Biofumigation with species of the *Brassicaceae* family: a review

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ABSTRACT: *Biofumigation involves the release of volatile biocidal compounds in the soil through the incorporation of certain plants and their residues. Species of the Brassicaceae family are the most widely used plants for biofumigation. These plants contain glucosinolates, which produce compounds, such as isothiocyanates, following enzymatic hydrolysis, with scientifically proven fungicidal effects. The most commonly used brassica species belong to the genera Brassica, Raphanus, Sinapis, and Eruca. In addition to the release of compounds in the soil, complementary mechanisms, such as the supply of organic matter and nutrients, and improvement of the soil structure, also play a role in biofumigation. In the past two decades, several studies on the use of brassica residues in biofumigation have been published, showing promising results in the management of soil pathogens (fungi and oomycetes, nematodes, bacteria, and protozoa), weed seeds, and insects. Usage of new biofumigation compounds has also been validated in recent years, including the development of patented technological products such as liquid formulations and pellets. The objective of this article was to review these new developments, beginning with concepts related to biofumigation, and to discuss the mechanisms of action of compounds involving brassica species and the recommendations on usage. Promising examples of the use of this technique are also presented, further detailing the advances in basic and applied knowledge on the subject.*
Key words: *Brassica spp., soil pathogens, glucosinolates, isothiocyanates, green manure.*

Biofumigação com espécies da família *Brassicaceae*: uma revisão

RESUMO: *A biofumigação consiste na liberação de compostos biocidas voláteis no solo a partir da incorporação de determinadas plantas e de seus resíduos. As espécies da família Brassicaceae são as plantas mais utilizadas na biofumigação. Em sua constituição, possuem os glucosinolatos que, após hidrólise enzimática, produzem compostos como os isotiocianatos com efeito biofungicida comprovado cientificamente. As espécies de brássicas mais utilizadas pertencem aos gêneros Brassica, Raphanus, Sinapis e Eruca. Além da liberação de compostos no solo, mecanismos complementares como o fornecimento de matéria orgânica, nutrientes e melhoria da estrutura do solo, também desempenham papel complementar na biofumigação. Diversos estudos foram publicados nas últimas duas décadas com a utilização de resíduos de brássicas na biofumigação e apresentaram resultados promissores no manejo de patógenos de solo (fungos e oomicetos, nematóides, bactérias e protozoários), sementes de plantas daninhas e insetos. Novas formas de utilização também foram validadas nos últimos anos, inclusive com o desenvolvimento de produtos tecnológicos patenteados como formulações líquidas e pellets. Nesta revisão, objetivamos apresentar estes novos desdobramentos iniciando com os conceitos relacionados à biofumigação. Em seguida, apresentamos os mecanismos de ação e compostos envolvidos; as espécies de brássicas, produtos e recomendações para sua utilização; e exemplos promissores de adoção da técnica a nível mundial. Pretende-se, dessa forma, detalhar os avanços no conhecimento básico e aplicado do assunto.*
Palavras-chave: *Brassica spp., patógenos de solo, glucosinolatos, isotiocianatos, adubação verde.*

INTRODUCTION

The management of soil-borne phytopathogens is a challenge in agricultural production (DIXON & TILSTON, 2010; GAMLIEL & BRUGGEN, 2016; LOBO JÚNIOR et al., 2018; DUTTA et al., 2019). Under intensive conventional cultivation systems, volatile chemical compounds of nonspecific action are used frequently in a practice known as soil fumigation (LADHALAKSHMI et al., 2015; LEITE & LOPES, 2018). Methyl bromide (CH_3Br) is a classic example of a product used for this purpose and, until it was prohibited, around three-quarters of its consumption worldwide was associated with soil fumigation for cultivation of vegetable species (EPSTEIN, 2014). Its prohibition led to limitations in some production sectors, such as vegetables, flowers, and seedlings, and a race began in the search for CH_3Br substitutes (BAKER et al., 1996; EPSTEIN, 2014; PRASAD et al., 2015). These substitutes included metam sodium ($C_2H_4NNaS_2$) and dazomet ($C_5H_{10}N_2S_2$) (AGROFIT, 2020), both

of which are precursors of the volatile compound methyl isothiocyanate.

However, regardless of the product used, fumigation aims to sterilize the soil, which is incompatible with the philosophy and principles of production systems that value the biological activity of the soil, such as organic or agroecological systems (LADHALAKSHMI et al., 2015; BRUGGEN & FINCKH, 2016; GAMLIEL & BRUGGEN, 2016). Since the 1990s, studies have investigated alternative proposals or techniques that may be used to replace fumigation with synthetic chemical compounds (KIRKEGAARD et al., 1993; MATTHIESSEN & KIRKEGAARD, 2006; KIRKEGAARD, 2009; MORRIS et al., 2020). One of these techniques is biofumigation, which involves the application or incorporation of residues from plant species capable of releasing gases with bioactive or biofumigant action. Biofumigation is effective at controlling phytopathogens that cause disease in plants (fungi, oomycetes, nematodes, and bacteria), insects, and weed seeds (KARAVINA & MANDUMBU, 2012; LADHALAKSHMI et al., 2015; PRASAD et al., 2015; GUREL et al., 2018; DUTTA et al., 2019).

Plant species from the *Brassicaceae* family have been widely used and studied for biofumigation due to the presence of compounds, including glucosinolates (GSLs), which, after enzymatic hydrolysis, release bioactive gases such as isothiocyanates (GIMSING & KIRKEGAARD, 2009; NTALLI & CARBONI, 2017). Brassica or cruciferous plants, a generic name given to species of the *Brassicaceae* family, are widely consumed by humans and animals, and used for the production of edible and industrial oils (RAHMAN et al., 2018; UGRENOVIĆ et al., 2019). Generally, these plants grow quickly and produce large amounts of biomass. They also provide good soil coverage and are highly efficient at absorbing nutrients (UGRENOVIĆ et al., 2019). Among the *Brassicaceae* family, species of the genera *Brassica*, *Raphanus*, *Sinapis,* and *Eruca* are the most commonly used for biofumigation (MATTHIESSEN & KIRKEGAARD, 2006; CLARKSON et al., 2015; HANSCHEN & WINKELMANN, 2020; MORRIS et al., 2020).

The technique can be summarized with a sequence of events, beginning with the cultivation of brassica, followed by cutting and fragmentation, incorporation of biomass into the soil, and the addition of water. This process results in the release of bioactive substances, nutrient cycling, increased levels of organic matter in the soil; and consequently, improved physical, chemical, and biological

properties (KIRKEGAARD, 2009; CLARKSON et al., 2015). Substantial technological advances have already been achieved in terms of tests and validation, and commercial products based on the cake obtained from pressing brassica seeds are available for oil extraction, liquid formulations, pellets, leaf extracts, and brassica-based oils (LORD et al., 2011; NICOLA et al., 2013; CURTO et al., 2016; WEI et al., 2016; SERRANO-PÉREZ et al., 2017; RONCATO et al., 2018; RUBAYET et al., 2018; PONTES et al., 2019). To date, species of the *Brassicaceae* family have been tested and used in various countries such as Italy, India, Australia, China, the United Kingdom, Spain, the United States, Argentina, South Africa, Germany, Switzerland (MATTHIESSEN & KIRKEGAARD, 2006; KIRKEGAARD, 2009; DONALD et al., 2010; PERNIOLA et al., 2012; LAZZERI et al., 2013; MICHEL, 2014; LADHALAKSHMI et al., 2015; WEI et al., 2016; PAN et al., 2017; RÍOS et al., 2017; DANEEL et al., 2018; DUTTA et al., 2019; JIN et al., 2019; CAMPANELLA et al., 2020; HANSCHEN & WINKELMANN, 2020; MORRIS et al., 2020), and Brazil (OLIVEIRA et al., 2011; BARROS et al., 2014; RONCATO et al., 2018). Results obtained around the world have been promising. A meta-analysis including information from 934 biofumigation tests with brassica residues, reported a decreased incidence of diseases and an increase of around 30% in the productivity of different crops (MORRIS et al., 2020).

Studies on the use of *Brassicaceae* family plants in biofumigation have advanced in recent decades. This literature review was conducted to collate this information and encourage research and the use of these plants in biofumigation. This review was organized into the following six sections: (1) biofumigation concepts, benefits, and mechanisms of action; (2) main volatile compounds released in the soil following decomposition of residues of *Brassicaceae* family species involved in the suppression of phytopathogens, insects, and weed species; 3) main brassica species used in biofumigation, their forms of use, and management; 4) main controlled agents and examples of successful and promising experiences; 5) limitations of the technique and current knowledge gaps; 6) non-brassica species and organic residues with similar potential and future prospects.

Biofumigation - definition, benefits, and mechanisms of action

The term biofumigation was initially proposed to describe a disease control technique that incorporated plants or plant residues into the

soil to release volatile biocidal compounds during their decomposition (KIRKEGAARD et al., 1993; TSROR et al. 2007; LADHALAKSHMI et al., 2015; PRASAD et al., 2015; HANSCHEN & WINKELMANN, 2020). This term was originally proposed by J. A. Kirkegaard to describe the process of cultivation, fragmentation, and incorporation of brassica residues (generic name given to species belonging to the *Brassicaceae* family) with the aim of releasing volatile compounds by the hydrolysis of GSLs present in the tissues of these plants (KIRKEGAARD et al., 1993). Isothiocyanates (ITCs) are among the biologically active products obtained from GSL hydrolysis (GIMSING & KIRKEGAARD, 2009; KIRKEGAARD, 2009; CLARKSON et al., 2015; MAWAR & LODHA, 2015; PRASAD et al., 2015). Notably, the volatile compound (methyl isothiocyanate) released by the synthetic fumigants (metam sodium and dazomet) currently sold in Brazil is also an ITC (AGROFIT, 2020).

The term biofumigation integrates a broader phenomenon by involving a series of allelopathic effects that have been empirically observed in brassica for centuries (PRASAD et al., 2015). Currently, a more detailed approach to biofumigation research has demonstrated its positive effects on disease control, thereby improving our understanding of the processes and mechanisms involved, and its practical application (DIXON & TILSTON, 2010; LAZZERI et al., 2013; CURTO et al., 2016; WEI et al., 2016; MORALES-RODRÍGUEZ et al., 2018; DUTTA et al., 2019; JIN et al., 2019; CAMPANELLA et al., 2020; HANSCHEN & WINKELMANN, 2020; MORRIS et al., 2020). This concept has been expanded and popularized to cover the use of a series of organic vegetable materials and animal production residues (GIMSING & KIRKEGAARD, 2009; KIRKEGAARD, 2009, PRASAD et al., 2015; KUMAR et al., 2018; DUTTA et al., 2019). Therefore, the concept of biofumigation has been extended to include the aerobic disinfestation of soil by the addition of residues or organic matter, resulting in the release of volatile compounds during decomposition that exert toxicity on undesirable soil organisms (MATTHIESSEN & KIRKEGAARD, 2006; BRUGGEN & FINCKH, 2016; GAMLIEL & BRUGGEN, 2016).

Biofumigation is different from another similar technique known as anaerobic biodesinfestation or anaerobic soil disinfestation. In this case, easily decomposed organic materials are incorporated in the soil in high amounts to stimulate rapid microbial growth. This leads to oxygen depletion due to increased microbial respiration (BRUGGEN & FINCKH, 2016; GAMLIEL & BRUGGEN, 2016; WEI et al., 2016) and the release of toxic compounds and gases from the decomposition of organic matter (LARREGLA et al., 2015). Different types of residue can be used in this process, including brassica (ROSSKOPF et al., 2015). Despite the consensus among most experts and researchers, there are some disagreements about these terminologies and the differences between the two methods, which can lead to misinterpretation.

Aerobic biofumigation can be combined with other techniques, such as soil solarization (LADHALAKSHMI et al., 2015; GAMLIEL & BRUGGEN, 2016), in which the soil temperature is increased by utilizing the incident solar energy on previously moistened soil covered with transparent polyethylene film (GAMLIEL & KATAN, 2009). In this case, some researchers prefer to name the combination of these two techniques as 'soil biosolarization' (ROS et al., 2008; VILLALOBOS et al., 2013). This combination enhances their effects, especially in tropical regions where solar radiation is abundant (ROS et al., 2008; BARRAU et al., 2009; VILLALOBOS et al., 2013; GAMLIEL & BRUGGEN, 2016; ROS et al., 2016).

Biofumigation with the incorporation of brassica residues has other effects in addition to the release of ITCs (MATTHIESSEN & KIRKEGAARD, 2006; GIMSING & KIRKEGAARD, 2009; HANSCHEN & WINKELMANN, 2020), which may enhance the suppression of soilborne phytopathogens (CLARKSON et al., 2015). These effects include increased organic matter content in the soil, with consequent improvements in physical properties and water retention, improved soil fertility due to nutrient cycling and supply, and improved microbial activity (CLARKSON et al., 2015; PRASAD et al., 2015; NTALLI & CARBONI, 2017). However, a full understanding of this multiplicity of effects on disease suppression remains to be elucidated (CLARKSON et al., 2015).

The efficiency of biofumigation in disease control varies according to the phytopathogen and its sensitivity to the ITCs released during GSL hydrolysis (FAN et al., 2008; MAWAR & LODHA, 2015). For example, *Pythium* species have been reported to be least sensitive to these compounds (MAWAR & LODHA, 2015). Efficiency can also vary according to the life cycle phase of the pathogen and is higher during the active phases, such as fungal mycelia, and lower in survival structures (KUMAR et al., 2018). However, effects on survival structures, especially on *Verticillium* spp., have also been reported (DIXON &

TILSTON, 2010; NEUBAUER et al., 2014; MAWAR & LODHA, 2015).

Glucosinolates and isothiocyanates

Glucosinolates (GSLs) are secondary metabolites of some plants compounds containing sulfur and nitrogen (KIRKEGAARD, 2009; LADHALAKSHMI et al., 2015), especially those of the *Brassicaceae*, *Capparidaceae*, *Tropaeolaceae*, *Moringaceae*, and *Amaryllidaceae* families (KARAVINA & MANDUMBU, 2012; LADHALAKSHMI et al., 2015). More than 200 GSLs are reported to occur in about 3,500 plant species of the *Brassicaceae* family (DUTTA et al., 2019). These compounds can be divided into three groups according to the type of side-chain in their molecules: aromatic, aliphatic, or indole, the hydrolysis of which result in products with different biological activities (ROSA et al., 1997; KIRKEGAARD, 2009; GIMSING & KIRKEGAARD, 2009).

Plants containing GSLs also produce a hydrolytic enzyme (thioglucosidase hydrolase), commonly known as myrosinase. In intact tissues, there is a physical separation between GSLs and hydrolytic enzymes (ROSA et al., 1997; GIMSING & KIRKEGAARD, 2009; LADHALAKSHMI et al., 2015; NTALLI & CARBONI, 2017; HANSCHEN & WINKELMANN, 2020). However, with tissue maceration or degradation due to insect attack, mechanical damage, or phytopathogen infection, contact between the enzyme and GSLs is increased. This contact triggers GSL hydrolysis and the consequent release of ITCs, such as organic cyanides and thiocyanates (LADHALAKSHMI et al., 2015). The ITCs released through GSL hydrolysis with aliphatic and aromatic chains (KIRKEGAARD, 2009) have high bioactivity and are often associated with efficient disease control (GIMSING & KIRKEGAARD, 2009). Other compounds can be released during the decomposition of brassica tissues (GIMSING & KIRKEGAARD, 2009; HANSCHEN & WINKELMANN, 2020), such as methyl sulfide, dimethyl sulfide, dimethyl disulfide, carbon disulfide, and methanethiol, which can also improve the efficiency of biofumigation (LORD et al., 2011).

Both the disruption of plant tissues and the presence of water are essential for ITC release (GIMSING & KIRKEGAARD, 2009; KIRKEGAARD, 2009). Hydrolysis reactions occur in the presence of water, and increased soil moisture can increase the efficiency of ITC generation and release (PRASAD et al., 2015; KUMAR et al., 2018). Notably, the use of dry or dehydrated vegetable tissues

does not affect GSL and myrosinase conservation in brassica tissues (MICHEL, 2014).

GLS hydrolysis occurs rapidly, and ITCs and other hydrolysis products generally have a short lifespan in the soil, with a rapid decrease in their concentration within a few days and a mean soil persistence of up to 14 days (KIRKEGAARD, 2009). However, residues with high GSL levels can inhibit the growth of microorganisms for up to two weeks after use (MARSCHNER & RENGEL, 2010).

Main brassicas and products used, and forms of usage

The brassica species used in biofumigation must possess high GSL levels. However, the procedures of using brassicas can vary according to the species, target organism to be controlled, and context of the production system. Worldwide, the main forms of use included: previous cultivation followed by incorporation as green manure; rotational cultivation; addition and incorporation of fresh or dry vegetable residues (MATTHIESSEN & KIRKEGAARD, 2006; KIRKEGAARD, 2009; CLARKSON et al., 2015; CAMPANELLA et al., 2020; MORRIS et al., 2020); and addition of industrial residues, such as cakes from seed pressing, for oil extraction (KIRKEGAARD, 2009; CURTO et al., 2016; PAN et al., 2017). Commercial products and formulations registered as Biofence™ (pellets and liquids), which contain high GSL levels (LAZZERI et al., 2013; NICOLA et al., 2013; WEI et al., 2016; SERRANO-PÉREZ et al., 2017), have also been used and evaluated in Italy, England, and Spain. Likewise, some companies have marketed seeds of certain *Brassica juncea* ('ISCI 20' and 'ISCI 99') and *Eruca sativa* ('Nemat') cultivars, as they contain high GSL levels, and are recommended for cultivation and for use as biofumigants (TRIUMPH, 2015). At experimental levels, the use of brassica essential oils (DHINGRA et al., 2013; PONTES et al., 2019) and leaf extracts (LORD et al., 2011; RONCATO et al., 2018; RUBAYET et al., 2018) are also promising (Tables 1, 2, 3, 4, 5 and 6).

The strategy to be used should be chosen considering local conditions, farm planning, and the fragmentation and incorporation methods of the biomass produced, or availability of residues or commercial products in nearby locations, in addition to acquisition and transportation costs (KIRKEGAARD, 2009).

The *Brassicaceae* family species most used for biofumigation belong to the genera *Brassica*, *Raphanus*, *Sinapis*, and *Eruca* (Tables 1 to 6). Of these, several mustard species are highlighted,

Table 1 - Promising experimental results obtained for the control of fungi using biofumigation with plant species of the *Brassicaceae* family.

Biofumigant	Target	Host	Strategy	Test type	Comments	Reference
Brassica oleracea	*R. solani*	Tomato	Use of fresh residues	Bioassay, greenhouse, field	Reduced symptoms and increased yield.	TSROR et al. (2007)
Various species	Various species	N/A	Powder	*In vitro*	Reduced *Ceratobasidium fimbriata* by 68.6% and *V. dahliae* by 68.7%.	FAN et al. (2008)
Brassica napus, B. juncea, B. campestris	*Sclerotinia sclerotiorum*	N/A	Macerated and dry tissues	*In vitro*	Growth reduction and sclerotia formation.	OJAGHIAN et al. (2012)
N/A	*Sclerotium rolfsii, S. sclerotiorum*	N/A	Mustard synthetic essential oil	*In vitro*, field	Delayed sclerotia germination.	DHINGRA et al. (2013)
B. juncea	*R. solani*	Sugar beet	Planting and incorporation	Field	Consistent control of primary infection.	MOTISI et al. (2013)
Various species	*V. dahliae*	N/A	Residue incorporation	*In vitro*, field	*B. juncea* significantly reduced the number of viable microsclerotia.	NEUBAUER et al. (2014)
Various species	Various species	N/A	Dry powdered plants	*In vitro*	Released volatiles showed inhibitory effects.	PRASAD et al. (2016)
Various species	*S. sclerotiorum*	N/A	Dry and ground residues	*In vitro*	Effect on mycelial growth and germination.	WARMINGTON & CLARKSON (2016)
B. carinata	*V. dahliae*	N/A	*Pellets* (DSM), liquid formulation	*In vitro*, field	67% efficiency using the liquid formulation.	WEI et al. (2016)
B. juncea	*Sclerotinia homoeocarpa*	Grass	(DSM)	*In vitro*, field	Reduced mycelial growth and incidence.	PAN et al. (2017)
B. nigra, B. oleracea	*R. solani*	Potato	Leaf extract	*In vitro*, field	*B. nigra* was the most effective at inhibiting growth.	RUBAYET et al. (2018)

N/A – Not applicable. DSM - defatted seed meals.

including *Brassica juncea*, *B. carinata*, *B. nigra*, *B. campestris*, and *Sinapsis alba*; radish (*Raphanus sativus*); arugula (*Eruca sativa*) (OJAGHIAN et al., 2012; CLARKSON et al., 2015; HANSCHEN & WINKELMANN, 2020; MORRIS et al., 2020); and *Brassica oleracea*, mainly cabbage (*B. oleracea* var. *capitata*) (MATTHIESSEN & KIRKEGAARD, 2006; TSROR et al., 2007; VILLALOBOS et al., 2013; BANDYOPADHYAY & KHALKO, 2016). These species control several target organisms; although, the level of suppression and sensitivity can also vary (KIRKEGAARD & MATTHIESSEN, 2004; FAN et al., 2008; CAMPANELLA et al., 2020).

Some important aspects must be considered when the previous brassica cultivation is used as green manure, or is included in a crop rotation program, such as species versus climatic conditions, time of year, and susceptibility to target organism (KIRKEGAARD, 2009; DONALD et al., 2010; LU et al., 2010; LAZZERI et al., 2013; KRASNOW & HAUSBECK, 2015). Species and cultivars with good climatic adaptation to local conditions, rusticity, high biomass yield, and that non-host the pathogen or pest to be controlled should be prioritized.

Aspects that may improve the efficiency of the technique must be considered when biomass

Table 2 - Promising experimental results obtained in the control of *Fusarium* spp. using biofumigation with plant species of the *Brassicaceae* family.

Biofumigant	Target	Host	Strategy	Test type	Comments	Reference
B. oleracea	*Fusarium oxysporum*	N/A	Crushed residues	*In vitro*	Reduced the population of *F. oxysporum*.	IRIARTE et al. (2011)
B. juncea, S. alba	*F. graminearum*	N/A	Crushed residues	*In vitro*	Suppressed the growth of *F. graminearum*.	PERNIOLA et al. (2012)
B. juncea	*Fusarium* sp.	Bitter melon, calabash	Macerated leaves	Bioassay	Reduced mycelial growth and incidence.	RELEVANTE & CUMAGUN (2013)
B. juncea	*F. graminearum*	N/A	Crushed residues	*In vitro*	Biofumigation combined with *Trichoderma* spp. had a synergistic effect against the pathogen.	PERNIOLA et al. (2014)
B. carinata	*F. circinatum*	Pine	Pellets (DSM)	Bioassay	Inoculum control and reduced seed mortality.	MORALES-RODRÍGUEZ et al. (2018)
B. juncea, Diplotaxis tenuifolia	*F. oxysporum* f. sp. *cucumerinum*	Cucumber	Planting and incorporation	Greenhouse	Suppressed *Fusarium* wilt.	JIN et al. (2019)
B. carinata	*Fusarium* spp.	Wheat	Planting and incorporation	Field	Reduced incidence and severity; increased yield.	CAMPANELLA et al. (2020)

N/A – Not applicable. DSM - defatted seed meals.

is incorporated into the soil. These aspects included: the species to be incorporated and their cycle phase, fragmentation and incorporation method, soil moisture, and method of waterproofing of the soil surface. The accumulation of GSLs in brassica depends on the developmental stage of each species. However, in general, higher levels are observed during vegetative growth with decreasing levels observed after flowering (KIRKEGAARD, 2009). Thus, in most cases, the pre-flowering phase is the most suitable for cutting and incorporation (DONALD et al., 2010; KARAVINA & MANDUMBU, 2012; DUTTA et al., 2019; CAMPANELLA et al., 2020). Biomass cutting and fragmentation procedures can also interfere with the efficiency of the technique; the more uneven and larger the fragments, the more heterogeneous the distribution and release of volatile compounds. This decreases biofumigation efficiency (KARAVINA & MANDUMBU, 2012; MAWAR & LODHA, 2015). Vegetal materials cultivated in the area or brought from other places should be well-fragmented and immediately incorporated into the soil, at a depth of 15–20 cm, using a rotary hoe or disk harrow (KUMAR et al., 2018). Another essential

factor is soil humidity, since water is essential for GSL hydrolysis following cell rupture; therefore, the soil must be irrigated up to field capacity immediately after the incorporation of residues to optimize this reaction (MATTHIESSEN & KIRKEGAARD, 2006; GIMSING & KIRKEGAARD, 2009; KIRKEGAARD, 2009; DONALD et al., 2010; KUMAR et al., 2018; DUTTA et al., 2019). Additionally, as many of the products released during GSL hydrolysis are volatile, losses can be reduced if the soil is covered with transparent plastic film after incorporation (CLARKSON et al., 2015; ROS et al., 2016; DUTTA et al., 2019), or if the surface is sealed with a roller and/or irrigation (DONALD et al., 2010). According to Kirkegaard (2009), the use of plastic is not mandatory, despite increasing the efficiency of volatile compound retention (KIRKEGAARD, 2009). The incorporation of residues into the soil can also coincide with standard plastic mulching, which is commonly used for some crops, including strawberry-growing systems (KIRKEGAARD, 2009; BRUGGEN et al., 2016).

Signs of phytotoxicity have been reported in crops introduced soon after the incorporation

Table 3 - Promising experimental results obtained in the control of oomycetes using biofumigation with plant species of the *Brassicaceae* family.

Biofumigant	Target	Host	Strategy	Test type	Comments	Reference
Brassica juncea, Iberis amara	*Pythium* spp.	N/A	Dehydrated plants (pellets)	*In vitro*	After adding water, the dried plants showed fungitoxic activity against *Pythium* spp.	LAZZERI et al. (2004)
Brassica carinata	*Phytophthora cactorum*	Strawberry	Residue incorporation	Field	Biofumigation, combined with solarization reduced the occurrence of the pathogen and increased yield.	BARRAU et al. (2009)
B. oleracea	Various species	Sweet pepper	Fresh residues + solarization	Field	Reduction of plants with symptoms and increased yield.	VILLALOBOS et al. (2013)
B. napus	*Phytophthora capsici*	Pepper	Ground seeds	Field	Reduced incidence through changes in the structure of the soil microbial community.	WANG et al. (2014a)
B. napus	*P. capsici*	Pepper	Ground seeds	Bioassay	Reduced disease and increased bacterial diversity in the soil.	WANG et al. (2014b)
B. carinata	*P. cinnamomi*	*Quercus cerris*	Pellets (DSM)	*In vitro*, bioassay	Inhibited mycelial growth and germination of chlamydospores and zoospores, and reduced inoculum potential.	MORALES-RODRÍGUEZ et al. (2016)
B. carinata, B. juncea, B. napus	*P. cinnamomi*	Yellow lupin	Ground seeds	*In vitro*, bioassay	*B. carinata* and *B. juncea* reduced mycelial growth and decreased *P. cinnamomi* viability in the soil.	RÍOS et al. (2017)
B. carinata	*P. nicotianae*	N/A	Pellets (DSM)	*In vitro*	Reduced chlamydospore germination.	SERRANO-PÉREZ et al. (2017)

N/A – Not applicable. DSM - defatted seed meals.

of brassica; therefore, it is advisable to wait for a minimum of 2 weeks between biofumigation and the planting of subsequent crops to ensure the dissipation of phytotoxic compounds (KIRKEGAARD, 2009; CLARKSON et al., 2015; MAWAR & LODHA, 2015). When plastic film is used, it can be removed after 3–4 weeks, and the soil should be slightly stirred to allow gases to be exhausted from the soil. The next crop can be planted 24 hours after this procedure (KUMAR et al., 2018).

In addition to fresh biomass, such as green manure, brassicas have been used in different ways for soil biofumigation in commercial production areas (LAZZERI et al., 2013). Examples included the use of residues as the cake resulting from seed pressing for oil extraction (CURTO et al., 2016),

liquid formulations (NICOLA et al., 2013), and pellets (WEI et al., 2016; SERRANO-PÉREZ et al., 2017). The cake is an interesting form of use, because the seeds tend to accumulate GSL during ripening; the concentration of these compounds is 8–10 times higher than that in other parts of the plants (LAZERRI et al., 2013). One example is the use of residues from Abyssinian mustard seed pressing *(Brassica carinata)* to produce biodiesel and derived products, which are rich in GSLs and can be used in the production of biofumigants (KIRKEGAARD, 2009; LAZZERI et al., 2013; NICOLA et al., 2013; LADHALAKSHMI et al., 2015; CURTO et al., 2016; WEI et al., 2016; SERRANO-PÉREZ et al., 2017). Cakes resulting from brassica seed pressing (defatted seed meal - DSM), as well as their derived technological products,

Table 4 - Promising experimental results obtained in the control of nematodes using biofumigation with plant species of the *Brassicaceae* family.

Biofumigant	Target	Host	Strategy	Test type	Comments	Reference
Raphanus sativus, B. juncea, Sinapis alba	Various species	Onion, celery	Planting and incorporation	Field	Reduced parasitic nematodes in celery.	WANG et al. (2006)
B. carinata	*M. chitwoodi*	Potato	Pellets (DSM)	Field	Reduced nematode damage in the root and increased yield.	HENDERSON et al. (2009)
Various species	*Globodera pallida*	N/A	Leaf extract	*In vitro*	Presented toxicity to nematodes and inhibited the activity of juveniles.	LORD et al. (2011)
B. juncea	*M. incognita*	Tomato	Various	*In vitro*, greenhouse	Reduced number of galls, egg mass, and eggs in tomato plants by over 90%.	OLIVEIRA et al. (2011)
B. carinata	*M. incognita*	Tomato	Liquid formulation	Bioassay	Statistically significant dose-effect correlations related to isothiocyanate release.	NICOLA et al. (2013)
B. juncea	*M. incognita*	N/A	Leaf macerates	*In vitro*	Macerate and volatile organic compounds released have nematicidal effect.	BARROS et al. (2014)
B. juncea, R. sativus, Eruca sativa	*G. pallida*	Potato	Planting and incorporation	Field	*B. juncea* and *R. sativus* showed to be promising for integrated nematode management systems in potatoes.	NGALA et al. (2015)
Various species	*M. incognita*	Tomato	DSM	Greenhouse	Better results with *Eruca sativa*, *Barbarea verna,* and *Brassica nigra*.	CURTO et al. (2016)
R. sativus, B. juncea, S. alba	*M. incognita*	Sweet pepper	Planting, incorporation + solarization	Greenhouse	Biosolarization with brassicas reduced the population of juveniles in the soil.	ROS et al. (2016)
R. sativus, E. sativa	*M. arenaria*	Tomato	Planting and incorporation	Greenhouse	Galls and egg masses decreased significantly.	AYDINLI & MENNAN (2018)
E. sativa, R. sativus, B. juncea	*M. incognita, M. javanica*	Tomato, potato	Planting and incorporation	Greenhouse	Reduced inoculum density and increased yield.	DANEEL et al. (2018)
Crambe abyssinica	*M. incognita*	Tomato	Leaf extract	Greenhouse	Crambe extract weekly incorporated into the soil was promising for *M. incognita* management in tomato.	RONCATO et al. (2018)

N/A – Not applicable. DSM - defatted seed meals.

can be used for the production of vegetable species both in Brazil and globally. These materials contain high GSL levels and are sources of nitrogen and other nutrients (MATTHIESSEN & KIRKEGAARD, 2006; LADHALAKSHMI et al., 2015; CURTO et al., 2016; DUTTA et al., 2019; HANSCHEN & WINKELMANN, 2020).

There are several practical examples and technological *Brassica* spp. products patented for use as biofumigants to control pathogens (LAZZERI et al., 2013; NICOLA et al., 2013; LADHALAKSHMI et al., 2015). Likewise, it is possible to buy mustard cultivars that are selected and marketed for cultivation to incorporate biomass for soil biofumigation (TRIUMPH, 2015).

Table 5 - Promising experimental results obtained in the control of phytopathogenic bacteria and protozoa using biofumigation with plant species of the *Brassicaceae* family.

Biofumigant	Target	Host	Strategy	Test type	Comments	Reference
Bacteria						
B. oleracea	*S. scabies*	Potato	Incorporation of dry and ground residues	Field	Disease suppression by 90%.	GOUWS & WEHNER (2004)
B. juncea, R. sativus, B. oleracea	*R. solanacearum*	Potato	Residue incorporation	Field	Significant suppression of wilt by up to 50%.	KIRKEGAARD (2009)
B. oleracea	*R. solanacearum*	Ginger	Residue incorporation	Field	Lowered incidence of wilt and higher crop yield.	BANDYOPADHYAY & KHALKO (2016)
B. juncea	*R. solanacearum*	Tomato	Mustard essential oil	*In vitro*	Cell mortality and inhibition of colony	PONTES et al. (2019)
Protozoa						
B. rapa, B. napus	*P. brassicae*	Chinese cabbage	Planting and incorporation	Greenhouse, field	Reduced severity in roots with density greater than 8 plants m^{-2}.	CHEAH et al. (2001)
B. rapa, B. napus	*P. brassicae*	Various	Planting and incorporation	Field	*B. rapa* reduced severity and increased mass in cauliflower plants.	CHEAH et al. (2006)

In general, the principles of disease control using biofumigants based on industrial residues or press cakes for oil extraction are consistent with those using organic fertilizers. However, they are optimized in terms of time and agricultural space, as they do not involve the pre-cultivation of brassica for later incorporation (KIRKEGAARD, 2009). Another technology being development is pellets obtained from plants or parts of the plant rich in GSLs. The *in vitro* bioactivity of these pellets against *Pythium* and *Rhizoctonia* was reported by LAZZERI et al. in 2004. For example, *B. carinata* seed cake pellets were obtained after pressing for oil extraction. This product is registered in Italy as BioFence™ (Triumph Italia SPA, Cerealetoscana Group) and has demonstrated satisfactory results in the control of *Phytophthora nicotianae* (SERRANO-PÉREZ et al., 2017).

The hydrophobic nature of GSL degradation products has enabled the development of liquid formulations based on vegetable oil emulsion in water and residues from *B. carinata* seed oil extraction (LAZZERI et al., 2011; LAZZERI et al., 2013; NICOLA et al., 2013). This formulation was developed for use via drip irrigation, and has demonstrated good results in the control of *Meloidogyne incognita* and *Verticilium dahliae* (NICOLA et al., 2013; WEI et al., 2016). According to WEI et al. (2016), depending on the characteristics of this formulation, a liquid version of BioFence™ may be more efficient at releasing ITCs than the efficiency observed with pellets, as it facilitates the dispersion of the active ingredient through the soil.

Promising results using brassicas in biofumigation

Fifty reports published over the last 20 years were selected to systematically analyze the accumulated data on the use of brassicas for

Table 6 - Promising experimental results obtained in the control of weeds and insects using biofumigation with plant species of the *Brassicaceae* family.

Biofumigant	Target	Host	Strategy	Test type	Comments	Reference
Weed						
B. juncea, S. alba	Various species	N/A	Crushed residues	*In vitro*	At the evaluated doses, *B. juncea*, significantly inhibited weed germination.	PERNIOLA et al. (2016)
B. juncea	Various species	N/A	Powder	Bioassay	Biofumigation showed potential to be included in weed management programs.	LEFEBVRE et al. (2018)
B. juncea	Various species	N/A	Incorporation of crushed residues. Powder	Bioassay, field	Highly effective (mortality > 85%) against small seeds, (0% to 20%) against hard and large seed species. Propagule mortality > 90%.	CAUWER et al. (2019)
B. juncea	Various species	N/A	Crushed residues	Greenhouse	Biofumigation with 2.5 kg m^{-2} was an alternative tool for integrated weed management.	PERNIOLA et al. (2019)
Insects						
E. sativa	Stored product pests	N/A	ITCs extracted from seeds	Bioassay	Effects of isothiocyanates on adults and larvae of stored product pests.	SHAAYA & KOSTYUKOVSKY (2010)
B. carinata; B. juncea	*Agriotes brevis, A. sordidus, A. ustulatus*	Lettuce, corn	DSM, chopped residues	Bioassay, field	Insecticidal effect with high larval mortality and crop protection.	FURLAN et al. (2010)

N/A – Not applicable. ITCs – Isothiocyanates. DSM - defatted seed meals.

biofumigation (Tables 1 to 6). Some important results reported in periods prior to this can be found in KIRKEGAARD & MATTHIESSEN (2004) and MATTHISSEN & KIRKEGAARD (2006).

The present survey included tests that used brassica species to control fungi (Tables 1 and 2), oomycetes (Table 3), nematodes (Table 4), phytopathogenic bacteria, and protozoa (Table 5); weeds; and insects, including some species that are stored product pests (Table 6). Notably, in this analysis, the pathogen *Plasmodiophora brassicae* was grouped as a protozoan, considering different publications and recent phylogenetic analyses that included this species in the protist supergroup Rhizaria (BURKI et al., 2010; SCHWELM et al., 2015; BHERING et al., 2020).

Most reports involved the use of biofumigation or tests with species or products obtained from plants of the *Brassicaceae* family to control diseases in vegetables, such as tomatoes (*Solanum lycopersicum*), potatoes (*S. tuberosum*), peppers, and sweet peppers (*Capsicum* spp.) (GOUWS & WEHNER, 2004; TSROR et al., 2007; HENDERSON et al., 2009; KIRKEGAARD, 2009; OLIVEIRA et al., 2011; NICOLA et al., 2013; VILLALOBOS et al., 2013; WANG et al., 2014a,b; NGALA et al., 2015; CURTO et al., 2016; ROS et al., 2016; AYDINLI & MENNAN, 2018; DANEEL et al., 2018; RONCATO et al., 2018; RUBAYET et al., 2018; PONTES et al., 2019; MORRIS et al., 2020). Studies have also reported the use of biofumigation to control diseases in less known species with promising results, such as bitter melon (*Momordica charantia*) and calabash (*Lagenaria siceraria*) (RELEVANTE & CUMAGUN, 2013); ginger (*Zingiber officinale*)

(BANDYOPADHYAY & KHALKO, 2016); oak (*Quercus cerris*) (MORALES-RODRÍGUEZ et al., 2016); creeping bentgrass (*Agrostis stolonifera*) (PAN et al., 2017); yellow lupin (*Lupinus luteus*) (RÍOS et al., 2017); and pine (*Pinus radiata*) (MORALES-RODRÍGUEZ et al., 2018).

There have been positive reports on the suppressive effect of *Brassicaceae* species in relation to soilborne phytopathogens, such as *Rhizoctonia solani*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii, Fusarium* spp., and *Phytophthora* spp. (Tables 1 to 3). The growth of these agents has been reported to decrease *in vitro*, and the incidence of diseases they cause in the investigated host species has also been reported to decrease (TSROR et al., 2007; BARRAU et al., 2009; OJAGHIAN et al., 2012; PERNIOLA et al., 2012; DHINGRA et al., 2013; NEUBAUER et al., 2014; WANG et al., 2014 a,b; RUBAYET et al., 2018; CAMPANELLA et al., 2020).

The control of a series of phytoparasitic nematodes with biofumigation was reported by NTALLI & CARBONI (2017) and DUTTA et al. (2019). Most studies have involved the action of brassica residues or derived products on species of the genus *Meloidogyne* (Table 4) (HENDERSON et al., 2009; OLIVEIRA et al., 2011; NICOLA et al., 2013; BARROS et al., 2014; CURTO et al., 2016; ROS et al., 2016; AYDINLI & MENNAN, 2018; DANEEL et al., 2018; RONCATO et al., 2018). The use of *E. sativa*, *Barbarea verna,* and *Brassica nigra* DSM (defatted seed meals) reduced the occurrence of *M. incognita* and increased the development of tomato plants (CURTO et al., 2016). Crambe leaf extract (*Crambe abyssinica*) weekly incorporated into the soil reduced *M. incognita* second stage juveniles (J2) and eggs in tomato roots by up to 61.57% (RONCATO et al., 2018).

There have been few reports on the use and biofumigation potential of phytobacteria (Table 5); although, the technique is commonly associated with changes in bacterial community, structure, and diversity (WANG et al., 2014a,b; JIN et al., 2019; HANSCHEN & WINKELMANN, 2020). Some promising results have been obtained with the incorporation of dry and ground *B. oleracea* residues to control bacterial wilt (*Ralstonia solanacearum*) in potatoes, ginger, and tomatoes (KIRKEGAARD, 2009; BANDYOPADHYAY & KHALKO, 2016; PONTES et al., 2019), and common mange (*Streptomyces scabies*) in potatoes (GOUWS & WEHNER, 2004). For common mange, a reduction of up to 90% in disease intensity was reported (GOUWS & WEHNER, 2004). The incorporation of

cabbage residues reduced the incidence of bacterial wilt (*R. solanacearum*) and increased yield in ginger (BANDYOPADHYAY & KHALKO, 2016). The use of mustard essential oil (*B. juncea*) increased cell mortality and inhibited the growth of *R. solanacearum* colonies *in vitro*, thereby reducing the incidence of bacterial wilt in tomatoes (PONTES et al., 2019).

In New Zealand, the prior cultivation and incorporation of *B. rapa* and *B. napus* residue reduced the severity of clubroot caused by the protozoan *Plasmodiophora brassicae* in Chinese cabbage (*B. rapa*), cauliflower (*B. oleracea* var. *botrytis*), and broccoli (*B. oleracea* var. *italica*), mainly with the use of *B. rapa*. These species positively influenced the yield of the respective crops (CHEAH et al., 2001; 2006). In this case, it is notable that the species used for biofumigation are also pathogen hosts (DIXON & TILSTON, 2010). Thus, despite the positive reports by some authors, its use requires further analysis.

Biofumigation with brassica residues has also shown promising results in reducing weed seed banks and propagules in the soil (Table 6). The use of *B. juncea* residue (2.5 kg m^{-2}) reduced the population of mono and dicotyledonous species, especially *Digitaria sanguinalis*, *Portulaca oleracea*, and *Taraxacum officinalis* (PERNIOLA et al., 2019). Crushed residues of the same species also inhibited the germination of *Anoda cristata*, *Picris echiodes*, and *P. oleracea* seeds (PERNIOLA et al., 2016). The incorporation of *B. juncea* crushed residues effectively controlled weeds (> 85% mortality of small seeds) and decreased hard and large seed species (0–20%) (CAUWER et al., 2019).

Fresh *B. juncea* residues and *B. carinata* DSM were evaluated against *Agriotes brevis*, *A. sordidus*, and *A. ustulatus* larvae by FURLAN et al. (2010). The *B. carinata* cake resulted in high larval mortality and prevented the larvae from damaging the crops (FURLAN et al., 2010). SHAAYA & KOSTYUKOVSKY (2010) evaluated the toxicity of isothiocyanates extracted from *E. sativa* seeds against stored product pests. The authors reported that the use of isothiocyanates significantly increased the mortality of larvae and adults of the insect species evaluated.

Technique limitations

The literature also contains studies reporting negative results or with little consistency in the use of brassicas in biofumigation (LU et al., 2010; SMOLIŃSKA & KOWALCZYK, 2014; KRASNOW & HAUSBECK, 2015; MAZZOLA et al., 2017; DUTTA et al., 2019). The factors associated with these variations include the brassica species,

method and management used in biofumigation, the sensitivity of the target species, the phase of the target species cycle, and the influence of chemical, physical, and biological factors in the soil (KIRKEGAARD & MATTHIESSEN, 2004; FAN et al., 2008; KIRKEGAARD, 2009; CLARKSON et al., 2015; LADHALAKSHMI et al., 2015; MAWAR & LODHA, 2015; KUMAR et al., 2018; CAMPANELLA et al., 2020). Thus, the methodology and products must be adjusted according to different situations and objects of control: pathogen, pest, or weed. Hence, specific studies are needed to elucidate the species and products most appropriate to each context and production system (CLARKSON et al., 2015), especially in Brazil, where limited information is currently available. The susceptibility of biofumigant species to target organisms or other pathogens and crop pests that are part of the production system can also be a limitation (DONALD et al., 2010; LU et al., 2010; KRASNOW & HAUSBECK, 2015).

Additionally, most studies on biofumigation have been conducted in laboratory or greenhouse environments (Tables 1 to 6) (DUTTA et al., 2019; MORRIS et al., 2020). Despite the importance of studies performed under controlled conditions to better understand the phenomena involved, studies under field conditions are also essential (DUTTA et al., 2019). Only results from these studies can provide subsidies to improve and implement the technique in commercial crops.

Additionally, the influence of biofumigation on beneficial microorganisms present in the soil should also be highlighted, since it is not a selective technique. It can change the soil microbial community, and some authors believe it affects beneficial invertebrates present in the soil (HANSCHEN & WINKELMANN, 2020). Studies showing the effects of this technique on the effectiveness of associated strategies, such as the use of biocontrol agents, are also necessary (HENDERSON et al., 2009). WANG et al (2014b) reported that the combination of biofumigation with *Brassica* spp. residues and the antagonist *Bacillus amyloliquefaciens* increased the bacterial diversity of the soil and influenced certain microbial populations, both positively and negatively. The increased bacterial diversity may have played a significant role in the suppression of *Phytophthora capsici* in pepper (*Capsicum annuum*). Nevertheless, the authors suggested that further studies should be conducted before recommending this integrated approach.

Potential use of non-brassica plants and agricultural residues in biofumigation

In addition to plants of the *Brassicaceae* family, other species and residues can be investigated and explored for use in biofumigation (ARNAULT et al., 2013; CURTO et al., 2016; GAMLIEL & BRUGGEN, 2016; WEI et al., 2016). The effects of these materials are related to the production of volatile biotoxic compounds by increasing the microbial activity in the soil and changing its structure (GAMLIEL & BRUGGEN, 2016). Some species of the genus *Allium* exert biocidal activity and are recommended for use in biofumigation (ARNAULT et al., 2013) such as, lavender (*Lavandula* sp.) (WEI et al., 2016), neem (*Azadirachta indica*) (BARROS et al., 2014), medicinal plants such as citronella (*Cymbopogon nardus*) and wormseed (*Dysphania ambrosioides*) (SILVA et al., 2020), and papaya seeds (*Carica papaya* L.) (NEVES et al., 2008).

The use of organic fertilizers, manure, residues, and nitrogen-rich compounds can also be considered in soil biofumigation due to the release of allelochemicals (MEGHVANSI & VARMA, 2015; ZEIST et al., 2019). Some examples of residues with promising results are castor bean cake and poultry litter or chicken manure, with or without soil solarization. In this case, it may be classified as soil biosolarization (ROS et al., 2008; VILLALOBOS et al., 2013; ROS et al., 2016).

CONCLUSION

Biofumigation with plant residues from the family *Brassicaceae* can be an important strategy in the integrated management of soil organisms that are harmful to crops. Positive results have been reported when the methodologies are correctly used. In biofumigation, brassicas are the most commonly used plants for accumulating GSLs, which release ITCs after enzymatic hydrolysis, with strong biocidal action. However, other plant species (non-brassicas) and organic residues already used in agriculture have potential for use in this way; however, further studies are needed to validate and optimize their use in agriculture.

Biofumigation controls harmful agents by releasing volatile biocidal compounds, with some indirect benefits related to the supply of organic matter to the soil. Therefore, this technique represents a comprehensive management system involving chemical, physical, and biological soil changes, with multiple effects on phytopathogenic agents, pests, and weed species, the soil environment, and plants of economic interest. This technique may be of particular interest for organic production systems and can be integrated with other alternative strategies, such as soil solarization, with projections of improved effects.

However, biofumigation is not a selective practice. Studies investigating the effects of biofumigation on the beneficial soil microbiota, and on the interaction between biofumigation and biological control with microorganisms remain inconclusive.

Owing to the multiplicity of factors involved, such as biofumigant species, amount of biomass, form of use, physical characteristics of the soil and the target organisms, many studies are required, especially under tropical conditions. There is also a need to fill knowledge gaps and understand the processes and mechanisms involved, the specifics of each material used, and the effects on different groups of phytopathogens, pests, and on the soil microbiota and microfauna. Finally, the cultural component should also be considered, with reluctance to replace chemical fumigants with biofumigation, which is a less harmful option to the environment.

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DECLARATION OF CONFLICTS OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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