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Biofumigation: Success and Prospects in Soilborne Plant Disease Management.

Dr. Pramod Prasad[1], Dr. J. Kumar[2], Dr. Shailesh Pandey[3]
[1]*Scientist, Regional Station, Directorate of Wheat Research, Shimla, H.P. India*
[2]*Dean, College of Agriculture, G.B. Pant University of Agriculture & Technology, Pantnagar- India*
[3]*Rain Forest Research Institute, Jorhat,Assam*

Abstract

Over the last decade the phasing-out of methyl bromide has brought out the need for alternative strategies for the management of soilborne pests and diseases in to sharp focus. Among the different alternative control methods being touted to replace methyl bromide are the use of other fumigant-like pesticides such as the methyl isothiocyanate generator metam sodium, 1,3-dichloropropene (1,3-D), chloropicrin, or mixtures of these, and various biologically-based options. The attention for bioactive natural molecules has been strongly augmented because public opinion considers them as a mild, safe and reliable option to prevent or to fight several human, animal and plant diseases. Among several bioactive molecules, glucosinolates (GSLs) from Brassicaceae and their enzymatic degradation products especially isothiocyanates (ITCs) via myrosinase as biofumigant stands out as a promising alternative for the management of a variety of pests including weeds, insects and plant pathogens as reported through several researches. However, there are issues such as dose validation, response of other beneficial microbes in the soil to these molecules, etc. which needs to be further addressed.

Keywords:* *Biofumigation, Brassicaceae, Glucosinolates, anti-microbial properties, soilborne plant disease management

I. Introduction

Diseases caused by soilborne pathogens are the major constraint in establishing sustainable agriculture systems in which the use of chemical pesticides and fertilizers are to be limited to protect the environment for the future. Generally, these diseases are severe and often a limiting factor in conventional production systems, but are rare in undisturbed natural ecosystems (**Cook and Baker, 1983).** There is generally little scope for radical change in the underlying approach to management of soilborne pests and diseases. Effective control of soil-borne pathogens requires an understanding of the behavior of the target pest in the soil and soil characteristics in order to tailor the optimal utilization of resources. This includes all the aspects of the physicochemical characteristics of the soil conditions especially soil temperature, soil moisture, pH, soil organic matter, soil texture and soil microorganisms, and their behavior in soil under field conditions. A crucial factor in the management of diseases caused by these pathogens is to reduce their inoculum level below the critical threshold level before a susceptible crop is planted. Since the 1950s, chemical soil disinfestations have commonly been used for this purpose. Soil fumigants, especially Methyl Bromide (MB) is the most effective soil fumigant used by farmers around the world for this purpose. Despite being a broad-spectrum pesticide, MB was identified as a risk to the stratospheric ozone layer in 1992 and was targeted for worldwide phase-out in 1997 by means of the Montreal Protocol, an international treaty (**Stapleton et al, 2000**). The imminent loss of MB as a soil fumigant has stimulated rigorous efforts to develop and execute suitable replacement strategies.

Today, there is the need for diversified options and alternatives to fill different roles across the soil borne pest and disease management spectrum. Apart from various synthetic chemical alternatives, numerous nonchemical tactics have also been explored, field authenticated and in some cases implemented commercially. For certain situations, biofumigation and soil solarization are among the most useful of the non-chemical disinfestations methods. Biofumigation is the beneficial use of Brassica green manures that release Isothiocyanates (ITCs) chemically similar to methyl isothiocyanate, the active agent from the synthetic fumigant metam sodium, which is used as a substitute for methyl bromide in some systems (**Matthiessen and Kirkegaard, 2006**). This was later expanded to include animal and plant residues also. These advances have led to commercial adoption of biofumigation when applied to appropriate production systems.

Special focus has been made on members of the Brassicaceae family, which have been shown to act as suppressors of pathogens, insect pests and weeds. This effect is generally attributed to a range of biocidal compounds released into the soil when glucosinolates are transformed into bioactive fungicidal, insecticidal, nematocidal and herbicidal compounds like ITCs. Glucosinolates (GSLs), found in Brassica spp, are of interest due to the potential for using their degradation products as fumigants.

1.1 Soilborne plant disease management

Management of soilborne pests and diseases in intensive cropping systems is often highly challenging in implementation of acceptable methodologies and in dealing with secondary problems. Since the beginning of agriculture, generations of farmers have tried to develop practices for combating plagues suffered by their crops. A growing understanding of the interaction of pathogen and host has enabled the development of various methods for the control of specific plant diseases. Like all other diseases, the control of soilborne pathogens is based on the traditional principles of plant disease control strategies formulated as early as 1929 i.e. Avoidance, Exclusion, Eradication, Protection and Therapy. One of the principal strategies used by growers of high-value horticultural crops to combat these organisms is pre-plant soil disinfestation using pesticides or other physical and biological methods. Soil fumigants are the most effective soil disinfestation chemicals and methyl bromide (MB) is the most important and frequently used soil fumigant adopted by farmers around the world. Fumigants are pesticides which, when applied to soil, form a gas to control pests that live in the soil and can disrupt plant growth and crop production. The fumigants are either volatile chemicals that become gases at relatively low temperatures, around 40°F, or they are chemicals that react to produce such a gas (e.g., diazomet and metam-sodium converting to methyl isothiocyanate or MITC). Fumigants are usually heavier than air and commonly contain one or more halogen (Chlorine, Bromine or Fluorine). Over the last few decades, soil fumigation has been the most widely used method for soil-borne pest control. The use of fumigants to control soil pests has, therefore, become a common agricultural practice to maximize the yield of various crops, especially in warmer regions. However, environmental concerns have been raised because of negative attributes of fumigants such as high volatility, toxicity or carcinogenicity **(Baker et al., 1996**). In particular, methyl bromide (MeBr) was found to contribute to stratospheric ozone depletion. These concerns in combination with the frequent detection of fumigants in ambient air (**Van den Berg et al., 1994)** mandate that the processes and factors that affect fumigant behavior in the environment be better understood.

1.2 Methyl Bromide and ozone depletion

Methyl bromide is a colorless, non-flammable, low boiling point chemical with high vapor pressure (1,600 mm Hg at 20°C) and reasonable water solubility (13.4 g/L) (**Yates et al., 1996**). The first use of methyl bromide as a soil fumigant occurred in France in the 1930s**.** Since its discovery and implementation, methyl bromide has been consistently effective for control of nematodes, fungi, insects and weeds and has been used on more than 100 crops worldwide (**Yates et al., 1996**). Methyl bromide's high vapor pressure allows for rapid and thorough distribution through the soil, enhancing its effectiveness as a fumigant.

Despite being a broad-spectrum pesticide, MB was identified as a risk to the stratospheric ozone layer in 1992 and was targeted for worldwide phase-out in 1997 by means of the Montreal Protocol, an international treaty **(Stapleton et al., 2000).** Ozone loss is caused by heterogenous chemical processes in which chlorine and bromide molecules are converted to reactive forms by chemical reactions that take place on particles found in polar stratospheric clouds. Because of large economic consequences the loss of methyl bromide, EPA has made it a priority to find and register its replacement. To this end some progress has been made. The chemical 1,3-dichloropropene (Telone®) was registered in 2001 for preplant soil fumigation in strawberries and tomatoes. Other chemicals such as chloropicrin, 1, 3-dichloropropene, propagyl bromide, metam sodium, methyl iodide and sodium azide were evaluated as alternatives to methyl bromide, but were not considered for registration in the USA. The fumigants 1,3-D and Methyl isothiocyanate (MITC) are considered to be viable alternatives for MeBr. Although 1,3-D is effective against nematodes and MITC is effective against nematodes and a variety of weeds and fungal pathogens **(Dungan and Yates, 2003).**

1.3 Organic amendments and pathogen suppression

The use of organic amendments such as animal manure, green manure (the incorporation of crop residues into the soil), composts and peats has been proposed both for conventional and biological systems of agriculture, to improve soil structure and fertility (**Cavigelli and Thien, 2003**), and decrease the incidence of disease caused by soilborne pathogens (**Litterick et al., 2004**). During the past century, the introduction of synthetic inorganic fertilizers, disease-resistant varieties and fungicides has allowed farmers to break the link between organic amendments and soil fertility (**Hoitink and Boehm, 1999**). As a result, organic materials such as crop residues and manure from essential resources became solid wastes. After the reduction of the organic input, soil organic matter decreased over time, soil fertility declined, and a large number of diseases caused by soilborne plant pathogens spread in agro-ecosystems (**Bailey and Lazarovits, 2003**). A renewed interest in application of organic matter (OM) to soil, for control of soilborne pathogens, has been stimulated by public concern about the adverse effects of soil fumigants and fungicides on the environment, and the need for healthier agricultural products (**Lazarovits, 2001**). Several studies have shown that organic amendments can be very effective in controlling diseases caused by pathogens such as *Fusarium* spp. (**Szczech, 1999**), *Phytophthora* spp. (**Szczech and Smolinska, 2001**), *Pythium* spp. (**Veeken et al., 2005**), *Rhizoctonia solani* (**Diab et al., 2003**), *Sclerotinia* spp. (**Boulter et al., 2002)**, *Sclerotium* spp. (**Coventry et al., 2005**), *Thielaviopsis basicola* (**Papavizas, 1968**) and *Verticillium dahliae* (**Lazarovits et al., 1999**).

Different complementary mechanisms have been proposed to explain the suppressive capacity of organic amendments such as enhanced activities of antagonistic microbes **(Hoitink and Boehm, 1999)**, increased competition against pathogens for resources that cause fungistasis (**Lockwood, 1990**), release of fungitoxic compounds during organic matter decomposition (**Tenuta and Lazarovits, 2002**), or induction of systemic resistance in the host plants (**Pharand et al., 2002**).

1.4 The Biofumigation Concept Synthetic Isothiocyanates

Broad-spectrum true gaseous fumigants with high vapor pressure that diffuse and penetrate rapidly and far through soil such as methyl bromide have historically not found a place in intermediate production systems because of their high cost, need for plastic trapping of the soil to retain the gas and more importantly environmental risks associated with them. However, the less potent and less costly broad-spectrum pesticide like metam sodium (sodium *N*-methyldithiocarbamate) has been widely used world-wide since the 1950s to control a range soilborne pests and diseases in high value crops. Metam sodium generates the compound methyl isothiocyanate upon contact with moist soil that possesses broad-spectrum biological activity against nematodes, fungal pathogens, insects, and weeds. The toxic effects of pure isothiocyanates have been known for many years. Despite the broad-spectrum efficacy of methyl isothiocyanate, however, metam sodium is too costly for use in many production systems (**Matthiessen and Kirkegaard, 2006**). This logically provides richer opportunities for researchers seeking to develop non-pesticide-based management options than do highly intensive systems.

1.5 Glucosinolates: chemistry

Glucosinolates, once known as mustard oil glucosides, have been part of human life for thousands of years because of the strong flavors and tastes they elicit in cabbage, broccoli, and other *Brassica* vegetables. In the past few decades, the importance of these nitrogen and sulfur containing plant secondary metabolites has increased further following discovery of their potential as cancer-prevention agents, crop-protection compounds in agriculture. Glucosinolates have been reported almost exclusively from the order Capparales, which contains 15 families, including the Brassicaceae, Capparaceae, and Caricaceae. Curiously, glucosinolates are also known from the genus *Drypetes* of the family Euphorbiaceae, a genus completely unrelated to the other glucosinolates containing families (**Barbara and Jonathan, 2006**).

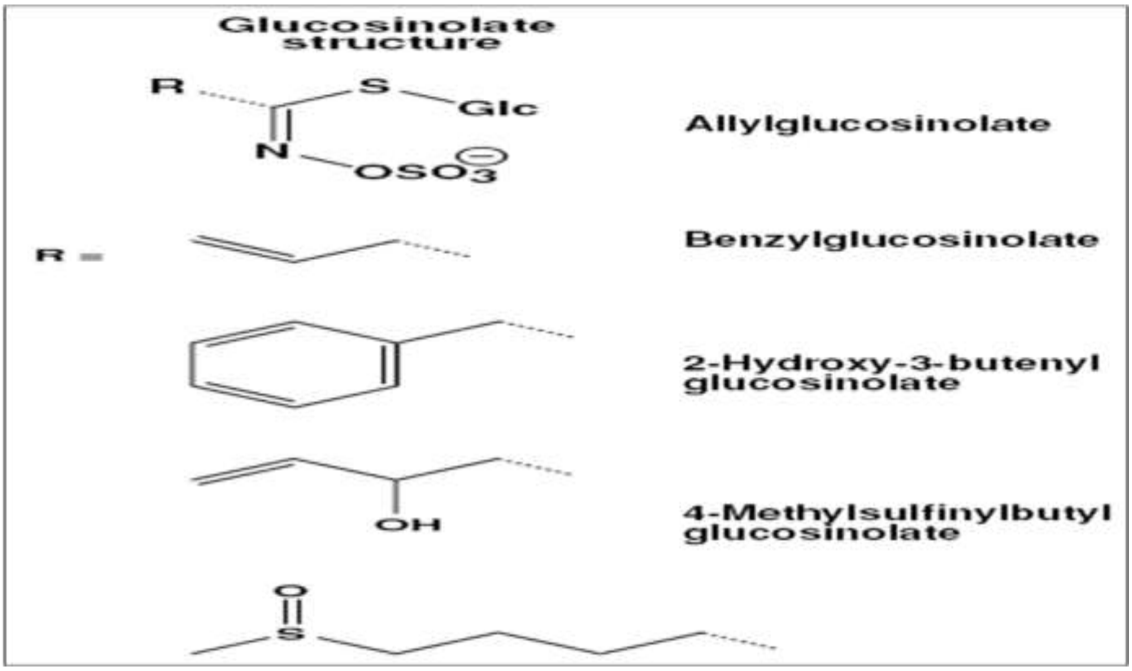

Figure 1. Chemical structures of glucosinolates. The common structure is shown, as well as examples of some specific glucosinolates cited in the text that show typical variation in the structure of the side chain (R).

The approximately 120 described glucosinolates share a chemical structure consisting of a β-D-glucopyranose residue linked via a sulfur atom to a (*Z*)-*N*-hydroximinosulfate ester, plus a variable R group (**Figure 1)** derived from one of eight amino acids (**Fahey et al., 2001**). Glucosinolates can be classified by their precursor amino acid and the types of modification to the R group. Compounds derived from Ala, Leu, Ile, Met, or Val are called aliphatic glucosinolates, those derived from Phe or Tyr are called aromatic glucosinolates, and those derived from Trp are called indole glucosinolates. The R groups of most glucosinolates are extensively modified from these precursor amino acids, with methionine undergoing an especially wide range of transformations (**Fahey et al., 2001**). Plants accumulating glucosinolates always possess a thioglucoside glucohydrolase activity known as myrosinase, which hydrolyzes the glucose moiety on the main skeleton (**Rask et al., 2000**). The products are glucose and an unstable aglycone that can rearrange to form isothiocyanates, nitriles, and other products. Most of the biological activities of glucosinolates are attributed to the actions of their hydrolysis products (**Wittstock et al., 2003**).

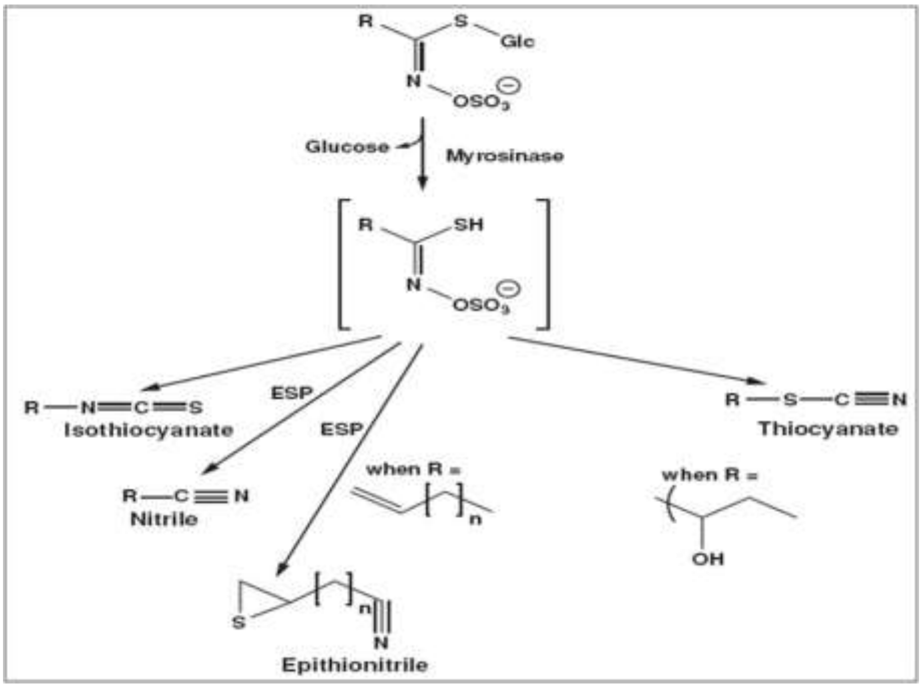

Figure 2. Glucosinolate hydrolysis. Abbreviations: ESP, epithiospecific protein; R, variable side chain.

1.6 The Glucosinolate-Myrosinase System

Glucosinolates in themselves have no or very limited biological activity. But they are important because of the wide variety of active products that derive from them as a result of myrosinase action, a dynamic link that has led to the interaction commonly being dubbed the "glucosinolate-myrosinase system (**Rask et al., 2000**). It is generally believed that the glucosinolate-myrosinase system is part of plant defense against insects and possibly pathogens and that it evolved from the more prevalent system of cyanogenic glucosides (**Rask et al., 2000**). Glucosinolates are very stable water-soluble compounds, while myrosinase is sequestered in vacuoles within myrosin cells, with both compounds separately coexisting distributed throughout the plant (**Fahey et al., 2001**). It is only upon physical disruption of the plant tissue that the physical separation of the myrosinase from its glucosinolate substrate is overcome, triggering in the presence of water the hydrolysis reactions that release a variety of biologically-active products such as isothiocyanates, organic cyanides, oxazolidinethiones, nitriles and ionic thiocyanates (**Brown and Morra, 1997**) **(Figure 2).** Among these isothiocyanates have been generally reported as the most biologically active. The type of isothiocyanate generated corresponds to the type of glucosinolates substrate, as characterized by its side-chain (**Chew, 1988).**

Table 1. Glucosinolates identified in *Brassicaceae* and their major hydrolysis products (Rosa et al., 1997).

Group	S. No.	Chemical name	Trivial name	Hydrolysis products
Aliphatic	1	3-Methylthiopropyl	Glucoiberverin	ITC, nitriles
	2	3-Methylsulphinylpropyl	Glucoberin	ITC, nitriles
	3	2-Propenyl	Sinigrin	ITC, nitriles
	4	4-Methylthiobutyl	Glucoerucin	ITC, nitriles
	5	4-Methylsulphinylbutyl	Glucoraphanin	ITC, nitriles
	6	3-Butenyl	Gluconapin	ITC, nitriles
	7	2-Hydroxy-3-butenyl	Progoitrin	Oxazolidine-2-thiones
	8	5-Methylsulphinylpentyl	Glucoalyssin	ITC, nitriles
	9	4-Pentenyl	Glucobrassicanapin	ITC, nitriles
	10	2-Hydroxy-4-pentenyl	Gluconapoleiferin	Oxazolidine-2-thiones
Aromatic	11	2-Phenylethyl	Gluconasturtiin	ITC
	12	2-Hydroxy-2-phenylethyl	Glucobarbarin	ITC
	13	Benzyl	Glucotropaeolin	ITC
	14	*p-Hydroxybenzyl*	Glucosinalbin	ITC
Indolyl	15	3-Indolylmethyl	Glucobrassicin	Indolyl-3-carbinol
	16	4-Hydroxy-3-indolylmethyl	4-ydroxyglucobrassicin	Thiocyanate
	17	4-Methoxy-3-indolylmethyl	4-ethoxyglucobrassicin	Auxins?
	18	1-Methoxy-3-indolylmethyl	Neoglucobrassicin	Phytoalexins

1.7 Brassicaceae and biofumigation

Mustards (Cruciferae or *Brassicaceae*) are broadleaf, cool-season spring annuals with large, deep taproots. They are native to the Mediterranean region of Europe and were domesticated about 4,000 years ago as a source of oil, spice and medicines. Today, they are grown around the world as specialty oilseed crops, green manures and forage crops for animals (**Barbara and Jonathan, 2006**). Special focus has been made on members of the *Brassicaceae* family, which have been shown to act as suppressors of pests and disease organisms. This effect is generally attributed to a range of biocidal compounds released into the soil when glucosinolates are transformed into bioactive or nematocidal, fungicidal, insecticidal and herbicidal compounds. Glucosinolates (GSL), found in *Brassica* species, are of interest due to the potential for using their degradation products as fumigants. When hydrolyzed by the enzyme myrosinase, GLS produce D-glucose, sulfate, isothiocyanates (volatile mustard oils), thiocyanates and nitriles. Isothiocyanates (ITC) and nitriles have been demonstrated to control fungi (**Sarwar et al., 1998**) bacteria (**Delaquis and Mazza, 1995**), nematodes (**Mojtahedi et al., 1993**), insects (**Noble et al., 1999**) and some weed seeds in laboratory experiments (**Sarwar et al., 1998**). Allyl isothiocyanate (AITC) is the predominant ITC produced by indian mustard (*B. juncea*). There are about 18 different types of GSLs (**Table 1**) commonly found in brassicas which vary in their structure depending on the type of organic side chain (aliphatic, aromatic or indolyl) on the molecule. The profile, concentration and distribution of these GSLs (**Table 2**) vary within and between *Brassica* species and in different plant tissues, and consequently the concentration and type of biocidal hydrolysis products evolved also varies **(Kirkegaard and Sarwar, 1998).**

Table 2. Concentration of individual glucosinolates (µmol/g) found in field grown *Brassica* and related species at flowering. Glucosinolates are numbered as previous (Table 1).

Species	Aliphatic							Aromatic	Indolyl			
	2	3	5	6	7	9	10	11	15	16	17	18
B. napus oleifera	–	–	0.5–0.8	0.3–8.7	0.5–5.9	0.5–4.5	0.8–2.9	0.4–0.9	0.1–0.6	0.1	0.1	0.1–0.4
B. napus rapifera	–	–	–	0.4–0.6	0.5–5.1	0.6	0.5–3.7	0–1.4	0.1–0.4	–	–	0.1–0.4
B. campestris oleifera	–	–	–	1.6	1.7	3.8	1.8	1.6	0.2	0.1	–	–
B. campestris rapifera	–	–	–	0.7–4.0	0.9–4.6	1.9–6.5	1.1–2.1	0.6–1.7	0.1–0.2	0.1	–	0.1–0.3
B. oleracea	2.2	1.3	1.0	0.5	0.5	–	–	–	2.9	0.2	–	0.3
B. carinata	–	10.0–20.2	–	–	–	–	–	–	0.4–0.9	0–0.1	–	0–0.1
B. nigra	–	10.7–26.4	–	–	–	–	–	0.3–0.4	0.1	–	–	–
B. juncea	–	0.1–18.7	–	7.5	–	0.1–2.0	0.1–0.3	0–1.3	0–0.2	0–0.1	–	0.1–0.2

Kirkegaard and Sarwar (1998) investigated the variation in GSL production in the roots and shoots of 76 entries from 13 *Brassica* and related weed species grown in the field. Total plant GSL production on ground area basis ranged from 0.8 to 45.3 mmol/m^2. They found that the types of GSLs present in the tissues varied considerably between species but were consistent within species. In contrast, the concentration of individual and total GSLs in both root and shoot tissues varied four to ten-fold both between and within all species. Shoots contained predominately aliphatic GSLs, while aromatic GSLs, particularly 2- phenylethyl GSL, were dominant in the roots of all entries. Moreover; the growth stage of the mustard plant also decides the glucosinolate content. Most of the studies suggest that it is the flowering stage during which maximum glucosinolate can be detected (**Kirkegaard and Sarwar, 1998**).

The term biofumigation coined by J. A. Kirkegaard for that part of the suppressive effects of *Brassica* species on noxious soilborne organisms that arose quite specifically through liberation of isothiocyanates from hydrolysis of the glucosinolates that characterize the *Brassicaceae* (**Kirkegaard and Matthiessen, 2004**). Since isothiocyanates are biologically active, and the methyl isothiocyanate generator metam sodium is widely used as a broad-spectrum fumigant like pesticide, there existed a logical link and an impetus to the notion of harnessing, and further developing, a biological source of isothiocyanates for suppression of soilborne pests and diseases (**Brown and Morra, 1997**). The term biofumigation attempted to ascribe, in a simplified way, a mechanistic name to this particular part of a general phenomenon of allelopathic effects that have been observed in the Brassicaceae for centuries and given them a reputation as poor companion plants (**Chew, 1988**).

1.8 Brassica used in plant disease management

The antifungal effects of pure ITCs vary with different organic side chain structures (**Drobnica et al., 1967).** Fungicidal concentrations of ITCs are also known to differ by an order of magnitude for different fungal species (**Brown and Morra, 1997**). However, generalizing with regard to the toxicity of different ITCs is made difficult by the different experimental approaches used in previous studies. Some studies have investigated the toxicity of pure ITCs in headspace experiments where the volatility of the compound may influence its activity (**Angus et al., 1994**), while others have used ITCs dissolved in the growing media (**Drobnica et al., 1967**).

The superior growth and yield of wheat following *Brassica* crops such as canola (*B. napus* L.) and Indian mustard {*B. juncea* (L.) Czern & Coss} is thought to be due to suppression of soil-borne fungal pathogens by ITCs released from the *Brassica* crop residues (**Angus et al., 1994; Kirkegaard et al., 1994**). Several fungal root pathogens including *Sclerotium rolfsii, Sclerotinia sclerotium, Gaemannomyces graminis, Rhizoctonia solani* (Kuhn), *Bipolaris sorokiniana*, *Pythium irregulare* and *Fusarium graminearum* cause significant losses in crop yield worldwide. **Angus et al. (1994)** and **Kirkegaard et al. (1996)** have established the toxicity of volatiles released from *Brassica* tissues to these fungi but the contribution of individual ITCs is not known, and is important in order to develop selection criteria to develop brassicas with enhanced biofumigation potential.

1.9 Pathogen suppression through biofumigation

Maintaining high levels of organic matter on the soil surface and incorporated into soil generally is associated with lower incidence and severity of root diseases (**Cohen et al., 2005**). However, numerous reports have indicated that the management of crop residues or the addition of various types of organic amendments had either enhanced or suppressed diseases caused by soilborne pathogens. Meanwhile, there also are many examples of the value of incorporating organic matter, such as dry or green oat, barley, and maize; tree bark; and chicken manure for the control of such pathogens as

Fusarium, *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Thielaviopsis* spp. (**Cook and Baker, 1983**). A number of recent reports also pointed out that various kinds of crop residues or composts incorporated into soil were shown to be effective in suppressing soilborne pathogens: hairy vetch (*Vicia villosa*) for *Fusarium oxysporum* (**Zhou and Everts, 2004**), green manure or composts for *Pythium graminicola* (**Craft and Nelson, 1996**), compost tea for *P. ultimum* (**Scheuerell and Mahaffee, 2004**), cotton-gin trash for *Sclerotium rolfsii* (**Bulluck and Ristaino, 2002**), and residue of broccoli for *Verticillium dahliae* (**Koike and Subbarao, 2000**).

Here we are presenting some of the reports where biofumigation using *Brassica* species and other crops have really proved promising in managing soil borne plant diseases. **Mazzola et al., (2001)** indicated that soil amendment with *Brassica napus* seed meal suppressed apple root rot caused by *R. solani* regardless of the glucosinolate content, suggesting that proliferation of soil microbes after seed meal amendment was responsible for disease suppression. Thus, glucosinolates in cruciferous plants may be related to growth inhibition of *R. solani*, however; their effects on disease suppression in the natural soil need to be examined in a controlled, sensitive experimental system. **Diab et al., (2003)** specifically examined various cultivars of *B. rapa* for their biofumigation activity. Glucosinolate content of plant residues was determined and correlated to the resulting incidence of disease. The effects of plant residues incorporated into the soil on suppression of *R. solani* were evaluated by measuring damping off and pericarp colonization, representing the pathogenic and saprophytic activities of the pathogen, respectively, in order to assess the relationship between these two parameters and the site or sites of microbial interaction responsible for disease suppression. **Hiddink et al. (2005)** studied the effect of mixed cropping on disease suppressiveness of soils in Brussels sprouts-barley and triticale-white clover cropping systems and observed increased disease suppressiveness in field soils cropped barley against *F. oxysporum* f. sp. *lini* than soils cropped to Brussels sprouts or the mixture of Brussels sprouts and barley. **Motisi et al. (2009)** investigated the differences in efficacy observed in the field, by analyzing the mechanisms by which a Brassica cover crop can act as a biofumigant crop in the prevention of soil-borne disease development and their finding suggests that mustard residue incorporation was consistently effective at decreasing disease incidence from year to year. **Bensen et al. (2009)** conducted several experiments in commercial lettuce fields to evaluate the effects of mustard cover crops on lettuce drop caused by *Sclerotinia minor* and on weed density and seed viability and concluded the incidence of lettuce drop was lower in mustard-cover-cropped and head lettuce yield was significantly higher in mustard-cover-cropped plots compared with a fallow control. **Cohen et al. (2005)** applied a low glucosinolate content (21.8 micromoles/g) Brassica napus seed meal (RSM) to orchard soils which altered communities of both pathogenic and saprophytic soil micro-organisms. RSM amendment reduced infection by native and introduced isolates of *Rhizoctonia* spp.. **Debode et al. (2005)** screened several crop residues for their ability to reduce the viability of microsclerotia of *Verticillium dahliae* var. *longisporum* when incorporated into soil and observed that the effectiveness in reducing the viability of microsclerotia depended on the soil sample and on the type of residue. Broccoli, cauliflower and ryegrass incorporation significantly reduced the inoculum level by more than 90%, while Indian mustard significantly reduced numbers of viable microsclerotia by 50%. **Kirkegaard et al. (2000)** conducted field experiments to investigate the hypothesis that biofumigation by *Brassica* break crops would reduce inoculum of the take all fungus *Gaeumannomyces graminis* var. *tritici* (Ggt) to lower levels than non-*Brassica* break crops, and thereby reduce Ggt infection and associated yield loss in subsequent wheat crops. Ggt inoculum declined more rapidly under *Brassica* crops than under linola and this reduction coincided with the period of root decay and reduced root glucosinolate concentrations around crop maturity. **Deadman et al. (2006)** reported that under commercial conditions solarization and biofumigation (solarization following cabbage residue incorporation) both reduced *Pythium* *aphanidermatum* inoculum levels in soil relative to untreated controls under greenhouse conditions. Both treatments also reduced the level of damping-off disease in greenhouse seedlings. **Fayzalla et al. (2009)** conducted laboratory, glasshouse and field experiment to evaluate the efficacy of mustard (*B. juncea*) seed meal against soil borne pathogenic fungi of soyabean i.e. *F. oxysporum, R. solani, Macrophomina phaseolina*, *S. rolfsii* and found that the activity of these pathogens was hampered in all the experiments.

II. Integration of biofumigation and biological control in IPM approach Integrated pest management

The direction taken for pest management over the last 50 years has been influenced by a variety of factors, including the availability of pest control technologies, priorities of governments to provide adequate food supplies, the growth of agribusiness and the value placed on scientific endeavor. These factors and their impact have been tempered by increasing public concern for food safety and the global realization of the need to use resources more sustainably. These combinations of driving forces form the foundation for pest management in the future. IPM is essentially a holistic approach to pest control that seeks to optimize the use of a combination of methods to manage a whole spectrum of pests within a particular cropping system.

2.1 Integrating biological control with biofumigation

Biofumigation involves enrichment in carbon source that may alter or even stimulate the resident or introduced microflora, both beneficial and pathogenic. More recently, disease control was even related to functional mechanisms other than biofumigation, but occurring as a consequence of green manures or seed meal incorporation, involving stimulation of resident streptomycetes or actinomycete**.** One point which still needs to be elucidated concerns the effects of the biocidal compounds derived from glucosinolate degradation, mainly isothiocyanates, on the beneficial soil microflora naturally occurring or artificially introduced as biological control agents. In particular, unclear effects of rapeseed meal on *Trichoderma* have been reported, suggesting incorporating the meal prior to *Trichoderma* inoculation, since the direct combination seemed non-compatible (**Dandurand et al., 2000**). **Galletti et al., (2008)** tested *Trichoderma* spp. for tolerance to toxic volatiles released from *Brassica carinata* seed meal (BCSM) and in direct contact with the meal. They reported that all *Trichoderma* spp tested were less sensitive to BCSM than the assayed pathogens (*Pythium ultimum*, *Rhizoctonia solani* and *Fusarium oxysporum*). Moreover; most of the isolates were also able to grow on BCSM and over the pathogens tested. BCSM incorporation increased pathogen population, but reduced sugar beet damping off disease incidence, probably due to indirect mechanisms (**Galletti et al., 2008**).

The nutritive base incorporated with the antagonist into soil has various effects on soil microflora. It may act as an organic amendment. In some cases, biocontrol is the result of the incorporation of organic material and not the result of the activity of the introduced antagonist**.** In some situations root exudates may replace additional food to stimulate the antagonist. Before extensive application of antagonists as a biocontrol agent, the strain used should be checked for its compatibility with the biofumigant crop, innocuousness to the host plants of the target pathogens, and also to many other plants. This suggestion is valid for any potential antagonist, including mycoviruses, actinomycetes and bacteria. Further investigations are needed to evaluate the effect of this integrated approach with different Brassica or non Brassica meals, on other patho-systems and finally in field conditions.

III. Conclusion

Soilborne plant pathogens are among the major factors limiting the productivity of agro-ecosystems. They are often difficult to control with conventional strategies such as the use of resistant host cultivars and synthetic fungicides. The lack of reliable chemicals, the occurrence of fungicide resistance in pathogens and associated environmental risks, and the breakdown or circumvention of host resistance by pathogen populations are some of the reasons underlying efforts to develop new disease control measures. Over the last decade, the attention for bioactive natural molecules is strongly increased because public opinion considers them as a mild, safe and reliable option to prevent or to fight not only several diseases in humans, but even different plant pathogens, thus limiting the use of synthetic pesticides in agriculture. Among several bioactive molecules, glucosinolates from Brassicaceae and their enzymatic degradation products especially isothiocyanates (ITCs) via myrosinase stand out as very promising alternative for the management of variety of pests including weeds, insects and plant pathogens. Significant progress has been made towards plant disease suppression through biofumigation in various plant-pathogen systems. However, we are still lacking reliable guidelines to predict the impact of any type of organic amendment on specific soilborne diseases. In this context, further studies would improve our understanding of this subject.

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