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RESEARCH ARTICLE

BIOFUMIGATION: A NEW STRATEGY FOR DISEASE MANAGEMENT IN ORGANIC FARMING SYSTEM

*Smita Puri

Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Regional Agriculture Research Station, Bhopal Road, Sagar-470002 (M.P.), India

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ABSTRACT

Soil borne diseases are very difficult to control, traditionally chemical soil fumigants were used to manage them but they are harmful to the environment and human health. For the management of soil borne diseases in organic production system use of various eco-friendly methods viz. green manures, mulches, organic amendments and composts etc. was recommended and practiced. In this context biofumigation is a new concept, which is gaining attention of the researchers and shown some potential in management of soil borne disease in Europe and Australia. Biofumigation is an agronomic technique that makes use of some plants' defensive systems and biofumigant plants are mainly Brassicas. It is an eco-friendly process and important strategy of disease management in organic production system in the developed countries. The present review was prepared in an effort to compile different research works conducted worldwide regarding various aspects of biofumigation.

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INTRODUCTION

Organic farming is the eco-friendly production system which relies on biological methods for soil nutrition as well as disease and pest management with no use of chemical inputs. Management of soilborne plant pathogens and pests is a major problem faced by organic farmers. Virulent and aggressive pathogens could significantly affect the crop production and reduce the yield. Therefore, in modern organic farming a reliable and sustainable pathogen management strategy is required. Soil solarisation, green mauring, compost and biological control etc. are different methods used for the management of soil borne plant pathogens. Biofumigation has also shown some potential in the management of soil diseases in organic production system in various parts of the world. The viability of biofumigation process for management of plant pathogens has been investigated for many years. This paper describes the concept of biofumigation, its mechanism, biofumigants, and instance of disease management and its future prospects.

Biofumigation: Bio-fumigation is a popular concept for the management of soil-borne plant pathogens in the developed countries. J. A. Kirkegaard coined the term biofumigation for the suppressive effects of *plant* species on noxious soilborne organisms that arose quite specifically through liberation of isothiocyanates from hydrolysis of the glucosinolates that is a characteristic feature of the Brassicaceae (Kirkegaard and Matthiessen, 2004). In a simplified way biofumigation attempted to ascribe, a mechanistic name to a particular part of a general phenomenon of allelopathic (Whittaker and Feeny, 1971) effects that have been observed in the Brassicaceae for centuries and given them a reputation as poor companion plants (Chew, 1988).

It is an agronomic technique based on the use of some compounds of plants' defensive systems, in which volatile chemicals (allelochemicals) released from decomposing plant tissues are used to suppress soil-borne pests and pathogens. The main plant species in which these volatiles found are the Brassicaceae (cabbage, cauliflower, kale, mustard), Capparidaceae (cleome), Moringaceae (horse-radish) species and Sorghum species (Rodman *et al.,* 1996). Since being coined, the initially adjectival term biofumigation has morphed into a noun and has rapidly entered the pest management lexicon in a much broader and more popularized way to encompass any beneficial effect arising from green manure or rotation crops, and even composts.

Corresponding author: Smita Puri
Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Regional Agriculture Research Station, Bhopal Road, Sagar-470002 (M.P.), India

Biofumigants

For using plants as effective biofumigant, ensure that the crop has good growth to maximize biomass and toxins production. It should be well chopped to release biofumigant chemicals and incorporated immediately following chopping to avoid vapour loss. It should be mixed into moist soil to seal in biofumigant and should not be allowed to go to seed. Sorghum species - The biofumigation affect of Sorghum spp. (*Sorghum bicolor* (L.) Moench) and Sudangrass (*Sorghum bicolor* subsp. *sudanense* (P.) Stapf), is due to the production of a cyanogenic glucoside *p*-hydroxy-(*S*)-mandelonitrile-β-D-glucoside compound called Dhurrin, as a substrate of its secondary defensive system that breaks down to release toxic cyanide when plant tissue is damaged due to biotic or abiotic factors. Mojtahedi *et al.,* (1993) reported that certain sudangrass (*Sorghum sudanense* (Piper) Stapf) and sorghum-sudangrass hybrids (*S. bicolor* (L.) Moench×*S. sudanense*) were rated non-hosts for *Meloidogyne hapla* under greenhouse conditions.

- *Solanaceae (Capsicum* spp.) –Chili and marigold etc. are also known to behave as biofumigants. Mexican marigold, also known as *Tagetes*, has been successfully *used in the co*ntrol of *root-kno*t nematode in roses by a Kenyan Farmer. It is also used as a trap *c*rop and its root cells react to mechanical and biotic damage by producing terthiophenes which block the development and metabolism of plant pathogens.
- C*r*op residues and composts- 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).
- Swine manure - Soil or potting mixes amended with swine manure also suppressed *R. solani* (Diab *et al.,* 2003) and *S. rolfsii* (Bulluck and Ristaino, 2002), and volatile fatty acids released from liquid swine manure killed microsclerotia of *V. dahliae* (Tenuta and Lazarovits, 2002 b).
- *Muscodor albus* -An endophytic fungus, *M. albus* is also used as a biofumigant for the management of post harvest diseases of fruits and vegetable. It is closely related to another endophytic fungus *Xylaria* (Ascomycetes) and was first isolated from a cinnamon tree (Worapong *et al.,* 2001). *M.albus* is a sterile mycelium and can grow readily on ordinary culture media such as potato dextrose agar. It is effective against a wide range of storage pathogens and controlling fungal decay. Biofumigation for 24 h or longer with rye grain culture of *M.albus* controlled brown rot of peaches, caused by *Monilinia fructicola*, and gray mold and blue mold of apple, caused by *Botrytis cinerea* and *Penicillium expansum*, respectively and postharvest lemon diseases also (Mercier and Smilanick, 2005). Biofumigation of greenhouse soilless mix with rye grain culture of *M. albus* was also effective in controlling soil-borne diseases of vegetable seedlings (Mercier and Jiménez, 2004; Mercier and Manker, 2005). *M. albus* was reported to produce 28 organic volatile compounds which together inhibited and killed various species of fungi, oomycetes, and bacteria (Strobel *et al.,* 2001).
- Ceratocystis fimbriata- C.fimbriata Ellis & Halsted is a soilborne ascomycete fungus. Recently, it is found that a variety of volatile organic compounds (VOCs) produced by C. fimbriata have strong bioactivity against a wide range of fungi, bacteria and oomycetes (Li *et al.,*, 2015) thus making it a potential player in control of post harvest diseases of fruits through biofumigation. Unlike some traditional biological control agents, which must colonize wounds or some other susceptible sites to be effective (Janisiewicz and Korsten, 2002), the VOCs from C. fimbriata, acting as a biofumigant, does not require contact. Butyl acetate, ethyl acetate and ethanol were identified as VOC isolated from this fungus.
- *Brassicaceae* - The Family Brassicacea (*Brassicas*) contains more than 350 genera with 3000 species, of which many are known to contain glucosinolates. Mustards are native to the Mediterranean region of Europe; plants have broad leaf with large, deep taproots. They were domesticated about 4,000 years ago and now grown worldwide as a source of oil, spice and medicines. (Barbara and Jonathan, 2006). In addition to the fresh mass of *Brassica* (seeds, shoots and roots), different parts of *Brassica* plant like its meal as a cake or powder can be incorporated into the soil and may be used as mulch. Volatile oil of Mustard (VOOM), which is a mixture of different edible oils of canola can be used as pre-planting application as an alternative to methyl bromide. *Brassicas* are the most widely used plant species as biofumigants. The profile, concentration and distribution of different glucosinolates varies within and between *Brassica* species and in different plant tissues, and consequently the concentration and type of biocidal hydrolysis products evolved also varies (Mithen, 1992). In some cases where *Brassica* species is susceptible to some pathogens i.e. root knot nematode, the use of *Brassica* species has reduced their applicability as biofumigant green manure (Mcleod *et al.,* 2001). Therefore, it is desirable to select biofumigants those are poor or non hosts of the pathogen.

Mechanism of biofumigation

Special emphasis has been given to *Brassica* to describe the mechanism of biofumigation because detailed studies have been conducted on *Brassicas (*Kirkegaard and Matthiessen, 2004*)* mainly to explore and explain the same. In *Brassicas*, biofumigation is based on its most important enzymatic defensive systems the myrosinase-glucosinolate system (against insects and possibly pathogens) (Rask *et al.,* 2000). With this system, tissues of these plants can be used as a soft, eco-friendly alternative to chemical fumigants and sterilants. Mustards possess glucosinolate compounds in their seeds and foliage that upon soil-incorporation act as ‘‘biofumigants”, hydrolyzing to form isothiocyanates and other volatile compounds toxic to many soil-borne plant pathogens as well as pests. Previously known as mustard oil glucosides, Glucosinolates (GSLs), 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. The occurrence of glucosinolates has been reported from the order

Capparales, which have 15 families, including the Brassicaceae, Capparaceae, and Caricaceae. Moreover, glucosinolates are also known from the genus *Drypetes* of Euphorbiaceae family, a genus which is completely unrelated to the other glucosinolates containing families (Barbara and Jonathan, 2006). In fact, glucosinolates are not confined to *Brassicas* alone. At least 500 species of non-*brassica* dicotyledonous angiosperms have also been reported to contain one or more of the over 120 known GSLs (Fahey *et al.,* 2001). In the cells of *Brassica* plants, glucosinolates and hydrolysing enzyme myrosinase are found separately in vacuoles and myrosin cells, respectively.

Once attacked by pests or due to mechanical damage glucosinolates from vacuoles come in contact with the enzyme. After which, glucosinolates (GSLs) are hydrolyzed by the endogenous enzyme thioglucosidase hydrolase, (myrosinase) at neutral pH, to release isothiocyanates (ITCs) (Rosa *et al.,* 1997). Thiohydroximate O-sulfonate is the intermediate product of this reaction which on the basis of medium pH, metal ions and presence of protein factors could be converted in to isothiocyanates (ITC), thiocyanates, epithionitriles or nitriles (Kirkegaard and Sarwar, 1998; Fahey *et al.,,* 2001). Glucosinolates in themselves have no or very limited biocidal activity. But they are important because of the wide variety of active products such as isothiocyanates, organic cyanides, oxazolidinethiones, nitriles and ionic thiocyanates (Poulton and Moller, 1993) that derive from them as a result of myrosinase action (Brown and Morra, 1997), a dynamic link that has led to the interaction commonly being dubbed as the “glucosinolate-myrosinase system (Rask *et al.,* 2000).

Among the degradation products of glucosinolates, the isothiocyanates have been generally reported as the most biologically active, being recognized since early in the twentieth century as broad-spectrum biocides (Rosa and Rodrigues, 1999). ITCs are related to the active ingredient in the commercial fumigants metham sodium and dazomet and are highly toxic to pests and pathogens (Brown and Morra, 1997). Efficacy of ITCs is even comparable to the affectivity of synthetic pesticide MB and some antibiotics (gentamycin) (Lord *et al.,,* 2011; Aires *et al.,,* 2009; Lin *et al.,* 2000; Ward *et al.,* 1998; Lazzeri *et al.,* 1993, 2004; Lugauskas *et al.,* 2003). Isothiocyanates (ITC) and nitriles have been demonstrated to control fungi (Charron and Sams, 1999, 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*).

ITCs and their role in biofumigation

The biofumigation effect of *Brassicas* are due to ITCs that form from precursor glucosinolates (GSLs) when disrupted, such as when it is incorporated into soil (Kirkegaard and Sarwar, 1998). Till now researchers have identified over 100 Isothiocyanates, 20 of which are commonly produced by *Brassicas* and known to have a biocidal effect. Profiles of glycosinolates and the subsequent ITCs produced vary between *Brassica* species. Three main ITCs were identified and quantified from *B. juncea* roots (3-butenyl, 4-pentenyl, 2-phenyethyl) and five from the turnip/canola (*B. campestris/B.napus*) mix (3- butenyl, 4-pentenyl, 2-phenyethyl, 5-methylthiopentyl, benzyl). In total, the Bc/Bn mix produced 8 times more ITCs (7.5 µmol ITC/g root tissue) than *B. juncea* (0.9 µmol ITC/g root tissue). Most of the studies suggest that it is the flowering stage during which maximum glucosinolate can be detected (Manici *et al.,* 1997; Thurston, 1997; Kirkegaard and Sarwar, 1998). Identity and concentration of ITC also varies with the variety of mustard and with the soil condition (more sulphur = more glucosinolates = more ITC). The toxicity of an ITC sometimes differs among organisms, suggesting that specific plants could be utilized more successfully than others for biofumigant effects by matching them to particular pests or diseases. The difference in structure of individual GCs and ITCs depends on their organic side-chain (aliphatic, aromatic or indole), which also influences their antimicrobial activity (Drobnica *et al.,* 1967).

Disease management by biofumigation

Biofumigation of soil controls a number of weeds, nematodes and a variety of fungal soil-borne diseases but bacteria are less prone to it. Biofumigation is a novel method for controlling a range of post harvest diseases of fruits. For eg. volatiles produced by *Muscodor albus,* a mixture of low molecular weight compounds, are biocidal or biostatic to a broad variety of microorganisms (Strobel *et al.,* 2001; Worapong *et al.,* 2001), including *Botrytis cinerea, Geotrichum citri-aurantii, G. candidum,Monilinia fructicola*, *Penicillium digitatum,* and *P. expansum* (Mercier and Jiménez, 2004; Mercier and Smilanick, 2005). Placement of *M. albus* inside grape packages significantly controlled gray mold and may be a feasible approach to manage postharvest decay of table grape (Gabler *et al.,* 2006). Volatiles of *M.albus are* known to control brown rot of peach (Mercier and Jiménez, 2004). *Biofumigation by Muscodor albus* controlled green mold and sour rot of stored lemon (Mercier and Smilanick, 2005), *Monilinia fructicola*, and gray mold and blue mold of apple, caused by *Botrytis cinerea* and *P. Expansum (*Mercier and Jiménez, 2004*) and* soil-borne diseases of vegetable seedlings (Mercier and Manker, 2005). In Georgia, several cultivars of sorghum were found to suppress populations of *Criconemoides xenoplax* in the greenhouse (Nyczepir *et al.,* 1996).

Cultivation of marigolds on a nematode infested field resulted in a 50% increase in yield of tomatoes and melons Ploeg (2002). Significant reduction in the population of *Pratylenchus penetrans* in the soil and the roots of susceptible tomato and potato plants was reported when they double cropped with *Tagetes erecta* L. (Alexander and Waldenmaier, 2002). Galls caused by *Meloidogyne javanica* on the roots of tomatoes, were significantly reduced when the tomatoes were inter-cropped with *T. Erecta* (Abid and Maqbool ,1990). Both Castro *et al.,* (1990) and Akhtar and Alam (1992) reported that crop rotation with *T. erecta* and the incorporation of *T. erecta* in the soil, not only caused a reduction of the *M. incognita* population, but also resulted in a reduced gall index and increased tomato and chili yields. Akiew and Trevorrow (1999) took Brassica tissue, pulverize it and incorporated in soil, which was immediately irrigated before beds were formed and covered with reflective plastic for weed control, as is common in commercial practice. During crop growth all of the biofumigants delayed the onset and reduced the incidence and severity of wilting in the subsequently planted tomatoes and at harvest the mustard treatments were more effective than the rape treatments in reducing wilt severity and in increasing tomato yield. Hyphal growth of *Fusarium sambucinum* grown in agar plates was suppressed when exposed to volatile fungicidal compounds released from macerate green leaf tissues of various *Brassica* plants (Mayton *et al.,* 1996). It was reported for *R. solani,* that enzyme-degradation products of some glucosinolates were fungitoxic in *in-vitro* tests (Manici *et al.,* 1997). Lazzeri *et al.,* (2004) used dry biocidal pellets of some of the *Brassaica* spp. as organic treatments in addition or in alternative to biocidal green manure to limit, during drying, glucosinolate leakage and myrosinase activity loss.

These dried pellets, after water addition, showed a good fungitoxic activity on *Pythium* ssp. and *Rhizoctonia solani, in-vitro.* Charron *et al.,* (2002) evaluated the impact of glucosinolates on *Pseudomonas marginalis*, a causal agent of bacterial soft rot, in an *in-vitro* assay through simple linear regression analysis, and found that 48% of differences in suppression of *P. marginalis* growth was related to the differences in total glucosinolate content ($P \leq 0.01$). Kirkegaard *et al.,* (1996) investigated the effects of volatile compounds released from the root, shoot and seed meal tissues of canola (*Brassica napus*) and Indian mustard (*Brassica juncea*) on the mycelial growth of five soilborne pathogens of cereals—*Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani*, *Fusarium graminearum*, *Pythium irregulare* and *Bipolaris sorokiniana* by exposing them to volatiles released *in vitro*. They reported that the root and shoot tissues of both *Brassica* species were more suppressive at flowering than maturity and mustard tissues were generally more suppressive than canola. The degree of fungal suppression by the various *Brassica* tissues was directly related to the concentration and type of isothiocyanates released. Smolinska *et al.,* (1997) determined hyphal growth, germination of encysted zoospores, and oospore survival and inoculum potential of *Aphanomyces euteiches* f. sp. *pisi,* causing root rot of pea in the presence of volatiles produced from *B. napus* seed meal. Volatile compounds from *B. napus* meal completely suppressed the mycelial growth and germination of encysted zoospores on agar.

The superior growth and yield of wheat following *Brassica* crops such as canola (*B. napus* L.) and Indian mustard (*B. juncea* (L.) Czern & Coss) in Australia 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.,* 1996). The use of *Brassicas* such as canola (*Brassica napus*) as break crops to help control take-all (*Gaeumannomyces graminis*) in cereal rotations in Australia, is an example of success of biofumigation in the field (Kirkegaard *et al.,* 1996). Biofumigation using *Brassicas* has successfully controlled the diseases of Lettuce and potato disease (Matthiessen and Kirkegaard, 2002), Take-all (*Gaeumannomyces graminis*) in cereal (Kirkegaard, 1998) diseases caused by *Fusarium*, *Nectria*, and *Cladosporium (*Omirou *et al.,* 2011*),* Damping-off disease, *Pythium aphanidermatum (*Deadman *et al.,* 2006*),* Apple Replant Disease (Turnbull *et al.,* 2011), *Phytophthora capsici* and *Fusarium* spp. (Masiunas *et al.,* 2009) etc. Organic amendments and organic matters used as biofumigants were reported to control Grapevine fan leaf virus (GFLV) and its nematode vector, *Xiphinema index (*Bello *et al.,* 2004*) and Rhizoctonia* (Cohen *et al., 2005*). A number of nematodes were controlled by *Brassica* biofumigation viz. *Criconemoides xenoplax* and peach tree short life (PTSL) (Nyczepir and Kabana, 2007), Potato cyst nematode (*Globodera pallida)* (Turner*, et al.,* 2006), Nematodes and some seedling disease in cotton (Rothrock and Medders, 2011), Pale potato cyst nematodes (Lord *et al.,* 2011) and weed infestation in pea (Saeed *et al.,* 2012).

Biofumigation research in India

Biofumigation is a new concept in context of Indian agriculture. However, Indian farmers follow various cultural methods like intercropping with sorghum to control wilt, crop rotations, mulching, mustard as catch crop etc. but biofumigation is not a familiar and popular practice among farmers. In addition, plants have been used as green manure and organic amendments to increase soil fertility, improve its properties and also for reduction in plant pathogen and pests populations but incorporation of plants in soil as biofumigant is not a widely used practice. Biofumigation has good scope in Indian agriculture. Only a few studies were done in Indian condition on biofumigation and *Brassica* like cauliflower and cabbage were found most effective in reducing the incidence of carnation stem rot to 22.28 and 24.25% (Chandel and Sharma, 2014). Anita in 2012 found that radish leaf residue was effective in controlling root knot nematode *Meloidogyne hapla*, and causes 60.6 % reduction in their population in soil and 41.9% increase in celery green leaf and stalk yield.

Brassicaceous crops are widely grown and consumed in all parts of India in one way or another. It is grown as an oil seed crop and consumed as leafy-vegetables, fodder etc. by farmers. The cost benefit ratio for utilization of biofumigants in comparison to other methods of soil borne disease management must be calculated before making it an affordable and useful practice for the farmers. In many parts of the country, *Brassicas* are grown intercropped traditionally with wheat and legumes and main crops always perform better in the presence of *Brassica*. For example, in many parts of India mustard meal or cake is used to increase the crop health especially in vegetables cultivation such as in eggplant.

Future prospects

In a number of countries over the past few years, several experiments have been carried out to evaluate the effectiveness of the myrosinase-glucosinolate system, in particular using the glucosinolate-containing plants as a biologically active rotation and green manure crop for controlling several soil-borne pathogens and diseases. The use of this technique is growing, and it is studied in several countries at a full-field scale (USA, Australia, Italy, The Netherlands and South Africa), thus triggering the interest of some seed companies, with a positive effect on the "biofumigation" seed market, which is significantly growing year after year. New potential has also been found for the dehydrated plant tissues and/or for defatted meal pellets production and use. Another point which still needs to be elucidated concerns the effects of the biocidal compounds derived from glucosinolate degradation, mainly isothiocyanates, on the beneficial soil micro flora naturally occurring or artificially introduced as biological control agents. Some other areas which also need attention of researchers are as follows:

- Identification of appropriate species of biofumigants having high amount of active ingredients in their tissue.
- Breeding of superior cultivars having high active ingredients for the purpose of Biofumigation.
- Standardization of method (plant extract, green manure cop, inter-cropping etc.) of plant tissue incorporation in the soil.
- Long-term research to learn the susceptibility of *so*il microbes to the Biofumigation.
- Irrigation *schedul*ing coupled with plant biofumigant amendments.
- Ecological studies on the plant and pathogen *interact*ion with biofumigants including the non-target microbes population in the soil.

The various researches carried out worldwide describe the importance and benefits of bio fumigation in management of soil borne plant pathogens. In view of the advantages of bio fumigation, it could be concluded as an alternative management strategy for soil borne plant pathogens which is of utmost importance to the commercial and well developed agricultural sector in the developed countries. Seeing that the cost of chemicals is very high and input costs are accumulating each year it could be considered as an alternative strategy but more research is needed under Indian conditions to find out its usefulness for our farmers. This concept could be used for the management of soilborne diseases under protected cultivation and may also have a future in organic agriculture sector in India. The most important benefit arise from this research is the generation of an eco-friendly option which could be used as a control measure against a series of plant pathogens and pests in an integrated cropping system, perfectly suited to each individual farmer.

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