technique to use of the chemical fumigant methyl bromide. Though few reports are available on the combination of cultural practices along with biofumigation processes, research has shown that the effects of biofumigation could be further strengthened alongside solarization and in combination with ITC-resistant biocontrol agents to achieve maximum benefit in management of soil-borne pathogens.

References

- Angus, J.F., Gardner, P.A., Kirkegaard, J.A. and Desmarchelier, J.M. (1994) Biofumigation: isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. *Plant and Soil* 162, 107–112.
- Arnault, I., Mondy, N., Diwo, S. and Auger J. (2004) Soil behavior of natural sulfur fumigants used as methyl bromide substitutes. *International Journal of Environmental Analytical Chemistry* 84, 75–82.

- Auger, J. and Thibout, E. (2004) Sulfur compounds derived from *Allium* and *Crucifers* and their potential applications in crop protection. In: Tec and Doc (eds) *Biopesticides of Plant Origins*. France, pp. 77–95.
- Back, M. and Askew, M.F. (2011) Biofumigation: a significant opportunity for OSR and other *Brassica* species with high glucosinolate contents? Available at: <https://cdn.harper-adams.ac.uk/document/page/120911> (accessed 22 May 2015).
- Bello, A., López-Pérez, J.A. and García Álvarez, A. (2003) *Biofumigación de Agricultura Extensiva de Regadío Producción Integrada de Hortícolas*. CSIC-Caja Rural de Alicante, Mundi-Prensa, Madrid, 670 pp.
- Bensen, T.A., Smith, R.F., Subbarao, K.V., Koike, S.T., Fennimore, S. and Shem-Tov, S. (2009) Mustard and other cover crop effects vary on lettuce drop caused by *Sclerotinia minor* and on weeds. *Plant Disease* 93, 1019–1027.
- Besri, M. (2002) Alternatives to methyl bromide for tomato production in the Mediterranean area. In: Batchelor, T. and Bolivar, J. (eds) *Proceedings of International Conference on Alternatives to Methyl Bromide*, 5–8 March 2002, Sevilla, Spain, pp. 177–181.
- Bianchi, A., Zambonelli, A., D'Aulerio, A.Z. and Bellesia, F. (1997) Ultrastructural studies of the effects of *Allium sativum* on phytopathogenic fungi *in vitro*. *Plant Disease* 81, 1241–1246.
- Bjerg, B. and Sorensen, H. (1987) Quantitative analysis of glucosinolates in oilseed rape based on HPLC of desulfoglucosinolates and HPLC of intact glucosinolates. In: Wathelet, J.P. (ed.) *Glucosinolates in Rapeseeds: Analytical Aspects*. Lancaster Martinus Nijhoff, Boston, Massachusetts, pp. 125–150.
- Blok, W.J., Lamers, J.G., Termorshuizen, A.J. and Bollen, G.J. (2000) Control of soil-borne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology* 90, 253–259.
- Brown, P.D. and Morra, M.J. (1997) Hydrolysis products of glucosinolates in *Brassica napus* tissues as inhibitors of seed germination. *Plant and Soil* 181, 307–316.
- Burmeister, P.W., Cottaz, S., Driguez, H., Lori, R., Palmieri, S. and Henrissat, B. (1997) The crystal structures of *Sinapis alba* myrosinase and a covalent glycosyl-enzyme intermediate provide insights into the substrate recognition and active-site machinery of an S-glycosidase. *Structure* 5, 663–675.
- Cherr, C.M., Scholberg, J.M.S. and Mc Sorley, R. (2006) Green manure approaches to crop production: asynthesis. *Agronomy Journal* 98, 302–319.
- Chew, F.S. (1988) Biological effects of glucosinolates. In: Cutler, H.C. (ed.) *Biologically Active Natural Products: Potential Use in Agriculture*. American Chemical Society, Washington, DC, pp. 155–181.
- Chung, W.C., Huang, J.W., Huang, H.C. and Jen, J.F. (2002) Effect of ground *Brassica* seed meal on control of *Rhizoctonia* damping-off of cabbage. *Canadian Journal of Plant Pathology* 24, 211–218.
- Clarke, D. (2010) Glucosinolates, structure and analysis in food. *Analytical Methods* 4, 301–416.
- Cohen, M.F. and Mazzola, M. (2006) Resident bacteria, nitric oxide emission and particle size modulate the effect of *Brassica napus* seed meal on disease incited by *Rhizoctonia solani* and *Pythium* spp. *Plant Soil* 286, 75–86.
- Fahey, J.W., Zalcmann, A.T. and Talalay, P. (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56, 5–51.
- Fan, C.M., Xiong, G.R., Qi, P., Ji, G.H. and He, Y.Q. (2008) Potential biofumigation effects of *Brassica oleracea* var. *caulorapa* on growth of fungi. *Journal of Phytopathology* 156, 321–325.
- Fayzalla, E.A., El-Barougy, E. and El-Rayes, M.M. (2009) Control of soil-borne pathogenic fungi of soybean biofumigation with mustard seed meal. *Journal of Applied Sciences* 9, 2272–2279.
- Fennimore, S.A., Shem-Tov, S., Ajwa, H. and Weber, J.B. (2006) Retention of broadcast-applied fumigants with impermeable film in strawberry. In: *Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reduction*, 6–9 November 2006, Orlando, Florida, pp. 121–124.
- Fenwick, G.R. and Heaney, R.K. (1983) Glucosinolates and their breakdown products in Cruciferous crops, foods and feeding stuffs. *Food Chemistry* 11, 249–271.
- Ferary, S. and Auger, J. (1996) What is the true odor of cut *Allium* complementarily of various hyphenated methods: GC/MS, HPLC/MS with particle beam and atmosphere pressure ionization interfaces in sulfenic acids rearrangement components discrimination. *Journal of Chromatography* 750, 63–74.
- Friberg, H., Edel-Hermann, V., Faivre, C., Gautheron, N., Fayolle, L., Faloya, V., Montfort, F. and Steinberg, C. (2009) Cause and duration of mustard incorporation effects on soil-borne plant pathogenic fungi. *Soil Biology and Biochemistry* 41, 2075–2084.
- Galletti, S., Sala, E., Leoni, O., Burzi, P.L. and Cerato, C. (2008) *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. *Biological Control* 45, 319–327.
- Gamliel, A., Austerweil, M. and Kritzman, G. (2000) Non-chemical approach to soilborne pest management – organic amendments. *Crop Protection* 19, 847–853.

- Gardiner, J.B., Morra, M.J., Eberlein, C.V., Brown, P.D. and Borek, V. (1999) Alleochemicals released in soil following incorporation of rapeseed (*Brassica napus*) green manures. *Journal of Agriculture and Food Chemistry* 47, 3837–3842.
- Gimsing, A.L., Kirkegaard, J.A. and Hansen, H.C.B. (2005) Extraction and determination of glucosinolates from soil. *Journal of Agricultural and Food Chemistry* 53, 9663–9667.
- Handiseni, M., Brown, J., Zemetra, R. and Mazzola, M. (2013) Effect of Brassicaceae seed meals with different glucosinolate profiles on *Rhizoctonia* root rot in wheat. *Crop Protection* 48, 1–5.
- Hansen, Z.R. and Keinath, A.P. (2013) Increased pepper yields following incorporation of biofumigation cover crops and the effects on soil borne pathogen populations and pepper diseases. *Applied Soil Ecology* 63, 67–77.
- Henderson, D.R., Riga, E., Ramirez, R.A., Wilson, J. and Snyder, W.E. (2009) Mustard biofumigation disrupts biological control by *Steinernema* spp. nematodes in the soil. *Biological Control* 48, 316–322.
- Hoagland, L., Carpenter-Boggs, L., Reganold, J. and Mazzola, M. (2008) Role of native soil biology in Brassicaceae seed meal induced weed suppression. *Soil Biology and Biochemistry* 40, 1689–1697.
- Iapichino, G., Prinzivalli, C. and D'Anna, F. (2008) Soil solarization as an alternative to methyl bromide fumigation for annual strawberry production in a Mediterranean area. *Journal of Sustainable Agriculture* 32(2), 365–375.
- Jones, M.G., Hughes, J., Tregova, A., Milne, J., Tomsett, A.B. and Collin, H.A. (2004) Biosynthesis of the flavour precursors of onion and garlic. *Journal of Experimental Botany* 55, 1903–1918.
- Karavina, C. and Mandumbu, R. (2012) Biofumigation for crop protection: potential for adoption in Zimbabwe. *Journal of Animal and Plant Sciences* 14, 1996–2005.
- Kaskavalci, G., Tuzel, Y., Dura, O. and Öztekin, G. (2009) Effects of alternative control methods against *Meloidogyne incognita* in organic tomato production. *Ekoloji* 18(72), 23–31.
- Keusgen, M., Schultz, H., Glodek, J., Krest, I., Krüger, H., Herchert, N. and Keller, J. (2002) Characterization of some *Allium* hybrids by aroma precursors, aroma profiles and alliinase activity. *Journal of Agricultural Food Chemistry* 50, 2884–2890.
- Kirkegaard, J.A. and Matthiessen, J. (2004) Developing and refining the biofumigation concept. *Agroindustria* 3, 5–11.
- Kirkegaard, J.A. and Sarwar, M. (1998) Biofumigation potential of *Brassicas*. *Plant and Soil* 201, 71–89.
- Kirkegaard, A.J., Gardener, A.P., Desmarchelier, M.J. and Angus, F.J. (1993) Biofumigation – using *Brassica* species to control pests and diseases in horticulture and agriculture. In: Wratten, N. and Mailer, R. (eds) *Proceedings of 9th Australian Research Assembly on Brassicas*. Wagga Wagga, Australia, pp. 77–82.
- Kirkegaard, J.A., Sarwar, M., Wong, P.T.W. and Mead, A. (1998) Biofumigation by *Brassicas* reduces take-all infection. In: Michalle, D.L. and Pratley, J.E. (eds) *Agronomy – Growing a Greener Future: Proceedings of 9th Australian Agronomy Conference, Australia*, pp. 465–468.
- Kruger, D.H.M., Fourie, J.C. and Malan, A.P. (2013) Cover crops with biofumigation properties for the suppression of plant-parasitic nematodes: a review. *South African Journal of Enology and Viticulture* 34, 287–295.
- Lancaster, J.E., Dommissie, E.M. and Shaw, M.L. (1988) Production of flavour precursors [Salk(en)yl-L-cysteine sulfoxides] in photo callus of garlic. *Phytochemistry* 27, 2123–2124.
- Larkin, R.P. and Griffin, T.S. (2007) Control of soilborne potato diseases using *Brassica* green manures. *Crop Protection* 26, 1067–1077.
- Larkin, R.P. and Halloran, J.M. (2014) Management effects of disease-suppressive rotation crops on potato yield and soilborne disease and their economic implications in potato production. *American Journal of Potato Research* 91, 429–439.
- Larkin, R.P. and Honeycutt, C.W. (2006) Effects of different 3-year cropping systems on soil microbial communities and *Rhizoctonia* diseases of potato. *Phytopathology* 96, 68–79.
- Lazzeri, L. and Manici, L.M. (2001) Allelopathic effect of glucosinolate containing plant green manure on *Pythium* sp. and total fungal population in soil. *Horticultural Science* 36, 1283–1289.
- Lazzeri, L., Leoni, O. and Manici, L.M. (2004) Biocidal plant dried pellets for biofumigation. *Industrial Crops and Products* 20, 59–65.
- Lewis, J.A. and Papavizas, G.C. (1975) Survival and multiplication of soil-borne plant pathogens as affected by plant tissue amendments. In: Bruehl, G.W. (ed.) *Biology and Control of Soil-Borne Plant Pathogens*. American Phytopathological Society, St. Paul, Minnesota, pp. 84–89.

- Lodha, S. and Sharma, S.K. (2002) Effect of natural heating and *Brassica* amendments on survival of *Macrophomina phaseolina*. *Indian Phytopathology* 55, 342–344.
- Lord, J., Lazzeri, L., Atkinson, H. and Urwin, P. (2011) Biofumigation for control of potato cyst nematodes: activity of brassica leaf extracts and green manures on *Globodera pallida* *in vitro* and in soil. *Journal of Agricultural Food Chemistry* 59, 7882–7890.
- Mallek, S.B., Timothy, S. and James, J.S. (2007) Interaction effects of *Allium* spp. residues, concentrations and soil temperature on seed germination of four weedy plant species. *Applied Soil Ecology* 37, 233–239.
- Matthiessen, J.N. and Kirkegaard, J.A. (2006) Biofumigation and enhanced biodegradation: opportunity and challenge in soil-borne pest and disease management. *Critical Reviews in Plant Sciences* 25, 235–265.
- Mattner, S.W., Porter, I.J., Gounder, R.K., Shanks, A.L., Wren, D.J. and Allen, D. (2008) Factors that impact on the ability of biofumigants to suppress fungal pathogens and weeds of strawberry. *Crop Protection* 27, 1165–1173.
- Mazzola, M. and Brown, J. (2010) Efficacy of brassicaceous seed meal formulations for the control of apple replant disease in conventional and organic production systems. *Plant Disease* 94, 835–842.
- Mazzola, M. and Mullinix, K. (2005) Comparative field efficacy of management strategies containing *Brassica napus* seed meal or green manure for the control of apple replant disease. *Plant Disease* 89, 1207–1213.
- Mazzola, M. and Zhao, X. (2010) *Brassica juncea* seed meal particle size influences chemistry but not soil biology-based suppression of individual agents inciting apple replant disease. *Plant and Soil* 337, 313–324.
- Mazzola, M., Granatstein, D., Elfving, D. and Kent, M. (2001) Suppression of specific apple root pathogens by *Brassica napus* seed meal amendment regardless of glucosinolate content. *Phytopathology* 91, 673–679.
- Mazzola, M., Reardon, C. and Brown, J. (2012) Initial *Pythium* species composition and Brassicaceae seed meal type influence extent of *Pythium*-induced plant growth suppression in soil. *Soil Biology and Biochemistry* 48, 20–27.
- MBTOC (2010) United Nations Environment Programme (UNEP) Methyl Bromide Technical Options Committee (MBTOC) Assessment of the Alternatives to Methyl Bromide. United Nations Environment Programme, Nairobi, 331 pp.
- McLeod, R.W. and Steel, C.C. (1999) Effects of brassica-leaf green manures and crops on the activity and reproduction of *Meloidogyne javanica*. *Nematology* 1, 613–624.
- Medina, J.J., Miranda, L., Soria, C., Palencia, P. and López-Aranda, J.M. (2009) Non-chemical alternatives to methyl bromide for strawberry: biosolarization as case-study in Huelva (Spain). *Acta Horticulturae* 842, 961–964.
- Melakeberhan, H., Xu, A., Kravchenko, A., Mennan, S. and Riga, E. (2006) Potential use of arugula (*Eruca sativa*) as a trap crop for *Meloidogyne hapla*. *Nematology* 8, 793–799.
- Mojtahedi, H., Santo, S.G., Hang, N.A. and Wilson, H.J. (1991) Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. *Journal of Nematology* 23, 170–174.
- Mojtahedi, H., Santo, G.S., Wilson, J.H. and Hang, A.N. (1993) Managing *Meloidogyne chitwoodi* on potato with rapeseed as green manure. *Plant Disease* 77, 42–46.
- Mowlick, S., Yasukawa, H., Inoue, Takashi, Takehara, T., Kaku, N., Ueki, Katsuji and Ueki, A. (2013) Suppression of spinach wilt disease by biological soil disinfestations incorporated with *Brassica juncea* plants in association with changes in soil bacterial communities. *Crop Protection* 5, 485–493.
- Ndiaye, M., Termorshuizen, A.J. and Van Bruggen, A.H.C. (2007) Combined effect of solarization and organic amendment on charcoal rot caused by *Macrophomina phaseolina* in the Sahel. *Phytoparasitica* 35(4), 392–400.
- Nguyen, D., Seo, D.-J., Kim, K.-Y., Park, R.-D., Kim, D.-H., Han, Y.-S., Kim, T.-H. and Jung, W.-J. (2013) Nematicidal activity of 3,4-dihydroxybenzoic acid purified from *Terminalia nigrovenulosa* bark against *Meloidogyne incognita*. *Microbial Pathogenesis* 59–60, 52–59.
- Njoroge, S.M.C., Riley, M.B. and Keinath, A.P. (2008) Effect of incorporation of *Brassica* spp. residues on population densities of soilborne microorganisms and on damping-off and Fusarium wilt of watermelon. *Plant Disease* 92, 287–294.
- Oka, Y. (2010) Mechanisms of nematode suppression by organic soil amendments – a review. *Applied Soil Ecology* 44, 101–115.

- Omirou, M., Rousidou, C., Bekris, F., Papadopoulou, K.K., Menkissoglou-Spiroudi, U., Ehaliotis, C. and Karpouzas, D.G. (2011) The impact of biofumigation and chemical fumigation methods on the structure and function of the soil microbial community. *Microbial Ecology* 61, 201–213.
- Padilla, G., Cartea, M.E., Velasco, P., De Haro, A. and Ordas, A. (2007) Variation of glucosinolates in vegetable crops of *Brassica rapa*. *Phytochemistry* 68, 536–545.
- Patrick, Z.A., Toussoun, T.A. and Koch, L.W. (1964) Effect of crop-residue decomposition products on plant roots. *Annual Review of Phytopathology* 2, 267–292.
- Piedra Buena, A., Garcia-Alvarez, A., Diez-Rojo, M.A. and Bello, A. (2006) Use of cover crop residues for the control of *Meloidogyne incognita* under laboratory conditions. *Pest Management Science* 62, 919–926.
- Piedra Buena, A., Garcia-Alvarez, A., Diez-Rojo, M.A., Ros, C., Fernandez, P., Lacasa, A. and Bello, A. (2007) Use of pepper crop residues for the control of root-knot nematodes. *Bioresource Technology* 98, 2846–2851.
- Ploeg, A.T. and Stapleton, J.J. (2001) Glasshouse studies on the effect of time, temperature and amendment of soil with broccoli plant residues on the infestation of melon plants by *Meloidogyne incognita* and *M. javanica*. *Nematology* 3, 855–861.
- Porras, M., Barrau, C., Romero, E., Zurera, C. and Romero, F. (2009) Effect of biofumigation with *Brassica carinata* and soil solarization on *Phytophthora* spp. and strawberry yield. *Acta Horticulturae* 842, 969–972.
- Potter, M.J., Davies, K. and Rathjen, A.J. (1998) Suppressive impact of glucosinolates in *Brassica* vegetative tissues on root lesion nematode, *Pratylenchus neglectus*. *Journal of Chemical Ecology* 24, 67–80.
- Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C. and Moënne-Loccoz, Y. (2009) The rhizosphere playground and battlefield for soil borne pathogens and beneficial microorganisms. *Plant Soil* 321, 341–361.
- Rahman, L. and Somers, T. (2005) Suppression of root knot nematode (*Meloidogyne javanica*) after incorporation of Indian mustard cv. Nemfix as green manure and seed meal in vineyards. *Australian Plant Pathology* 34, 77–83.
- Rausch, T. and Wachter, A. (2005) Sulfur metabolism: a versatile platform for launching defence operations. *Trends in Plant Science* 10, 503–509.
- Rosa, A.S.E. (1997) Daily variation in glucosinolate concentrations in the leaves and roots of cabbage seedlings in two constant temperature regimes. *Journal of the Science of Food and Agriculture* 73, 364–368.
- Sarwar, M., Kirkegaard, J.A., Wong, P.T.W. and Desmarchelier, J.M. (1998) Biofumigation potential of *Brassicas* III. *In vitro* toxicity of isothiocyanates to soil-borne fungal pathogens. *Plant and Soil* 201, 103–112.
- Shaban, W.I., El-Barougy, E. and Zian, A.H. (2011) Control of lupine *Fusarium* wilt by biofumigation with mustard and canola seed meal. *Tunisian Journal of Plant Protection* 6, 87–98.
- Snapp, S.S., Date, K.U., Kirk, W., O'Neil, K., Kremen, A. and Bird, G. (2007) Root, shoot tissues of *Brassica juncea* and *Cereal secale* promote potato health. *Plant Soil* 294, 55–72.
- Stefania Galletti, S., Sala, E., Leoni, O., Luigi Burzi, P. and Cerato, C. (2008) *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. *Biological Control* 45, 319–327.
- Steffek, R., Spornberger, A. and Altenburger, J. (2006) Detection of microsclerotia of *Verticillium dahliae* in soil samples and prospects to reduce the inoculum potential of the fungus in the soil. *Agriciculturae Conspectus Scientificus* 71, 145–148.
- Taylor, F.I. (2013) Control of soil borne potato pathogens using *Brassica* spp. mediated biofumigation. PhD thesis, University of Glasgow, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, Glasgow, Scotland, UK.
- Timonin, M.I. and Thexton, R.H. (1950) The rhizosphere effect of onion and garlic on soil microflora. *Soil Science Society of America Proceedings* 15, 186–189.
- Traquair, J.A. (1984) Etiology and control of orchard replant problems: a review. *Canadian Journal of Plant Pathology* 6, 54–62.
- Van Os, G. and Lazzeri, L. (2006) Control of *Rhizoctonia solani* in lily by biofumigation with *Brassica* seed meal. In: *Second International Biofumigation Symposium*, University of Idaho, Moscow, Idaho, p. 63.
- Van Os, G.J., Bijman, V., van Bruggen, A.S., de Boer, F.A., Breeuwsma, S., van der Bent, J., de Boer, M. and Lzzeri, L. (2004) Biofumigation against soil borne diseases in flower bulb culture. *Agroindustria* 3, 285–301.

- Vera, C., McGregor, D. and Downey, R. (1987) Detrimental effects of volunteer *Brassica* on production of certain cereal, and oilseed crops. *Canadian Journal of Plant Science* 67, 983–995.
- Vianene, N.M. and Abawi, G.S. (1998) Management of *Meloidogyne hapla* on lettuce in organic soil. *Plant Disease* 82, 945–952.
- Walker, J.C., Moreil, S. and Foster, H.H. (1937) Toxicity of mustard oils and related sulphur compounds to certain fungi. *American Journal of Botany* 24, 536–541.
- Wang, D., Marschner, P., Solaiman, Z. and Rengel, Z. (2007) Below ground interactions between intercropped wheat and *Brassicaceae* in acidic and alkaline soils. *Soil Biology and Biochemistry* 39, 961–971.
- Wang, D., Rosen, C., Kinkel, L., Cao, A., Tharayil, N. and Gerik, J. (2009) Production of methyl sulfide and dimethyl disulfide from soil-incorporated plant materials and implications for controlling soilborne pathogens. *Plant and Soil* 324, 185–197.
- Wang, Q., Ma, Y., Wang, G., Gu, Z., Sun, D., An, X. and Chang, Z. (2014) Integration of biofumigation with antagonistic microorganism can control *Phytophthora* blight of pepper plants by regulating soil bacterial community structure. *European Journal of Soil Biology* 61, 58–67.
- Williams-Woodward, J.L., Pfleger, F.L., Fritz, V.A. and Allmaras R.R. (1997) Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches*. *Plant and Soil* 188, 43–48.
- Xu, Y., Wang, G., Jin, J., Liu, J., Zhang, Q. and Liu, X. (2009) Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. *Soil Biology and Biochemistry* 41, 919–925.
- Yulianti, Y., Sivasithamparan, K. and Turner, W.D. (2006) Response of different forms of propagules of *Rhizoctonia solani* AG2-1 (ZG5) exposed to the volatiles produced in soil amended with green manures. *Annals of Applied Biology* 148, 105–111.
- Zasada, I.A. and Ferris, H. (2004) Nematode suppression with *Brassicaceae* amendments: application based upon glucosinolate profiles. *Soil Biology and Biochemistry* 36, 1017–1024.
- Zasada, I.A., Masler, E.P., Rogers, S.T. and Halbrecht, J.M. (2009) Behavioural response of *Meloidogyne incognita* to benzyl isothiocyanate. *Nematology* 11, 603–610.
- Zurera, C., Romero, E., Porras, M., Barrau, C. and Romero, F. (2007) *Brassica carinata* as a biofumigant to control *Phytophthora* spp. in strawberry fields. In: XVI International Plant Protection Congress, Abstract. Glasgow, Scotland, 546 pp.