



## Biofumigation Potential of Brassicas to Control White Rot Caused by *Sclerotinia sclerotiorum* on Eggplant

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**T**HIS STUDY aimed to examine the biofumigation effect of cabbage, broccoli and cauliflower against *Sclerotinia sclerotiorum* (Lib.), the pivotal agent of white rot in eggplant under *in vitro* conditions. Also, field experiments were carried out to assess the effects of brassicas on the disease severity index of white rot in eggplant and plant growth parameters in two successive seasons using a hybrid eggplant Darko F1 cultivar. Autolysis products of leaves and roots of the three brassicas plants were analyzed by gas-liquid chromatography-mass spectrometry (GC-MS). The results confirmed that the tested biofumigation plants led to a significant decrease in the mycelial growth and number of sclerotia formation of *S. sclerotiorum* under laboratory conditions. Cabbage meal was the most effective treatment, resulting in the most reduction in mycelial growth and sclerotia formation. Under field conditions, the incorporation of cabbage, broccoli and cauliflower plants into soil decreased the DSI of white rot during both seasons. This led to a significant increase in plant growth parameters, total fruit yield per plant and total fruit yield per feddan compared to control. Eight glucosinolates were identified by the natural autolysis of leaves and roots of cabbage, broccoli and cauliflower. The autolysis products as isothiocyanates, nitriles and epithioalkane nitriles were analyzed using GC-MS and referred to sinigrin, glucoiberberin, gluconapin, glucoerucin, gluconasturtiin, glucoiberin, glucoraphanin and glucobrassicin glucosinolates. The highest percentage of autolysis products was detected in cabbage roots. Other sulfur compounds and volatile oils with biofumigant activity were also detected in the three investigated plants.

**Keywords:** Biofumigation, Brassicas, Eggplant, Glucosinolates, *Sclerotinia sclerotiorum*.

### Introduction

Eggplant (*Solanum melongena* L.) is one of the most economical solanaceous vegetable crops worldwide. Different pathogens and pests attack eggplant crop and other solanaceous crops causing economic losses to the total yield (Bletsos et al., 2003; Ibrahim, 2017; Sadik et al., 2022; Rao & Viswanath, 2023).

The disease symptoms of eggplant white rot disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, might include water-soaked lesions on the stem that increase in length and then covered with white mycelium, black sclerotia developed on the mycelium, lesions girdle the

stem, plant wilted and died (Hansda et al., 2014). *S. sclerotiorum* is widely distributed and it could infect many protected and open field crops causing white rot disease (Boland & Hall, 1994).

The control of white rot in eggplant by the available fungicides is extremely difficult. The excessive use of synthetic fungicides in controlling eggplant diseases causes serious risks to consumers and the environment. So, seeking alternative strategies is strongly urged to combat this risk. Biofumigation is a biologically inert control tactic that can be used as a tool to control several plant diseases particularly soil-borne diseases through the release of biocidal compounds called isothiocyanates (ITCs)

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(Rahmanpour et al., 2013; Mazzola et al., 2015; Rao & Viswanath, 2023). ITCs are released from brassicaceous crops on hydrolysis of glucosinolates (GSLs) by the myrosinase enzyme (Vivaldo et al., 2017). Ascomycetes, which are known as the largest group of true fungi (Larena et al., 1999) including *Sclerotinia*, are expected to be controlled by biofumigation methods (Ojaghian et al., 2012).

This study aimed to investigate the biofumigation potential of three tested brassicas plants (cabbage, broccoli and cauliflower) against *S. sclerotiorum* *in vitro*. Also, the effectiveness of brassicas plants against the disease severity index of eggplant white rot, plant growth characters and fruit yield were studied under field conditions. The autolysis products including volatiles from roots and leaves of brassicas plants were identified using GC-MS.

## **Materials and Methods**

### *Isolation and identification*

*S. sclerotiorum* was isolated from eggplant plants from Ismailia Governorate with typical symptoms of white rot disease. Plant samples and sclerotia collected from the diseased plants were disinfected in a 1.0% solution of sodium hypochlorite for one minute, washed in several changes of sterile water and dried on filter paper. Aseptically plant samples were inoculated on PDA medium in Petri dishes and kept in an incubator at  $20\pm 1^\circ\text{C}$  for 7 days (Iqbal et al., 2003). Fungus was purified and identified based on its morphological and cultural characteristics (Kohn et al., 1979). Verification of the target fungus voucher sample was done in the Mycological Research and Disease Survey Department, Plant Pathology Research Institute, ARC, Egypt.

### *In vitro experiment*

#### *Effect of green meals of brassicas on linear growth and sclerotia formation*

The studied brassicas included cabbage, (*Brassica oleracea* var. *capitata*), broccoli (*Brassica oleracea* var. *italica* L.) and cauliflower (*Brassica oleracea* var. *botrytis* L.). The roots and greens or the entire above-ground parts of the three brassicas were harvested from the experimental plots in February 2021, plant materials were chopped into small pieces. Sandy soil was used in this experiment, which was pulverized to fine structure before being used. *S.*

*sclerotiorum* was maintained on a PDA medium. A little modification of the methodology proposed by Handiseni et al. (2016) was used in this experiment.

Six hundred grams of the soil was placed in a plastic packing pan, then the soil was amended with brassicas plant tissue at 20, 40 and 60% (w/w). Two hundreds of distilled water were added to the mixed soil inside each plastic pan. Each plastic pan had six Petri dishes (9cm diameter), which were inoculated with a 5mm disc of *S. sclerotiorum* grown on PDA medium, sealed by parafilm sheet, and incubated at  $20\pm 1^\circ\text{C}$  in a tightly closed and sealed plastic pan. The control treatment consisted of a plastic pan containing 600g of soil mixed with 200mL of distilled water. When the growth of *S. sclerotiorum* completely covered the PDA in the control treatment, the inhibition percentage of *S. sclerotiorum* growth was calculated. The number of sclerotia formation and reduction % were calculated after 3 weeks from inoculation (El-Ashmony et al., 2017).

### *In vivo experiment*

#### *Effect of biofumigation on eggplant white rot under field conditions*

Field experiments with natural infection were conducted to evaluate the biofumigation potential of the three tested brassicas (cabbage, broccoli and cauliflower) as green manures against the eggplant white rot using a hybrid eggplant Darko F1 variety. These experiments were performed in two successive seasons (from October to July in both seasons) at the main Experimental Farm of SCU, Ismailia, Egypt. For the first season (2020/2021) trial, *Brassica* crops were planted using seeds in October 18, 2020 season. In the second season (2021/2022) trial, seeds were planted in October 24, 2021. Brassicas were incorporated into the soil on 15 February in both seasons. Eggplant seedlings at 30 day-old were sown on 22 March in both field trials.

Experiments were conducted as randomized complete block design (RCBD). Each RCB consisted of four treatments (three treatment of *Brassica* crops and untreated control treatment) with three experimental plots for each treatment in the two seasons. Each experimental plot (6 × 5m) contained 6 rows (1m width × 5m length). Agricultural practices (plowing, irrigation, fertilization and mechanical weed control) were

performed. At maturation stage, approximately in mid of February in both seasons, brassicas plants were incorporated into the top 20-cm layer of the soil. Plots were re-lined up at the same rate (6 rows for each plot) with 1m apart. Plots were covered by polyethylene sheets. The flooding irrigation system was used and performed at 3-day intervals to enhance the decomposition of the chopped plant tissues and increase the released isothiocyanates. Three weeks later, the polyethylene cover sheet was removed, and eggplant seedlings (Hybrid eggplant Darko F1 cv) were sown at the rate of 10 seedlings /row 50cm apart.

#### *Field study measurements*

Disease severity index (DSI) of white rot in eggplant plants for each treatment was recorded two and four months (the end of the experiment) post-sowing using a modified scale (from 0 to 4) described by Lesovoi et al. (1987). DSI (%) was calculated for each treatment by a formula suggested by Wheeler (1969). Vegetative growth measurements were also recorded at 60 and 120 days after sowing. The growth parameters included root length, whole plant length, number of leaves, total fresh weight and dry weight/plant. Mature eggplant fruits were collected at 4-day intervals or when they were marketable. The collected fruits from each replicate and treatment were counted and weighed.

#### *Natural autolysis of cabbage, broccoli and cauliflower*

Twenty g of fresh leaves or roots of each brassica plant was taken and homogenized separately with 250mL distilled water. The homogenized samples of each brassica crop were left for natural autolysis overnight (13h) at room temperature, followed by extraction in 50mL dichloromethane then they were shaken for 10min and centrifuged at 3000rpm for 5min. The organic layer was dried over anhydrous sodium sulfate and concentrated under nitrogen to about 1mL. The concentrated autolysate was kept at -20°C in a closed vial until being used for further analysis (Al-Gendy & Lockwood, 2003).

#### *Identification of the autolysis products and other volatiles using GC-MS*

A Shimadzu GC/MS-QP2020 (Kyoto, Japan) coupled with Rtx-1MS fused bonded column (30m length, 0.25mm internal diameter and 0.25µm film thickness, Restek, USA) was

used for the analysis. The oven temperature was initially set at 40°C for 2min, increased to 250°C at a rate of 5°C/min and then it was held isothermal at 250°C for 10min. The injector temperature was adjusted to 250°C. Helium was used as carrier gas with a flow rate of 1.44 mL/min. Mass spectra were acquired utilizing filament emission current (equipment current) of 60 mA, ionization voltage of 70eV and ion source temperature of 220°C. Samples were injected in split mode with split ratio 1: 10. Identification of glucosinolates was performed based upon comparison of mass fragments and spectra of autolysis products with available literature (Spencer & Daxenbicher, 1980; Al-Gendy & Lockwood, 2003; Vaughn & Berhow, 2005; Blažević & Mastelić, 2009; Al-Gendy et al., 2016; Mohammed et al., 2017, 2021). Other volatile constituents were identified (Al-Gendy et al., 2016; Adams, 20017) as well as a comparison with Nist (2017) spectral data. The autolysis was operated at Ain Shams University, Faculty of Pharmacy, Center for Drug Discovery Research and Development.

#### *Statistical analysis*

The obtained data were analyzed by Analysis of Variance (ANOVA) using CoStat software (version 6.311). Means were separated using Duncan Multiple Range Test (DMRT) at P= 0.05 level of significance.

## **Results**

#### *Effect of biofumigant plants as green meal on linear growth of *S. sclerotiorum* and sclerotia formation*

Green meals of the tested brassica crops (cabbage, broccoli and cauliflower) differed significantly in reducing the mycelial growth of *S. sclerotiorum* as compared to the control. Cabbage caused the greatest growth reduction in *S. sclerotiorum* mycelia at 51.32, 70.39 and 87.59% for the respective cabbage incorporation rates (20, 40 and 60%). In contrast, cauliflower caused the lowest reduction at 29.33, 42.43 and 54.14 %. (Fig. 1). The mean production of sclerotia number was recorded in the control treatment at 28 sclerotia / plate. Cabbage was the most effective brassica species in reducing sclerotia production with the highest reduction percentage being 43.09, 65.47, and 72.62% for the respective brassica incorporation rates (Fig. 2).

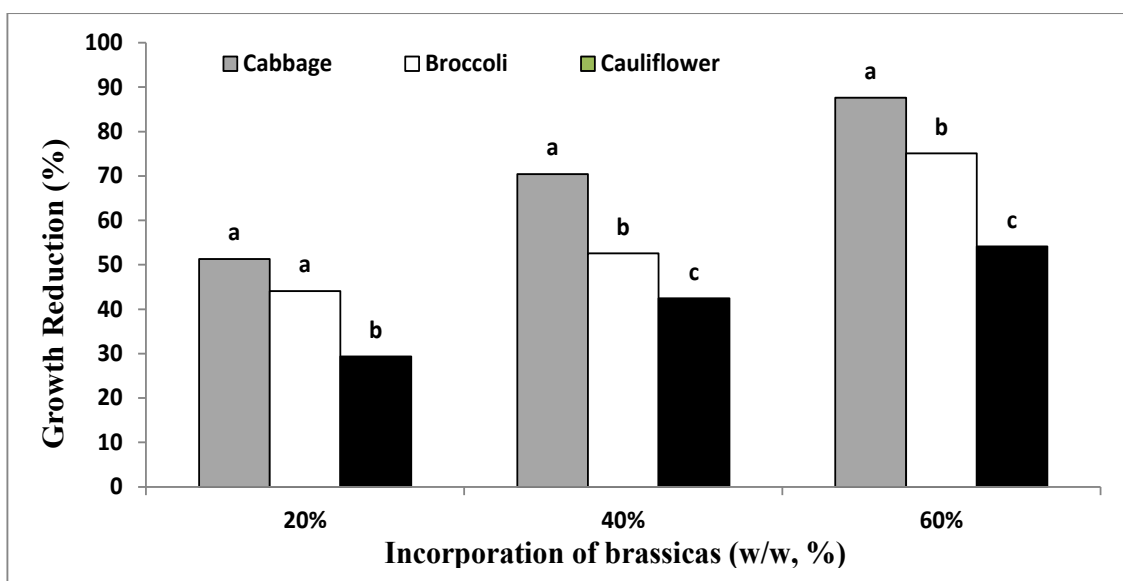


Fig. 1. Effect of brassicas green meal on mycelia growth of *S. sclerotiorum* [Bars with different letters are significantly different (DMRT,  $P \leq 0.05$ )]

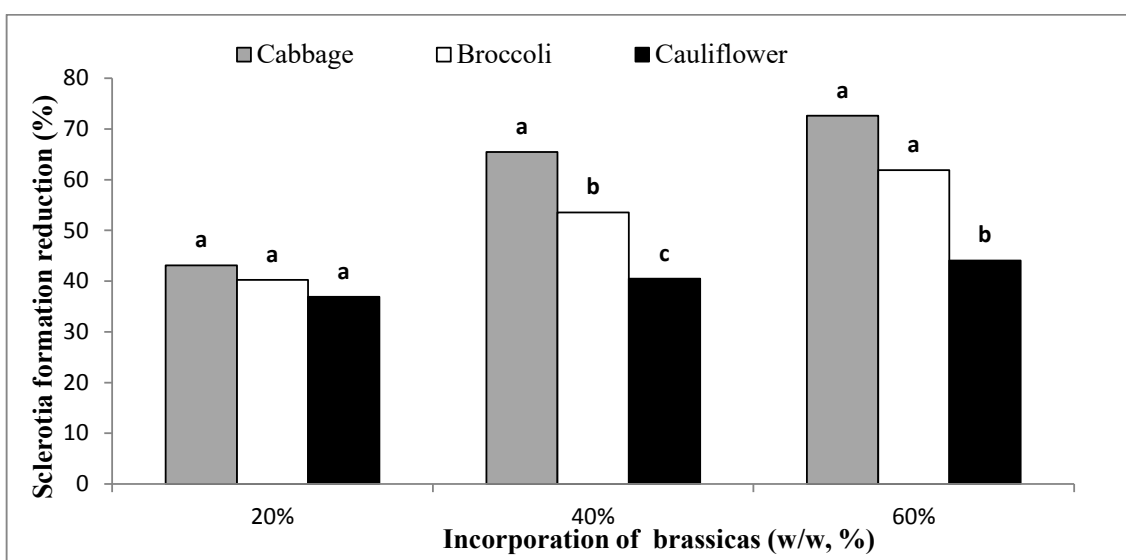


Fig. 2. Effect of brassicas green meal on number of sclerotia formation of *S. sclerotiorum* [Bars with different letters are significantly different (DMRT,  $P \leq 0.05$ )]

#### *Efficacy of biofumigation in controlling white rot under field conditions*

##### *Effects on disease severity index*

In the first season trial (2021) and after 60 days of planting, all brassicas (cabbage, broccoli, and cauliflower) treatments showed the lowest DSI of 1.87, 2.46 and 3.79% which were lower than that recorded in the control treatment at 10.85%. After 120 days of planting the respective treatments received the lowest DSI of 2.75, 3.08 and 5.31% as compared to non-treated control treatment

at 12.23% (Table 1). In the second season trial (2022), the obtained results showed the same trend of efficacy in reducing the DSI of eggplant white rot. Cabbage biofumigation treatment was the most effective with the lowest DSI followed by broccoli and cauliflower in both seasons. Statistically, significant differences existed among the DSI values for the studied treatments and control either in the first or the second season field trials (Table 1).

**TABLE 1. Effect of the three brassica cover crops on DSI of eggplant white rot under field conditions**

Treatment	(DSI) % of eggplant white rot			
	First season (2021)		Second season (2022)	
	60 days	120 days	60 days	120 days
Control	10.85a	12.23a	10.16a	14.88a
Cabbage	1.87c	2.75c	1.92c	2.33d
Broccoli	2.46bc	3.08c	2.15c	3.6c
Cauliflower	3.79b	5.31b	4.23b	5.53b

Means in the same column followed by different letters indicate significant differences (DMRT,  $P \leq 0.05$ )

DSI: Disease severity index

#### *Effects on plant morphological characters*

All the tested brassica-biofumigation treatments showed significant increases in the recorded plant growth characters compared to that of the control treatment (Table 2). For the first season, the cabbage treatment was superior and enhanced the growth characters of eggplant plants at 37.93cm, 94.74cm, 116.67 leaves, 406.49g and 95.52g for root length, whole plant length, leaves number, fresh weight and dry weight, respectively after 60 days of planting. The same trend was also observed for all plant characteristics 120 days post-planting. Regarding the second season, cabbage was also the superior treatment for both reading intervals. The recorded figures were 38.29cm, 107.76cm, 136.67 leaves, 611.24g and 124.56g for the respective plant characters 60 days post-planting. 40.47cm, 123.88cm, 267.66 leaves, 1218.79g and 279.14g after 120 days of planting. Meanwhile, broccoli and cauliflower biofumigation treatments also increased all morphological characters compared to the control treatment either after 60 or 120 days post-planting. Control treatment showed the lowest respective figures for the two studied seasons (Table 3).

#### *Effects on eggplant yield*

In first season trial, cabbage-biofumigation treatment showed the greatest yield at 11.66ton/ feddan with the highest weight of fruits /plant at 3.36kg/plant, followed by broccoli which was comparable with cabbage treatment at 11.28ton/ feddan and 2.84kg/plant. Cauliflower showed moderated results at 10.95ton /feddan and 2.91kg/ plant. In contrast, the control treatment achieved the lowest eggplant yield of 9.97ton/feddan and 2.03kg/plant (Table 4). The same trend of fruit yield was observed in the second season where the control treatment showed the lowest eggplant yield of 10.81ton /feddan with the subsequent lowest fruit yield/plant of 1.94kg/plant. Cabbage-biofumigation treatment had 12.64ton/feddan, and 4.1kg/plant.

Broccoli and cauliflower –biofumigation treatments were comparable (Table 4). Significant differences were found among tested brassicas and control treatment regarding the total fruit yield /feddan and total fruit yield/plant for both seasons.

#### *Identification of the autolysis products and other volatiles using GC-MS*

Eight glucosinolates were identified in the leaves and roots of cabbage, broccoli, and cauliflower through their autolysis products isothiocyanates, nitriles and epithioalkane nitriles by natural autolysis (Table 5). The percentage of glucosinolates autolysis products in leaves are much smaller than in roots. The highest percentage of glucosinolate autolysis products was detected in cabbage roots followed by broccoli roots and cauliflower roots. No glucosinolate autolysis products were detected in cabbage leaves and minimal amounts were detected in cauliflower leaves under the applied experimental conditions. Glucoerucin was the major glucosinolate of cabbage and broccoli roots that could be detected by its erucin nitrile while glucoiberberin was the major glucosinolate of cauliflower root which was identified by its iberberin nitrile. Glucoraphanin was the major compound of broccoli leaves as indicated by its autolysis products; sulphoraphane and sulphoraphane nitrile. Sinigrin, glucoiberberin, gluconapin, glucoerucin, glucoiberin and glucoraphanin were the identified aliphatic glucosinolates where gluconasturtin is the only identified aromatic glucosinolate and glucobrassicin was the indole glucosinolate found. The identification was based on a comparison of mass fragments and spectra of autolysis products. Other volatile constituents were identified as Sulphur-containing compounds e.g. di, tri and tetra sulfide as well as sulfurous acid, volatile oils (1,4-cineole and *p*-menthane) and fatty acid esters (palmitic acid methyl and isopropyl esters) (Table 6).

**TABLE 2. Effect of the three brassica cover crops on the morphological characters of eggplant plants under field conditions during 2021 season**

Treatment	Root length (cm)	Plant length (cm)	Leaves number	Fresh weight (g)	Dry weight (g)
<b>First season of 2021 (60 days post planting)</b>					
Control	21.76c	69.32d	76d	178.24c	49.85d
Cabbage	37.93a	94.74a	116.67a	406.49a	95.52a
Broccoli	29.38b	86.07b	105b	334.91b	86.63b
Cauliflower	31.45b	78.26c	97.3c	333.13b	80.32c
<b>First season of 2021 (120 days post planting)</b>					
Control	26.74c	78c	108.67d	321.87d	67.02d
Cabbage	41.64a	126.01a	187.33a	1304.83a	283a
Broccoli	36.24ab	112.53ab	161b	1012.99b	245.9b
Cauliflower	33.96b	103.58b	145.33c	882.1c	190.26c

Means in the same column followed by different letters indicate significant differences (DMRT,  $P \leq 0.05$ )

**TABLE 3. Effect of the three brassica cover crops on the morphological characters of eggplant plants under field conditions during 2022 season**

Treatment	Root length (cm)	Plant length (cm)	Leaves number	Fresh weight (g)	Dry weight (g)
<b>Second season of 2022 (60 days post planting)</b>					
Control	20.03d	66.51c	73.00c	187.46d	65.96d
Cabbage	38.29a	107.76a	136.67a	611.24a	124.56a
Broccoli	35.23b	97.06b	109.00b	421.8b	94.31b
Cauliflower	31.00c	99.14b	113.00b	327.38c	88.59c
<b>Second season of 2022 (120 days post planting)</b>					
Control	27.72c	80.48b	113.68d	218.00d	85.92d
Cabbage	40.47a	123.88a	267.66a	1218.79a	279.14a
Broccoli	39.25ab	119.49a	218.00b	1106.3b	250.05b
Cauliflower	37.09b	116.75a	186.67c	921.73c	209.93c

Means in the same column followed by different letters indicate significant differences (DMRT,  $P \leq 0.05$ )

**TABLE 4. Effect of the three brassicas cover crops on the yield of eggplant under field conditions**

Treatment	First season (2021)		Second season (2022)	
	Fruit yield/plant (kg)	Fruit yield/feddan (ton)	Fruit yield/plant (kg)	Fruit yield/feddan (ton)
Control	2.03b	9.97b	1.94b	10.81b
Cabbage	3.36a	11.66a	4.10a	12.64a
Broccoli	2.84ab	11.28a	3.57ab	12.12ab
Cauliflower	2.91ab	10.95ab	2.96ab	11.72ab

Means in the same column followed by different letters indicate significant differences (DMRT,  $P \leq 0.05$ )

TABLE 5. Glucosinolates autolysis products detected in leaves and roots of cabbage, broccoli and cauliflower

Parent compound Autolysis product (common name)	Rt (min)	Major mass fragments*	Relative % of hydrolysis products					
			Cabbage		Broccoli		Cauliflower	
			Roots	Leaves	Roots	Leaves	Roots	Leaves
<b><u>2-Propenyl glucosinolate (Sinigrin)</u></b>								
3,4-Epithiobutanenitrile	8.9	99(M <sup>+</sup> ), 72, 59 and 41	5.82		3.63		0.75	
<b><u>3-Methylthiopropyl glucosinolate (Glucoiberin)</u></b>								
4-(Methylthio) butanenitrile	11.73	115 (M <sup>+</sup> ), 61, 47 and 41	16.9		4.35		23.07 0.6	
<b><u>3-Butenyl glucosinolate (Gluconapin)</u></b>								
4,5-Epithiovaleronitrile	12.62	113 (M <sup>+</sup> ), 86, 73, 45 and 41	2.35				1.29	
<b><u>4-(Methylthio)butyl glucosinolate (Glucoerucin)</u></b>								
5-(Methylthio) penanenitrile (Erucin nitrile)	15.41	129 (M <sup>+</sup> ), 114, 82, 61, 55 and 41	29.04		21.13		0.52	
<b><u>2-Phenyl ethyl glucosinolate (Gluconasturtiin)</u></b>								
3-Phenyl propionitrile	16.58	131 (M <sup>+</sup> ), 91, 77, 65 and 51	13.87		6.07		4.28	
Phenyl ethyl isothiocyanate	23.05	163 (M <sup>+</sup> ), 105, 91, 72 and 65	2.1		8.91		0.8	
<b><u>3-(Methylsulphinyl) propyl glucosinolate (Glucoiberin)</u></b>								
3-(Methylsulphinyl) propyl isothiocyanate (Iberin)	25.76	163 (M <sup>+</sup> ), 130, 116, 100, 86, 72, 63 and 41					0.8	
<b><u>4-(Methylsulfinyl) butyl glucosinolate (Glucoraphanin)</u></b>								
5-(Methylsulfinyl)pentane nitrile (sulforaphane nitrile)	23.23	145(M <sup>+</sup> ), 128, 97, 82, 64, 55 and 41					10.15	
4-(Methylsulfinyl) butyl isothiocyanate (sulforaphane)	29.25	177 (M <sup>+</sup> , 0%), 160, 114, 72 and 55	1.08		7.15	7.77		
<b><u>Indol-3-ylmethylglucosinolate (Gluco Brassicin)</u></b>								
Indole-3-acetonitrile	30.58	156(M <sup>+</sup> ), 155, 130, 101, 77 and 51					2.48	
<b>Total % of identified compounds</b>			71.16		51.24	20.4	30.76 1.35	

TABLE 6. Other volatile constituents identified in leaves and roots of cabbage, broccoli and cauliflower

Compound Name	Rt (min)	Major mass fragments*	Relative % of volatiles						
			Cabbage		Broccoli		Cauliflower		
			Roots	Leaves	Roots	Leaves	Roots	Leaves	
Dimethyl disulphide	3.32	94(M <sup>+</sup> ), 79, 61 and 45	0.29		6.71	0.58	8.11		
Dimethyl trisulphide	8.82	126(M <sup>+</sup> ), 111, 79, 64 and 45	0.95		6.42		1.18		
Sulfurous acid	9.02	274 (M <sup>+</sup> , 0%), 97, 81 and 55					1.89		
1, 4- Cineole	9.17	154(M <sup>+</sup> ), 125, 111, 71, 55 and 43						0.88	
p-Menthane	9.7	140 (M <sup>+</sup> ), 97, 81 and 55		0.53					
Phenethyl alcohol	13.33	122(M <sup>+</sup> ), 91, 77, 65 and 51	0.47				0.77		
1,4-Dimethyl tetrasulphid	16.34	158 (M <sup>+</sup> ), 111, 94, 79, 64 and 45	0.74		0.78		1.34		
Palmitic acid methyl ester	34.63	270 (M <sup>+</sup> ), 256, 227, 143, 87, 74, 55 and 41	1.11				0.78		
Palmitic acid isopropyl ester	35.31	298(M <sup>+</sup> ), 256, 213, 157, 129, 115, 97 and 43					1.95		
Citric acid tributyl ester	40.62	360 (M <sup>+</sup> ), 185, 157, 129, 111, 57, 43 and 41	7.42				8.01		
<b>Total % of identified compounds</b>			10.98	0.53	23.9	3.47	12.06		

## Discussion

Brassicaceae are known to produce sulfur compounds called glucosinolates (GLS) that release biologically active products during enzymatic hydrolysis. Isothiocyanates (ITCs) are the key metabolites of the GLS hydrolysis that have the potential to reduce populations of soilborne pathogens (Larkin & Griffin, 2007; Rodríguez-Molina et al., 2016). Suppose there are pests or pathogens attack or mechanical wounding. In that case, the plant cells are ruptured and the GSLs and myrosinase will come into contact and glucosinolates are hydrolyzed in the presence of water to release various products, including ITCs (Vig et al., 2009).

In lab experiments, the three investigated brassicas (cabbage, broccoli and cauliflower) were able to cause a significant reduction in the linear growth of *S. sclerotiorum* at all tested brassicas incorporation rates. These results are in line with those of Warmington & Clarkson (2016) who tested five biofumigant crops and found that they showed potential management of sclerotia formation and mycelial growth of *S. sclerotiorum*

*in vitro*. Also, the application of vapor methyl, allyl and butyl isothiocyanates compounds at different concentrations from brassica crops inhibited mycelial growth and reduced the number of sclerotia of *S. sclerotiorum* (Ojaghian et al., 2012). Similarly, the effect of volatiles derived from Brassica tissues on *S. sclerotiorum in vitro* showed inhibition of growth and differed among varieties and species indicating that GLS contents in biofumigant crops have various toxic products (Rahmanpour et al., 2013). Also, mustard, broccoli, cabbage and cauliflower showed inhibition of mycelial growth for *F. oxysporum f.sp. melongenae* in eggplant (Rao & Viswanath, 2023).

In field experiments, the biofumigation treatments reduced DSI of eggplant white rot as compared to control treatment. These findings are in harmony with various reports of soilborne plant pathogens suppression such as *Aphanomyces*, *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinia* and *Verticillium* using biofumigant plants (Matthiessen & Kirkegaard, 2006; El-Sharkawy, 2015; Meng et al., 2022). Similarly, the suppressive effects of glucosinolate hydrolysis



products against soil-borne pathogens of sunflower including *S. sclerotiorum* under field conditions showed a significant reduction in disease severity (Ahmed et al., 2020). The effectiveness of biofumigant crops against soil-borne pathogens is known to be increased when used along with soil solarization. Solarization treatment (covering the soil with plastic after incorporation of biofumigant plants into the soil) detains volatile compounds and elevates the temperature inside the soil. Increasing temperature increases the production of ITCs indeed (Matthiessen & Kirkegaard, 2006; Gimsing & Kirkegaard, 2009).

Planting eggplant after the incorporation of brassicas into the soil was positively correlated to the improvement of vegetative growth parameters and the increase of eggplant yield over that of control treatment. This conclusion coincided with that reported by several earlier reports. The use of brassicas as seed meals or green manures reduce the population of soil-borne fungi leading to the increase of plant growth and yield mainly due to the emission of ITCs in the soil (Mazzola et al., 2015; El-Sharkawy, 2015). Similarly, Rao & Viswanath (2023) studied the effect of biocidal volatile compounds from six Brassica species on wilt incidence of *F. oxysporum* f.sp. *melongenae* on eggplant in greenhouses. Results further showed that mustard recorded lowest population followed by broccoli, cabbage, cauliflower, kohlrabi and radish tissues. Mustard biofumigant treatment had a significant biocidal effect on *V. dahliae* in the soil than oilseed rape compared to the control with subsequent significant increase in fruit yield of eggplant due to the lower disease index (Meng et al., 2022).

The percentage of glucosinolates autolysis products of leaves were much smaller than that of roots. These results are in line with Bhandari et al. (2015) who studied the profile and concentration of glucosinolate (Sinigrin) in various tissues of nine species of brassicas. In most crops, the concentrations of glucosinolates were in the order of seeds > roots > shoots. Similarly, the biocidal effects of the glucosinolate hydrolysis products active compounds were confirmed in laboratory studies and varied more in the field against *S. sclerotiorum* (Ahmed et al., 2020). Also, other methods using HPLC analysis were performed for separation of the Sinigrin from the leaf and root tissues in six Brassica species. The sinigrin concentration showed different quantities among

the six tested species in the roots and leaves of *F. oxysporum* f.sp. *melongenae* in eggplant (Rao & Viswanath, 2023). The concentration and the profiles of GSLs (aliphatic, aromatic and indole) according to the type of side chain also varied among Brassicas either their shoots or roots (Kirkegaard & Sarwar, 1998; Van Dam et al., 2009; Bhandari et al., 2015). Other volatile constituents were identified as sulfur-containing compounds, volatile oils and fatty acids are reported in this study. Dimethyl disulfide showed strong fungicidal activity such as the inhibition of mycelial growth, sclerotia formation, viability and reduction of disease symptoms in *Sclerotinia minor* on tomato plants (Tyagi et al., 2020).

### **Conclusions**

Based on *in vitro* and *in vivo* experiments, the tested biofumigants (cabbage – broccoli – cauliflower) proved to have great potential to control *Sclerotinia* rot in eggplant. Incorporation of brassicas into soil enhanced the tolerance of eggplant plants to *S. sclerotiorum* via plant vigor resulting in significant increase in the investigated growth parameters with subsequent increase in fruit yield. This study, in turn, might provide strong evidence for the possibility of using these brassicas crops as biofumigants to control *Sclerotinia* rot in eggplant. However, before any firm conclusions or recommendations are made, more field testing needs to be done.

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*Authors' contributions:* EEE contributed in all experiments, data analysis and interpretation and writing the manuscript. AAA performed the gas-liquid chromatography-mass spectrometry analysis, and revising the manuscript.

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## إمكانية استخدام التبخير الحيوى للمحاصيل الصليبية لمقاومة مرض العفن الأبيض المتسبب عن فطر *Sclerotinia sclerotiorum* فى نبات الباذنجان

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تهدف الدراسة إلى إختبار كفاءة التبخير الحيوى لثلاثة أنواع من نباتات العائلة الصليبية (الكرنب، البروكلى والقرنبيط) ضد فطر *Sclerotinia sclerotiorum* المسبب لمرض العفن الأبيض فى نبات الباذنجان تحت ظروف التجارب المعملية. أيضا اجريت التجارب تحت ظروف الحقل لإختبار تأثير هذه النباتات على شدة الإصابة بالمرض. كذلك التأثير على مقاييس النمو الخضريه خلال موسمين متتالين باستخدام صنف الباذنجان hybrid Darko F1. تم دراسة نتائج التحلل الذاتى لأوراق وجذور الثلاث أنواع النباتية من العائلة الصليبية بواسطة استخدام جهاز GC-MS. أكدت النتائج ان معاملات التبخير الحيوى أدت إلى انخفاض معنوى فى النمو الميسليومى وعدد الأجسام الحجرية (الأسكورشيات) المتكونه لفطر *S. sclerotiorum*. تحت ظروف التجارب المعملية. كانت معاملة التبخير الحيوى بمطحون نبات الكرنب أكثر كفاءة فى انخفاض النمو الميسليومى وعدد الأسكورشيات المتكونة للفطر. أوضحت النتائج أيضا انه تحت ظروف الحقل بعد تقطيع وتقليب نباتات الكرنب والبروكلى والقرنبيط بداخل التربة فإن ذلك أدى الى خفض شدة الإصابة خلال موسمى الدراسة، كما أدى إلى زيادة معنوية فى المقاييس الخضريه لنبات الباذنجان علاوة على زيادة كمية المحصول مقارنة بمعاملة الكنترول. تم تعريف ثمانية أنواع من مركبات الجليكوثيوسيانات بواسطة التحلل الذاتى الطبيعى لأوراق وجذور كلا من نباتات الكرنب والبروكلى والقرنبيط. كانت منتجات التحلل الذاتى مثل isothiocyanates, nitriles and epithioalkane nitriles والتي تم تعريفها بواسطة GC-MS، أشارت الى مركبات sinigrin, glucoiberin, gluconapin, glucoerucin, gluconasturtiin, glucoiberin, glucoraphanin and glucobrassicin glucosinolates. تم تسجيل أعلى نواتج للتحلل الذاتى فى جذور نبات الكرنب. أيضا تم اكتشاف أنواع أخرى من مركبات الكبريت والزيوت الطيارة ذات نشاط تبخير حيوى فى النباتات المختبرة الثلاثة.