***Review Article***

**Bio-fumigation: A promising approach for soil borne plant disease management**

**ABSTRACT**

Bio-fumigation involves the management of soil borne pathogens, pests, nematodes and weeds through the incorporation of certain plant residues releasing volatile biocidal products during their hydrolysis. Selected Brassicacea and non Brassicacea members are used for the management of plant pathogens. In addition to disease suppression, it also provides extra benefits including addition of organic matter to soil, improved aeration and increased water holding capacity of the soil. Bio-fumigant crops can be applied as fresh tissue, seed meals, pellets or liquid formulations. The present review details the importance of bio-fumigation, mode of action of hydrolysis products on plant pathogens, mode of application of bio-fumigants, their compatibility with bioagents and their effect on other soil microorganisms.

*Keywords: Bio-fumigation, Brassicacea plants, Pathogens, Volatile organic compounds.*

1. **INTRODUCTION**

Soil-borne phytopathogens such as *Rhizoctonia* spp., *Fusarium* spp., *Verticillium* spp., *Sclerotinia* spp., *Pythium* spp., and *Phytophthora* spp. result in substantial agricultural losses ranging from 50% to 75% [1]. These pathogens cause diseases like seed rot, wilts and root rot in several crops. Methyl bromide has been widely used since 1930’s as an effective fumigant to control soil-borne pathogens and pests in agriculture system. It has been utilized in plant nurseries, open fields, and greenhouses globally to ensure health of economically important crops like vegetables, fruits and flowers. However, the phase out of methyl bromide started under the montreal protocol due to its ozone depleting nature as well as detrimental effects on human life including damage to nervous and respiratory systems, eyes, skin *etc.* resulting in the search for alternative methods [3]. Soil solarisation and hot water treatments, due to expensive nature and practical difficulty, cannot be undertaken under all cases [4].This eventually resulted in the need for natural and ecofriendly plant derived compounds for disease management.

1. **BIO-FUMIGATION**

The term bio-fumigation was coined by Kirkegaardandit is the process of suppression of pests and diseases through the hydrolysis of glucosinolates resulting in the release of isothiocyanates with biocidal properties [5]. Bio-fumigation can be used as a natural alternative for methyl bromide, the most effective synthetic fumigant used for the management of soil borne phytopathogens, which was withdrawn due to its drastic effect on ozone depletion. Along with the pest and disease control, bio-fumigation offers additional advantages like improved soil structure, erosion control and increased organic matter. The commonly used Brassicaceae members for biofumigation include the genera *Brassica*, *Raphanus*, *Sinapis* and *Eruca* [6**, 7] In addition to Brassicaceae, several non Brassicacea members are also effective to be used for biofumigation.**

1. **GLUCOSINOLATES**

Glucosinolates, the secondary metabolites constitutively present in plant cells are synthesized and stored in vacuoles. They are characterized by a common chemical entity (β-thioglucoside with a sulphonated oxime moiety) and are distinguished by variable chemical side-chain [R] that differentiates one another (Fig. 1) [8]. These natural products are exclusively found in the order Capparales, which includes different families *viz*., Brassicaceae, Caricaceae, and Capparaceae [9]. Among these, Brassicacea family contains high concentration of glucosinolates, and are responsible for their distinct flavour. Their chemical and biological properties are attributed to the amino acid precursors from which they are derived [10]. Till date, more than 130 individual glucosinolates have been identified [11].



**Fig 1. Structure of glucosinolates.**

Glucosinolates are water stable compounds having limited biological activity, but the hydrolysis products derived from them by the action of myrosinase are of great importance due to their antimicrobial properties [12,13]. They are classified into three different groups which includes aromatic (derived from phenyl alanine or tyrosine), aliphatic (from methionine, alanine, valine, leucine and isoleucine) and indoyl (from tryptophan) side chains that influences their nature and biological activities [8,14]. Among them, only the aliphatic and aromatic glucosinolates release isothiocyanates upon hydrolysis. The major glucosinolates identified from the roots of broccoli and cabbage is Glucoerucin, whereas that from cauliflower root and broccoli leaves are glucoiberverin and glucoraphanin respectively [15]. In Brassicacea, the shoots in general are dominant in aliphatic GSL and roots contain aromatic GSL [16].

**3.1 Glucosinolate content and distribution in Brassicacea**

The developmental stage of Brassica determines the amount of glucosinolates accumulated in the plants. The concentration of GSL varies 3-10 fold depending on the environment in which it is grown and it increases with warm, long day conditions with maximum production reaching to 1100 moles ha–1 [17]. GSL concentration increases during vegetative stage, tends to decline with the onset of flowering and it declines to the lowest in mature tissues. The pre-flowering stage is the optimum period to apply as biofumigant, since it is the stage where the GSL production is maximum [17,18]. Although *Arabidopsis* *thaliana* has been identified with as many as 23 distinct glucosinolates, the majority of other species contain limited number of glucosinolates, typically less than one dozen [19,20]. Glucosinolates are found in almost all the organs of plants in Brassicacea family, but vary in their concentrations and constitute around 1 per cent of their dry weight [21]. About 3-4 glucosinolates can be found predominant in a single species of plant [22]. They are found abundantly in the seeds and siliques which are the reproductive organs, but their concentration is lower in older leaves. In Brassica plants, the concentration of GSL in reproductive organs are 10-40 times higher than that in the vegetative parts [23,24]. Within the same plant, the GSL concentration varies with the growth stage, which is evident from the seeds and sprouts having higher concentration of GSL when compared to adult plants [25]. Compared to shoots, sprouts contain higher concentration of glucosinolates during the same developmental stage in the Brassicacea crops because of difference in the regulatory mechanisms of GSL biosynthesis and turnover [26, 27].

**3.2 Breakdown of glucosinolate**

Glucosinolates are stable molecular structures found in plant cells that are typically regarded as harmless substances. The plants with glucosinolates produces an enzyme called myrosinase. Myrosinase is basically a β-thioglucosidase, with amino acid sequence and are similar to the glycosylhydrolase family [28]. There is a physical separation between cells containing glucosinolates and myrosinase in normal intact tissues [21, 29]. But, whenever there is a tissue damage due to chewing, heating or insect attack, the glucosinolates and myrosinase will come in contact with each other. For binding with glucosinolates the enzyme requires hydroxyl group at C-2 on the glucose moiety. Thus, upon tissue damage, the enzyme act on its substrate in the presence of water and the sulfate moiety is liberated non enzymatically from the aromatic and aliphatic chains of glucosinolates to generate the thiohydroxamate-O-sulfonate following the hydrolytic cleavage of the glucosyl moiety [29].Subsequently, depending on the glucosinolate substrate and the conditions in which reaction takes place (e.g. pH, or the presence of ferrous ion or epithiospecifer protein), the unstable intermediate undergoes a rearrangement and generates isothiocyanates or other products (such as ionic thiocyanates, nitriles, epithionitriles, oxazolidine-2-thiones and organic cyanides) [30,31].

ITCs are produced from thiohydroxamate-O-sulfonate under natural pH, warm temperature and high water dilution ratio. The less biologically active nitriles or EPTs are generated under acidic PH, in the presence of ferrous ions or is stimulated by other specifier proteins such as ESM and epithiospecifier protein (ESP) in lower temperature, and drier circumstances [21, 23, 31, 32]. Thiocyanates are formed in the presence of thiocyanate forming proteins (TFP). These primary decomposition products can act as starting point for the synthesis of other phytoalexins and other products (Fig. 2). Plants are equipped with an efficient defense mechanism against pathogens and herbivores through the utilization of the glucosinolate–myrosinase system [33].

The side chain of the glucosinolate substrate determines the kind of isothiocyanate that is produced [34]. Isothiocyanates are highly reactive compounds that respond to nucleophilic reagents. As a result, sulfhydryl groups, disulfide bonds and amines in the organic compounds may act as the targets of isothiocyanates [35].

In soil, the isothiocynate formation is influenced by different factors. These include the quantity of plant material, the myrosinase activity of both the soil and plant material, the extent of tissue damage, temperature of the soil, and the water content. In addition to all these, the initial glucosinolate concentration of the plant material which is usually highest prior to flowering also affect the ITC formation [17, 29, 36, 37]

**3.2.1 Fate and activity of ITCs in soil**

ITCs are short lived in soil and their concentration decreases within first few days. In soil they are subjected to microbial degradation [38] sorption to organic matter [35] and volatilisation losses [39].The specific structure of the ITC side chain and properties of the soil such as amount of organic matter, temperature and water content, influence the rates of these processes. The sorption of ITC is mainly due to their lipophilic character and it increases with lipophilicity [38]. Different techniques like rapid incorporation, covering of soil and watering reduces the loss of ITC, but higher soil temperature will lead to increased loss [36, 40, 41, 42].

The hydrophobic nature of ITC results in greater absorption to soil organic matter and thus increased organic matter content in soil can significantly reduce the disease control ability of ITC. This is particularly higher for long chained aliphatic or aromatic ITC [29, 43]. In amended soil, the concentration of ITC and its longevity are generally increased as the soil water content increases. This is likely due to the fact that the rate of GSL hydrolysis is facilitated, and the ITC concentration in solution is increased, which in turn reduces volatile losses [36, 40]. The behaviour of ITC in soil is generally less influenced by temperature, pH, and soil texture at ranges normally encountered in field soils [42].

**3.2.2 Persistence of ITC in soil**

The hydrolysis of glucosinolates occurs at a faster rate. In biofumigated soil, the concentration of ITC ranged from 1 to 100 nmol ITC/g soil [29]. The ITCs and other hydrolysis products have a short persistence in soil and within few days, their concentration reduces rapidly with mean persistence in soil for about 12 days [48]. In contrast, no ITC was detected after three days of biofumigation [48]. An increase in the ITC concentration after three days was reported due to increased water content in the soil [36].

Even though the thiocyanate is less toxic than isothiocyanates, through electrostatic interaction it can destroy the tertiary structure of proteins and at high concentration it might also act as soil sterilants [49]. When *B. juncea* tissue was used, rapid release of allyl ITC was observed within 12-24 h after treatment and it reduced to undetectable amounts by 72h irrespective of the quantity of tissue used [50].

Assessment of ITC release pattern in soil after the incorporation of *B. juncea* revealed thatmost of the ITC release occurred within 4 days after the application of biofumigant tissues [36]. Due to the highly volatile nature of all the hydrolysis products, covering the soil with plastic mulches can reduce their losses [51]. In some crops, soon after the application of Brassica, symptoms of phytotoxicity were reported. Thus, a waiting period of 2 weeks should be given between the application of biofumigants and growing of subsequent crops to enable the removal of toxic compounds [30, 52].



**Fig. 2. Hydrolysis of glucosinolates and the rearrangement products.**

**3.2.3 Possible targets of ITC**

The isothiocyanates react rapidly with thiols when compared to the alcohols and amines [53]. Thus, the two most important target sites of ITC can be the glutathione pool and the thiol side chains present in proteins. Increased level of ITC can deplete the GSH pool and destroy the fungi. In *Candida albicans*, oxidative stress is induced by ITC leading to increased superoxide content and upregulation of activities of glutathione reductase, glutathione peroxidase, catalase and superoxide dismutase. In *Alternaria alternata*, the less polar phenethyl ITC disrupted the cell membrane [54]. Similar results were observed when *A. alternata* was treated with benzyl ITC [55].

**3.3 MODE OF ACTION OF ISOTHIOCYANATES**

**3.3.1 Mode of action in fungi**

Isothiocyanates which are compounds produced from the hydrolysis of glucosinolate- myrosinase system possess broad antagonistic activity against plant pathogens. It hinders the mycelial growth of fungal pathogen and inhibits the spore germination thereby preventing their growth. In addition to this, downregulation of genes related to energy metabolism, melanin biosynthesis, and cell wall-degrading enzymes also contributes to their fungistatic effect [56]. Electrolyte leakage and hyphal deformity were observed in *F. solani* when treated with allyl ITC [57].

In plants, the role of glucosinolates in control of plant pathogen is to be clarified but their hydrolysis yields products including ITC which can control the fungal infection through the activation of host innate immune responses and cytotoxicity [58, 59,60]. When compared to aromatic ITCs, the aliphatic ITCs usually have greater inhibitory activity under *in vitro* conditions [61]. Due to antitumour activity, the ITCs were studied for their cell toxicity in mammalian cells. The ITC s can inhibit the growth of tumour cells through mechanisms like apoptotic and autophagic cell death [62,63,64]. When fungal cells were exposed to ITC, several genes which were involved in the protection of cell against oxidative damage were over expressed, and this response was similar to that observed during oxidative stress [65]. In *A. brassicola,* intracellular accumulation of ROS was observed upon exposure to ITCs. The other mechanisms involve disruption of mitochondrial membrane potential, decreased oxygen consumption rate and depletion of intracellular glutathione [66,67]. The toxic compounds released during biofumigation can render the sclerotia (*Sclerotium cepivorum*) weak which can further be parasitized by other fungi [61].

When compared to conidia, mycelium was found to be more susceptible to isothiocyanates in *Fusarium circinatum* [68]. This was supported by the several other studies in which the mycelium of *R. solani* and *S. sclerotiorum* were less tolerant to isothiocyanates than their sclerotia [69, 70]. On the contrary, the mycelium of *F. oxysporum* was less sensitive to isothiocyanates, than the germination of chlamydospores and conidia [71].

In *Cochliobolous heterostrophus*, the pathogenicity and germination of conidia are inhibited by the ITCs (allyl, 4-(methylthio)-butyl, and phenyethyl ITCs). The inhibition of the pathogen by allyl ITC was due to the downregulation of genes related to the biosynthesis of melanin, oxidoreductase activity, cell wall degrading enzymes and energy metabolism [56].

**3.3.2 Mode of action in plant pathogenic bacteria**

Several mechanisms of action of ITC was described against plant pathogenic bacteria. In Gram negative bacteria, the treatment with ITC can result in disruption of the outer cell membrane, which results in the change in cell membrane potential [72] and ultimately leading to cell metabolites leakage [73]. In addition to these ITC can also act on the bacterial enzymes *viz*., thioredoxin reductases and acetate kinases. ITC bind to these bacterial enzymes and damages the tertiary structure which will affect its functions. Some of the ITCs like the allyl-ITC are highly toxic to the phytopathogenic bacteria due to their multiple targets [74].

**3.4 MODE OF ACTION OF OTHER DECOMPOSITION PRODUCTS**

Compared to isothiocyanates, only few reports are available about the antifungal activity of nitriles against plant pathogenic bacteria. Compared to ITCs, nitriles are less efficient antifungal compound produced at low pH (<5), in the presence of Fe2+ ions and nitrile specifier proteins (NSPs) [75]. Along with isothiocyanates, nitriles were also found to be a major hydrolysis product of pure allyl glucosinolates [76]. In brown mustard Indole-3 aceto nitrile is an inducible metabolite and plays an important role in defense response against blackleg disease (*Leptosphaeria maculans*) [77]. Similarly indole-3-acetonitrile was shown to inhibit the most virulent isolates of *P. lingam* and *R. solani*. However, it had a much smaller impact on the growth and survival of *S. sclerotiorum* and did not inhibit *A. brassicae* [78]. The spore germination, appressorium formation and formation of infection hyphae of *M. grisea* were inhibited by nitriles [79].

Another glucosinolate hydrolysis product is epithionitrile. Due to the inherent instability of these chemicals, there is a scarcity of investigations conducted on them. When the importance of epithionitriles in defense response against the pathogen *Verticillium longisporum* was studied, it was found that they were less important than nitriles and ITCs. When *A. thaliana* was inoculated with *V. longisporum*, the plant lines dominant with epithionitrile exhibited an increased synthesis of about 31 decomposition products (both ITC and nitrile), but the level of epithionitriles did not changed [80].

**4. USE OF NON BRASSICACEA CROPS IN BIOFUMIGATION**

Due to the high concentrations of sulfur containing compounds in the organs of different *Allium* species, it has been suggested that they can be utilized for soil biofumigation [81]. They also contain S-alk(en)yl-cysteine sulfoxides (RCSOs), the precursors of the aromatic compounds in addition to methionine, cystine, glutathione, cysteine and other related peptide derivatives. The predominant RCSO in garlic is alliin (S-allyl-l-cysteine sulfoxide), which generates allicin (diallyl thiosulfinate) The characteristic odour of garlic is due to allicin, which rapidly produces diallyl disulfide (DADS) upon degradation. Onions and leeks are primarily composed of isoalliin (S-1-propenyl-l-cysteine sulfoxide) and propiin (S-propyl cysteine sulfoxide), which are the precursors to a variety of thiosulfinates and other volatile sulfur compounds known as zwiebelanes. The degradation of these molecules results in dipropyl disulphide (DPDS) [82]. Similarly, in bear's garlic (*Allium ursinum*) and Chinese chive (*Allium tuberosum*) the primary RCSO is methiin (S-methyl-l-cysteine sulfoxide). It undergoes degradation to form dimethyl thiosulfinate (DMTi), which in turn undergoes degradation into DMDS (dimethyl disulphide).

In addition to the bio-fumigant activity, the *Allium* by products stimulated vegetative growth and the DPDS which was frequently released after incorporation into soil was detectable for up to one month. The treatment with these *Allium* byproducts resulted in 15-20% increase in production of asparagus and strawberry, which was comparable with bio-fumigation using Brassicacea [83].

Allicin interacts with thiol groups in proteins and also they can diffuse across the lipid bilayers [84,85]. The antimicrobial action of allicin results from the inactivation of SH containing enzymes. In *Phytophthora nicotianae*, the application of garlic essential oil, containing DADS as a major component resulted in increased cell membrane permeability and cell death. It also reduced the population of pathogen present in the rhizosphere soil and incidence of black shank in tobacco [86].

Bio-fumigation with essential oils of palmarosa, eucalyptus and lemon grass were found to be effective in managing wilt in gingercaused by *R. solanacearum* race 4. Electron microscopy and Raman spectroscopy of the bio-fumigated cells of pathogen revealed that treatment with essential oils resulted in the breakage of cell debris, cell walls and cell membrane [87]. Similarly, *R. solanacearum* race 3 was subjected to treatment with essential oils of thyme, lavender, eucalyptus and cinnamon. It was evident from the study that sub lethal concentration of these EO can affect the pathogenicity of the bacteria by suppressing the biofilm formation, swarming and twitching mobility. Among all these EO of cinnamon with cinnamaldehyde and thyme with thymol as the predominant compounds were most effective in controlling the pathogen [88].

**5 MODES OF UTILISATION**

**5.1 Crop rotation or inter cropping**

In crop rotation or intercropping, either the plant parts above ground are harvested or they are kept as such undisturbed. In case of harvest of above ground parts, the suppression of plant pathogens mainly relies up on the release of GSLs, ITCs or other compounds through leaf washings. Along with these they can also alter the soil microbial population and this alteration may lead to suppression of soil-borne plant diseases, as certain beneficial microbes like Trichoderma show tolerance to ITCs [89]. Certain soil organisms possess myrosinase activity, enabling them to convert GSLs to ITCs [90]. This conversion process is crucial for the bioactivity of these compounds in soil. The inoculum concentration of take all disease of wheat was reduced by the 2- phenylethyl GSL which is produced in the roots of different varieties of canola [91].

**5.2 Bio-fumigant incorporation**

It is the most recognized use of bio-fumigant plants. Here the bio-fumigant crops are grown with the goal of releasing ITCs into the soil. For maximised ITC generation, the plant tissues are rapidly incorporated into soil after comprehensive maceration along with addition of water to ensure the complete hydrolysis. In order to prevent the escape of volatiles from soil it may be sealed or plastered. The glucosinolate concentration is at its peak during the flowering period, which is the most ideal stage for bio-fumigant incorporation [92]. High amount of propenyl isothiocyanate were discovered from the Brassica plants such as *B. nigra*, *B. carinata* and *B. juncea* which resulted in the suppression of *F. oxysporum* in soil of conifer seedling nursery [71].

**5.3 Incorporation of seed meals and other processed products**

**5.3.1 Seed meals, pellets, liquid formulation and Essential oils**

The seed meals or oil cakes after the extraction of oil contain high level of glucosinolates which can be used for the control of soil borne phytopathogens. These seed meals contain intact myrosinase enzyme which will help in the release of ITC upon addition of water. Besides its use as bio-fumigant, it can also be used as bio-pesticide or bio-fertiliser. Brassica seed meals inhibited the motility, EPS production and dehydrogenase activity of *Ralstonia solanacearum* [93]. Similarly, bio-fumigation with seed meals of mustard and canola, significantly reduced the growth of pathogens like F. oxysporum, S. sclerotiorum and R. solani [94]. The ground seed meal of mustard with the bioactive compound allyl isothiocyanate is reported to have fungicidal effect on Rhizoctonia. The treatment of cabbage seeds with ground seed meal of mustard and the carrier Biolan peat in the ratio 2:3 (w/w) significantly reduced the damping off caused by Rhizoctonia without any detrimental effect on the seed germination [95]. Along with disease control Brassicacea seed meals were reported to have inhibitory activity against nematodes [96].

BioFence is a processed product developed from B. carinata selection ISC17 by the partial defatting method. This method reduces the degradation of glucosinolates and myrosinase enzyme [97]. The use of this processed products helps to avoid the need to cultivate Brassica crops in huge quantity, their maintenance and soil incorporation. In pellets, there is no compartmentalization of the GSL- myrosinase system and hence, ITC will be quickly formed on addition of water in the treated soil.

Brassica carinata when applied as pellets (BioFence- 40 mg) has more fungicidal effect against P. nicotianae than the fresh tissue (320 g) [98]. Similarly, it reduced the growth and inhibited the germination of chlamydospores of P. nicotianae affecting the pepper plants [99]. It can also be used for seed treatment as the product is commercially available, easy to use and eco-friendly. In such BioFence treated seeds of Pinus radiata, there were no mycelial growth of the pathogen F. circinatum [68].

A liquid formulation was developed from the seed meal of B. carinata, which was suitable for drip irrigation. Sinigrin which is the most important glucosinolate, was hydrolysed to allyl isothiocyanate within about 30 minutes and was released from meal to liquid fraction. It was found to be effective in controlling M. incognita through drip irrigation at every 20-25 days [100].

Dried powder of bio-fumigants can be used in the control of soil borne plant pathogens. Plant tissue powders produced from the freeze dried materials of B. oleracea var. caulorapa (kohlrabi) and B. integrifolia (leaf mustard) suppressed the growth of *Fusarium* sp.,   
*F. oxysporum, P*. *aphanidermatum* [101]. The B. juncea seed meal followed by seed powder was found to be most efficient in controlling R. solani than the fresh plants [102].

Bio-fumigation with essential oils derived from the plants can be utilized as a substitute for the cruciferous plants. The essential oils can be either synthesized chemically or they can be produced from the macerated tissues by hydro-distillation. Essential oils derived from mustard increased the mortality and delayed the germination of sclerotia of S. rolfsii and S. sclerotiorum. These synthetic oils contain around 93 per cent of allyl isothiocyanate. The essential oil concentration and period of exposure determined the mortality of sclerotia of both the pathogens [103]. The essential oils from palmarosa increased the latency period, reduced the area under disease progress curve and bacterial wilt incidence of sweet pepper caused by R. solanacearum [104].

**5.3.2 Green manures and trap crops**

The addition of green manures provides significant reduction of plant pathogens and disease development [105]. In addition to disease suppression mediated by ITC, bio-fumigant crops also offer certain additional advantages. When incorporated into soil, they can offer a transient increase in soil organic matter, thereby improving soil health by enhancing microbial activity and nutrient recycling. The application of Brassica species as green manure helps in the capture of nitrogen which may otherwise be lost by leaching. It also adds organic nitrogen to soil which can be utilized by the subsequent crops. Along with increased soil fertility, they can also improve the soil structure, soil aeration, water infiltration and root penetration essential for plant growth and nutrient uptake.

Brassica green manure can also be used as trap crop for nematodes. Radish and mustard were used as trap crop for beet cyst nematode *Heterodera schachtii* Schmidt in sugar-beet. These crops stimulated the hatching of juvenile stage but were not suitable as hosts. As a result, they were unable to complete their life cycle in these crops, which subsequently lowered its population [106].

**6. EFFECT OF BIOFUMIGATION ON SOIL MICROORGANISMS**

Bio-fumigation can affect other microorganisms in soil. The growth and health of plants are significantly influenced by the microbial community in the soil, which includes both pathogenic and beneficial strains. Replant diseases of numerous plant species are associated with a change in the composition of the microbial community, which is characterized by the increase in pathogens and the absence of growth-promoting microorganisms [107].

Bio-fumigation has been observed to favour several bacteria genera, including *Pseudomonas*, which are known to possess advantageous characteristics. *Pseudomonas* species are helpful for plant growth because they improve sulfate uptake and function as antagonists against soil-pathogenic fungi [108, 109, 110]. Additionally, the suppression of soil-borne diseases is facilitated by the presence of numerous Actinobacteria phyla members that possess plant growth-promoting properties [111]. When essential oils derived from mustard was utilized for bio-fumigation there was an increase in the population of bacteria and Actinomycetes, but the fungal population decreased significantly [103]. Thus, a key factor for the efficacy of bio-fumigation appears to be the increase in disease-suppressive and growth-promoting bacteria that is brought about by bio-fumigation.

When bacteria such as *Pseudomonas aeruginosa* B1- SQU, *P. indica* B2- SQU and *Serratia marcescens*, isolated from the rhizosphere of cabbage grown in the bio-fumigated field were applied individually, there was a reduction in the cucumber damping off caused by *P. aphanidermatum*. However, when they were applied in combination with bio-fumigation using cabbage leaf residue, their effect was reduced considerably [112].

The bacterial and fungal diversities were unaffected by 1 per cent bio-fumigant after four weeks of fumigation. On the contrary, the microbial diversity and network complexity were diminished by 2–4% bio-fumigant amendments, irrespective of the fumigation period [113]. This bio-fumigant amendment establishes optimal conditions for growth of *Bacillus* and *Clostridium* [114, 115]. The resistance of these bacteria to the bio-fumigation may be due to the production of endospores which are highly resistant structures. However, the total number of Acidobacteria, was observed to decrease following the application of soil amendments.

A negative correlation was observed between the diversity of soil bacteria and Phytophthora blight incidence. The bio-fumigation with rapeseed meal increased the population of Bacillus and Actinobacteria, whereas it decreased the incidence of blight caused by *P. capsici* [116]. When soils treated with non Brassica based amendments were compared with soils subjected to repeated bio-fumigation with *Sinapis alba* and *B. carinata* pellets (Biofence®) an increase in population of bacteria, *Pseudomonas* sp. and Actinomycetes were observed. The potential to promote beneficial microbiota by glucosinolate hydrolysis products improves the efficiency of bio-fumigation. Organic amendments, such as defatted seed portions, introduce organic carbon and nitrogen into the soil, which are readily accessible for soil microbial degradation [108]. In comparison to control and fumigated soils, bio-fumigation with mustard resulted in an increase in the diversity of bacteria and fungi [117].

In several studies the inhibitory activity of ITC towards the nitrification process and nitrifying bacteria were studied [49, 118]. In soil tests, Brassicacea plants with high level of glucosinolates inhibited the nitrification process. When sandy and clay loam soils were compared for their durability of nitrification inhibition, it was found that the effect was longer in sandy loam than clay loam, because of the increased effect of bio-fumigation in such soil [118]. On contrary in another study, no nitrification inhibition was observed with the application of *B. juncea*. In addition to these the application of *B. juncea* resulted in increased abundance of Thermoactinomycetaceae, Nocardiaceae and Paenibacillaceae [119]. The application of Brassica seed meals resulted in increased population of Streptomyces and nitrification process, which led to the suppression of *R. solani,* which causes root rot of apple, irrespective of glucosinolate content of the meal [120,121,122].

**7. INTEGRATION OF BIOLOGICAL CONTROL WITH BIOFUMIGATION**

Several studies aimed at exploring the compatibility of bio-fumigants with biocontrol agents were conducted to understand the possibility of integrating bio-fumigation and biocontrol. Bio-fumigation with brasssicacea crops were found to be compatible with *Trichoderma* spp. and *P. fluorescens* [123]. About 40 strains of *Trichoderma* spp. were evaluated for their tolerance to bio-fumigation by placing it in the headspace in close contact with the seed meal. Trichoderma was found to be the most tolerant one compared to *P.* *ultimum*, *R. solani* and *F. oxysporum*, which were tested in parallel. In some cases *T*richoderma even grew on the tested seed meal, without compromising their antagonistic behaviour against *R. solani* and *F. oxysporum* [124]. Similarly, the native isolate *Trichoderma* sp. T-Nam was found to be tolerant to bio-fumigation treatment with *B. juncea* and when they were applied in combination there was a reduction in beetle vine collar rot incidence and inhibition of saprophytic colonization ability of *S. rolfsii* [125].

The different mechanism of tolerance includes adsorption of the isothiocyanate by the mycelium, emission of volatile compounds by Trichoderma which will interact with the AITC and the ability to metabolize AITC inside the mycelium which lowers the biocidal compound to a concentration not toxic for Trichoderma [124]. The efficiency of bio-fumigation with rapeseed meal against *P. capsici* was strengthened by integrating it with antagonistic *B.* *amyloliquefaciens* [116]. The increased growth of biocontrol agents in biofumigated soil may also be attributed to the decrease in competition by the soil pathogens.

**8. EFFECT ON MYCORRHIZAE**

Few studies suggested lack of effect of biofumigation on mycorrhizae. The addition of *Brassica* *napus* residues to *Zea mays* caused no negative effect on the colonization of mycorrhizae. Similarly winter crop forage radish (*Raphanus sativus* var. *longipinnatus*) also lacked any effect in Zea in a multi-year study [126]. However, in another study, ITC from Brassicaceae plants conferred allelopathic effects by their effects on ectomycorrhizal fungus (EMF) colonization [127].

**9. DEFENSE SYSTEM IN PATHOGENS AGAINST ITC**

Even though ITCs are effective antifungal agents, few fungi have developed strategies to detoxify it. There are different ways by which the plant pathogenic fungi manage to overcome the oxidative stress caused by the ITC and these are more profound in fungi that lives in close association with Brassicacea family. One such example is the detoxification of ITC by *S. sclerotiorum*. The fungi metabolizes ITCs to amines by a route previously un-described in fungi, with the help of sax A gene, and it makes up the dominant ITC degradation pathway. It is mediated by isothiocyanate hydrolase an enzyme that promotes the growth of fungi even in the presence of the toxins thereby contributing to the virulence of *S. sclerotiorum*. It is also found that induction of antioxidant defense response, catabolism of proteins, ergosterol synthesis and efflux pumps aids the pathogen to overcome the detrimental effects of ITC at sub lethal concentration [128, 129].

In another study, the fungus *Helminthoprium* species NRRL 4671 oxidizes I-isothiocyanato-4- (metbyltbio) butane to (->l-isothiocyanato-(4R)-(methylsulfinyl) butane (sulforaphane) [130]. The pathogens *viz*., *Colletotrichum dematium* and *C*. *higginsianum* metabolised Rapalexin A to its Cys conjugates, which were again converted into either sulphur containing heterocyclic ring or a cyclic dithiocarbamate through different transformation pathways. So the fungi was able to cope up with Rapalexin A by converting it into a product which was less toxic [131]. It was also evident that Rhizopus and Fusarium found in the rhizoplane of Brassicacea are more tolerant to ITC than those members of the same genus from other host plants [132].

In *A. brassicola*, upon exposure to ITC, the glutathione transferase was found to be over expressed. This particular enzyme was also upregulated during the infection of plants, thereby suggesting their possible role in ITC detoxification [133]. The plant pathogenic fungi *A.alternata*, when exposed to allyl ITC exhibited an increased expression of ABC multidrug CDR4 transporter, amino acid permeases and ATPases which are involved in toxin efflux aminoacid transport and fungicide resistance. Similarly, in *B.* *cinerea,* increased expression of Major Facilitator Superfamily transporter mfsG was observed in the presence of glucosinolates hydrolysis products. Those strains deficient in mfsG showed increased fluorescein ITC accumulation and susceptibility towards isothiocyanates [134].

The bacteria cope up with plant released antimicrobials such as coumarins by antimicrobial tolerance which gives them a selective advantage in the plant rhizosphere microbiome [135]. Prolonged exposure to ITC could result in the development of tolerance in bacterial communities and may be affected by the dose and duration of exposure to ITC. Among the different ITCs tested, Allyl ITC was found to be the most efficient one in controlling *R. solanacearum*. But prolonged exposure to allyl ITC can result in development of tolerance in the pathogen under laboratory conditions [136].

**10. CONCLUSION**

The serious threats posed by soil borne plant pathogens and the environmental concerns regarding the use of chemicals in their management have led to the search for adopting an alternative method. Biofumigation using plant residues can be used in the integrated management of soil borne pathogens. Brassicas are most commonly used as bio-fumigants due to the release of biocidal compounds upon the enzymatic hydrolysis of glucosinolates. Along with these Brassica members, certain non-Brassica members can also be employed in bio-fumigation. This particular technique can be utilized in organic production due to their more sustainable nature and the fact that, it can be integrated with biological control methods. They also have other beneficial effects like addition of organic matter to soil, improved soil structure and effect on soil microbial community. Since multiple components are involved, the type of bio-fumigant, the amount of its biomass, soil characteristics and targeted microorganisms are to be studied in detail. More studies are to be conducted to understand its effect on different pathogens, their efficacy and on how they can be utilized in large scale agricultural systems.

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