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Biocidal Efficacy of Plant Volatiles Obtained from Brassica species against *Fusarium* wilt of Eggplant caused by *Fusarium oxysporum* f.sp. *melongenae*

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ABSTRACT: Eggplant is the most traditional vegetable crop in India. It is susceptible to a number of diseases that reduce yield and quality. Among them, *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *melongenae* (FOM) is one of the most important soil borne diseases responsible for yield reduction in India. High doses of chemical fungicides are typically employed to treat this disease, but these chemicals have negative effects on both human and environmental health in addition to making disease-causing organisms more resistant to treatment. So, in the current study, a simple, inexpensive, and environmentally friendly method, i.e., soil incorporation of plant components having volatiles, was used to check the growth of a wilt-causing pathogen by testing the efficacy of plant leaf tissues of six cruciferous/Brassica species both in vitro and in earthen pots under laboratory and polyhouse conditions, respectively. During the in *vitro* study, mustard leaf tissue exhibited maximum mycelial inhibition (90.15 and 77.65 %) followed by Broccoli leaf tissue (65.50 and 73.86%) after five and seven days of inoculation (DAI) respectively. while radish leaf tissue recorded the least inhibitory effect (31.48% and 11.77 %) at 5 and 7 DAI, respectively. In the pot culture test, a similar trend was observed by increasing the germination percentage and decreasing the pre- and post-emergence seedling mortality in all Brassica leaf tissues tested. However, sinigrin concentrations estimated per 100 g of *Brassica* leaf and root tissues were correlated with the efficacy of different treatments, *viz*., mustard (512.00 mg and 38.20 mg), followed by broccoli leaf tissue (36.40 mg and 2.80 mg), cabbage (28.40 mg and 0.1 mg), cauliflower (17.20 mg and 0.1 mg), Knol kohl (2.60 mg and 0.1mg) and lastly radish (less than 0.1 mg in both leaf and root) led to a significant reduction of *F. oxysporum* f.sp. *melongenae* populations in pot experiment ranging from 12.83 × 10^6 CFU in mustard to 3.77 × 10^6 CFU in radish.

Keywords: *Fusarium* wilt, eggplant, biocidal volatiles, Isothiocyanates, Glucosinolates.

INTRODUCTION

Brinjal, a solanaceous vegetable crop, is the common name for eggplant (*Solanum melongena* L.). Numerous bacterial, viral, nematode, fungal, and viral diseases affect eggplant. Out of them, Fusarium wilt, which is caused by the fungus *Fusarium oxysporum f.* sp. *melongenae*, was the soil borne disease that caused the most severe damage to eggplant in India.

Since more than a century ago, chemical fungicides and soil fumigants have been a common practice in many crops, particularly fruits and vegetables for the control of soil borne diseases. However, environmental and safety concerns are putting more and more pressure on these products. In addition, the evidence is growing of their adverse effect on beneficial soil organisms and the rapid resurgence of soil-borne pathogens following fumigation. As limitations of chemical soil fumigants are becoming more apparent, interest in non-chemical approaches like bio-fumigation, especially plant volatile compounds, for managing soil-borne diseases has been rekindled with the recent concept of sustainable agriculture (Dangi *et al.*, 2017; Mazzola *et al.*, 2015; Watson *et al.*, 2017). Plant volatile compounds (VCs) are typically small, lipophilic, odorous, and low molecular mass compounds that can diffuse above and below ground through gas- and water-filled pores in soil and rhizosphere environments. Glucosinolates are plant volatile compounds (VCs) that are well-known as bio-fumigants for the control of soil borne pathogens and plant parasitic nematodes because they emit isothiocyanates (ITCs) as VCs during the biodegradation process. Additionally, ITCs are a key component of chemical fumigants like metam. Brassicaceae, Capparaceae, and Caricaceae are three plant families that all generate glucosinolates, and several of their genera have been investigated for their fungicidal effects on soil borne pathogens and nematicidal effects on plant parasitic nematodes (Kruger *et al.*, 2013). Glucosinolates will be digested to

release ITCs, which have broad-spectrum biological activity, after being macerated and incorporated. These ITCs are effective against numerous soilborne pathogens (Schroeder and MacGuidwin, 2010). According to Schulz-Bohm *et al.* (2017); Vivaldo *et al.* (2017), these VCs are the byproducts of secondary metabolisms in plants and microbes like bacteria and fungi. According to Effmert *et al.* (2012), a number of variables, including the development stage, nutrient availability, temperature, oxygen availability, pH, and soil moisture content, affect the emission of VCs from plants and microbes. Alkenes, alcohols, ketones, benzenoids, pyrazines, sulphides, and terpenes are some chemical classes into which VCs are divided. Glucosinolates of Brassicaceae crop residues have been reported to reduce propagules of soil borne pathogens and result in a concomitant decrease in the incidence of plant diseases caused by them (Villapudua and Munnecke 1988). The effects of crucifer residues have been attributed to the chemical breakdown of glucosinolates (GSLs), the characteristic sulphur-containing isothiocyanates (ITCs) constituents of members of the Brassicaceae responsible for their inherent pungent odour (Halkier and Gershenzon 2006). More than 200 glucosinolates were identified from 3,500 Brassica species and each Brassica species can contain various types and amounts of glucosinolates (Clarke, 2010). During the decomposition of crucifer residues, GSLs break down to produce sulphides, ITCs, thiocyanates and nitrile compounds, which have either fungistatic or fungicidal properties. Soil amendment with crucifer residue combined with solarization produce a greater variety of toxic volatile substances and improve the effectiveness of solarization in reducing pathogen population and thereby disease incidence (Prasad *et al*., 2015). The adverse effects of crucifer residues on soilborne diseases are reported to be positively correlated with the amount of GSL in the crop (Mayton *et al*., 1996). Therefore, evaluation of leaf tissues from six cruciferous/Brassica species was done in the current study to test their antifungal and biofumigant potential against the Fusarium wilt disease of eggplant both in vitro and in clay pots.

MATERIAL AND METHODS

***In vitro* evaluation of Biofumigation (volatiles released by *Brassica* species).** The experiment was conducted *in vitro* using the procedure given by Rahmanpour *et al.* (2009). Leaves of seven *Brassica* species were collected from 60 day old flowering plants. Leaf disks of 8 mm diameter were cut with cork borer. A thin layer of potato dextrose agar was poured on the upturned lid of a 90 mm Petri plates. These Petri plates were inoculated with a 5 mm diameter disc of five days old mycelium of the test pathogen and surrounded with twenty two leaf disks. The Petri plate inverted base was placed on top of the upturned lid. Control plates were kept without leaf disks. Petri plates were immediately sealed with parafilm tape. Petri plates were incubated for 72 hours at 28 ± 2°C in darkness. The diameter of the treatment colonies were measured at 7 days after incubation. Per cent inhibition of mycelial growth was calculated by using the formula given by Dennis and Webster (1971).

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent inhibition of mycelial growth
C = Colony diameter in control (mm)
T = Colony diameter treatment (mm)

Experimental details:
Design: Completely Randomized Design (CRD)
Replications: Three
Treatments: Seven

Treatment details

Tr. No.	Treatments
T_1	Leaves of Cabbage
T_2	Leaves of Cauliflower
T_3	Leaves of Knolkhol
T_4	Leaves of Mustard
T_5	Leaves of Radish
T_6	Leaves of Broccoli
T_7	Control (Untreated)

High Performance Liquid Chromatography protocol for Glucosinolate profiling in *Brassica* species

Sample preparation. Six Brassica species, *viz*., *Brassica oleracea* var. *capitata* (cabbage), *Brassica oleracea* var. *botrytis* (cauliflower), *Raphanus sativus* (radish), *Brassica nigra* (mustard), *Brassica oleracea* var. *italica* (broccoli), and *Brassica oleracea* (Knolkhol), grown at the Horticulture Research Scheme (vegetable) were uprooted at the 50-percent flowering stage and taken to the laboratory. The separated root, stem, and leaves were freeze-dried and stored at -20ºC till sample preparation. Samples for HPLC analysis were prepared by a new protocol, modified from the previously described methodology (Sarwar and Kirkegaard 1998). 500 mg of freeze-dried *Brassica* leaves were crushed into a fine powder using a pestle and mortar in liquid nitrogen. This powder was then added to a 50 ml plastic centrifuge tube containing 10 ml of 70 percent hot methanol. The tubes were caped, shaken vigorously, and placed in a water bath for 20 minutes at 70ºC. After cooling to room temperature, the tubes were centrifuged at 3000 rpm for 6 min. Three ml of the supernatant was then applied to a prepared 0.5 cm plug of Sephadex A-25 poly-prep columns (Biorad Laboratories, CA, USA).

Column preparation and desulfation. One gram of Sephadex A-25 (Sigma Aldrich Co., USA) was added to 20 mL of milli-Q water and left overnight. The next day, Sephadex was added up to a thickness of 0.5 cm to the poly-prep columns fitted with the stand. The sephadex was then washed with 1 mL milli-Q water followed by 1 mL 0.2 M sodium acetate (pH 5). After discarding water and buffer, a 100 µl aliquot of prepared sulfatase (Sigma Aldrich Co. USA) was added to the column. After one drop of buffer was displaced, 3 ml of prepared supernatant was added to each column for desulfation of GSLs, and the column was capped and left overnight. The next day, 1 ml of milli-Q water was added to the column, and the effluent was collected

in an HPLC vial. The samples were frozen until analysis.

Standard dilution and desulfation. As a glucosinolate standard, sinigrin (2-propenyl GSL), purchased from ChromaDex Inc. (Irvine, CA, USA), was used in the study. One mg of the standard was added to 1 ml of milli-Q water to prepare a stock solution of 1000 ppm, which was further diluted to 500, 400, 300, 200, and 100-ppm solutions for calibration purposes. The desulfation of the standards was also carried out by adopting the same protocol used for the samples.

HPLC analysis. HPLC experiments were performed on a Dionex Ultimate 3000 intelligent LC series HPLC instrument with an Ultimate 3000 VWD Series. Variable Wavelength Detectors and Ultimate 3000 × 2 Dual LC systems with Dual Gradient Pump were employed. A reversed phase Acclaim 120, C18 column (2.1 × 50 mm i.d., 3 µm Analytical) was used for separation of the Sinigrin in six *Brassica* species.

Ten µL of the aqueous sample extract was injected into the HPLC system by 1000 µL WPS-3000 and ACC-3000 Series Syringe. Individual Sinigrin were detected by the VWD Series variable wavelength detectors at a UV wavelength of 229 nm. Chromeleon version 6.80 was used to control the operation of the system. A gradient program was used for sufficient retention and baseline separation of the Sinigrin, in which the mobile phase consisting of a mixture of water (A) and Acetonitrile (B) was operated at a constant flow rate of 0.8 ml/min. During the initial time period (8 min) the fluent contained 0 per cent B, and from 8 to 24.5 min a linear fluent gradient to yield 20 per cent B was adjusted. From 24.5 to 28 min a further increase of B up to 25 per cent was set. From 28 to 33 min, per cent flow of B in the fluent was reduced to 0 per cent through a linear gradient. Finally, after a period of 33 min, the column was equilibrated for 10 min at the end of each run.

Determination of Sinigrin Concentration. The peak areas of the identified Sinigrin in the sample extracts were recorded and from this data calibration curve was prepared in Microsoft Excel. The correlation equation obtained from the curve was used for quantitative analysis of Sinigrin in plant extracts by comparing the peak areas of Sinigrin in sample with the peak area of Sinigrin in standard. Peaks other than that of Sinigrin were removed using Chromeleon programme itself. Each sample was replicated thrice to reduce chances of error. The experimental data was analyzed using software test procedure no.3.

Evaluation of biovolatiles from *Brassica* species in Pot culture. Earthen pots with a height of 27 cm and a radius of 13 cm were filled with 4 kg potting mixer consisting of three parts of red soil and one part of black soil and enough urea, single super phosphate (SSP) and muriate of potash.

Single plant of each *Brassica* species as mentioned in the treatments were grown for 60 days (flowering stage) in each earthen pot, cut into small pieces and incorporated into the soil at a depth of 15 cm from the top of the pot. Inoculum grown on Sand Maize medium was thoroughly mixed and applied to the top 5 cm soil surface @10 gm per kg soil. To hydrolyze the glucosinolates, a small amount of water was added and covered with polythene sheet for 15 days.

Later, in each pot, five seedlings were planted. Three replications were maintained in a completely randomized design for each treatment. Control was maintained without *Brassica* residues incorporation. Observations were recorded on per cent wilt incidence at 30 days after transplantation by using the following formula described by Mayee and Datar (1986).

$$\text{Per cent disease incidence} = \frac{\text{No. of dead plants}}{\text{Total number of plants}} \times 100$$

Experimental details:

Design: Completely Randomized Design (CRD)
Replications: Three
Treatments: Seven

Treatment details

Tr. No.	Treatments
T_1	Leaves of Cabbage
T_2	Leaves of Cauliflower
T_3	Leaves of Knolkhol
T_4	Leaves of Mustard
T_5	Leaves of Radish
T_6	Leaves of Broccoli
T_7	Control (Untreated)

RESULTS AND DISCUSSION

Evaluation of biocidal volatiles released from *Brassica* tissues.

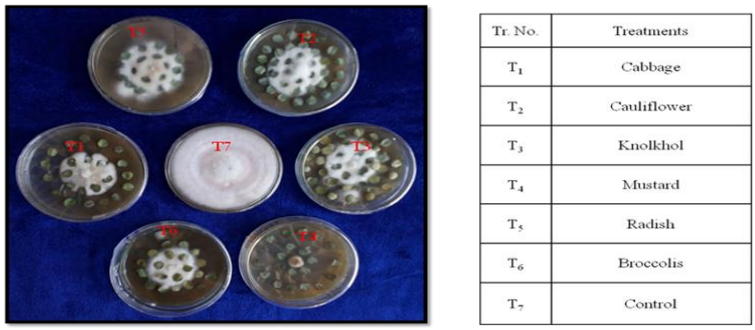
***In vitro* efficacy of biocidal volatiles released from *Brassica* leaf tissues on linear growth of *F.oxysporum* f.sp. *melongenae* .**Biocidal volatiles released by all the *Brasssica* species tested more or less significantly suppressed the radial mycelia growth of the pathogen at both of the durations recorded (Table 1, Fig. 1, Plates 1). Mustard leaf tissues have shown the greatest inhibition on mycelial growth of the pathogen at 5 days after incubation (7.37 mm) and 7 days after incubation (12.77 mm) as compared to the control (7.00 mm and 90.00 mm) with an inhibition percent of 90.15 and 77.65 percent, respectively. Broccoli leaf tissue (23.04 and 37.52 mm), cabbage leaf tissue (33.02 and 50.81 mm), cauliflower leaf tissue (35.27 and 62.96 mm), khol leaf tissue (38.94 and 63.26 mm), and radish leaf tissue (36.32 and 79.41 mm) were the next relatively effective biocidal treatments, with significant inhibition of mycelial growth (65.50 and 73.86%), (52.76 % and 43.53 %), (47.41% and 30.04 %) and (43.09 % and 29.71 %) at 5 DAI and 7 DAI, respectively. Radish leaf tissue showed the lowest colony diameter (36.32 mm and 79.41 mm) and percent inhibition effect (31.48 % and 11.77%) at 5 DAI and 7 DAI, respectively. These results were also in agreement with previous research findings (Olivier *et al*., 1996; Kirkegaard *et al.*, 1996; Relevante and Cumagun 2013; Sintayehu *et al*., 2011; Bharat and Jitender 2015).

Table 1: Efficacy of biocidal volatiles released by *Brassica* species against *Fusarium oxysporum* f.sp. *melongenae* under laboratory conditions.

Tr. No.	Treatments	5 DAI		7 DAI	
		Colony Dia. of test pathogen (mm)	Per cent Inhibition	Colony Dia. of test pathogen (mm)	Per cent Inhibition
T_1	Cabbage	33.02	52.76 (46.60)*	50.81	43.53 (41.26)
T_2	Cauliflower	35.27	47.41 (44.76)	62.96	30.04 (33.16)
T_3	Knol khol	38.94	43.09 (46.60)	63.26	29.71 (41.26)
T_4	Mustard	7.37	90.15 (71.04)	12.77	77.65 (67.88)
T_5	Radish	36.32	31.48 (43.82)	79.41	11.77 (20.02)
T_6	Broccoli	23.04	65.50 (54.98)	37.52	73.86 (49.78)
T_7	Control	70.00	0.00 (0.00)	90.00	0.00 (0.00)
	SE(m)±	**2.41**	**1.99**	**1.88**	**1.3**
	C.D.(P=0.01)	**7.38**	**6.1**	**5.78**	**3.98**

DAI: Days after incubation Dia.: Diameter
* Figures in the parentheses are angular transformed values

In vitro effect of volatiles released by Brassica Species against *Fusarium oxysporum* f.sp. *melongenae*



Tr. No.	Treatments
T_1	Cabbage
T_2	Cauliflower
T_3	Knolkhol
T_4	Mustard
T_5	Radish
T_6	Broccolis
T_7	Control

Plate 1.

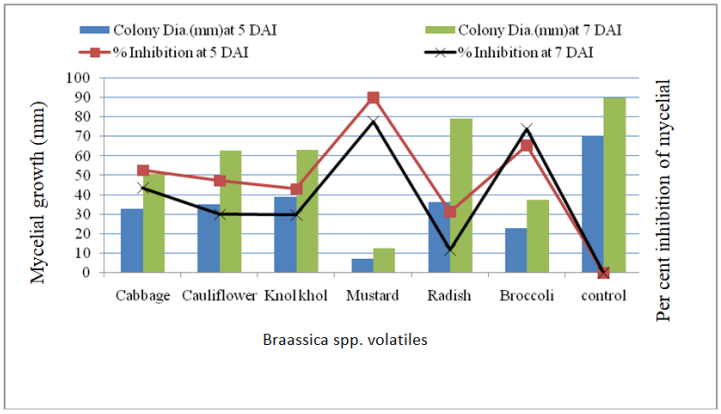


Fig. 1. *In vitro* efficacy of volatiles released by Brassica Species against *Fusarium oxysporum* f.sp. *melongenae.*

Efficacy of biocidal volatiles released from *Brassica* leaf tissues on *Fusarium* wilt (Pot culture)

Effect on seed germination. The results (Table 2, Fig. 2 and Plate 2) showed that all treatments improved seed germination compared to the untreated control, ranging from 61.66 to 93.00 percent versus 51.00 percent in the untreated control. However, mustard leaf tissue incorporation (93.00%) had the highest seed germination rate, followed by broccoli leaf tissue (86.00%), cabbage leaf tissue (83.00%), cauliflower leaf tissue incorporation (77.00%), khol leaf tissue incorporation (64.66%), and radish leaf tissue incorporation (61.66%), with percentage increases over control of 82.35, 68.62, 62.74, 50.98, 26.79, and 20.91 percent, respectively.

Effect on pre and post-emergence mortalities. Results (Table 2, Fig. 2 and Plate 2) revealed that all the treatments significantly influenced both pre-emergence seed rot (PREM) and post-emergence seedling mortality (POSM), caused by *F. oxysporum* f.sp. *melongenae* in eggplant. The pre-emergence seed rot recorded with all the treatments ranged from 7.00 to 38.33 percent, as against 49.00 percent in the untreated control. However, the treatment found most effective with the least significant PREM was mustard leaf tissue (7.00 %) followed by broccoli leaf tissue (14.0%), cabbage leaf tissue (17.00%), cauliflower leaf tissue (23.00%), knol khol leaf tissue (35.33 %) and radish leaf tissue (38.33%).

A similar trend with increased post-emergence seedling mortality was also observed, and it ranged from 5.53 percent to 32.44 percent, as against 44.66 percent in the untreated control. However, the treated mustard leaf tissue had the lowest POSM (5.53%), followed by

broccoli leaf tissue (13.44%), cabbage leaf tissue (17.50%), cauliflower leaf tissue (16.66%), knol khol leaf tissue (25.63%), and radish leaf tissue (32.44%).

The mean mortality recorded with all the treatments ranged from 6.27 to 35.39 percent, as against 46.83 percent in the untreated control. However, mustard leaf tissue (6.27%) had the lowest POSM, followed by broccoli leaf tissue (13.72%), cabbage leaf tissue (17.25%), cauliflower leaf tissue (19.83%), khol leaf tissue (30.49%), and radish leaf tissue (35.39%).

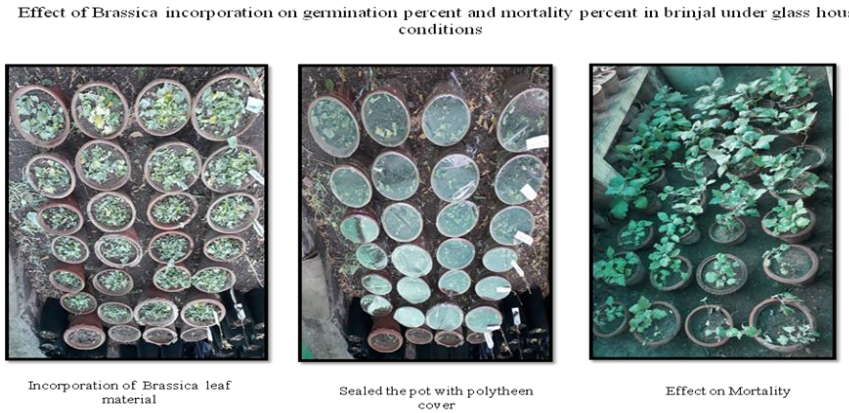


Plate 2.

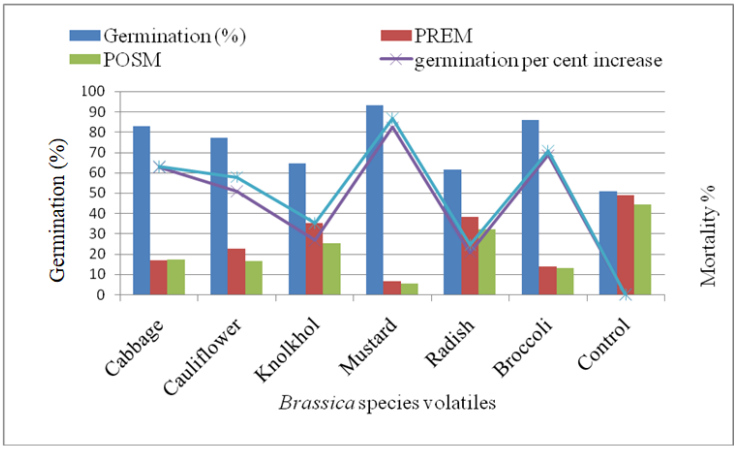


Fig. 2. *In vitro* efficacy of *Brassica* residues on *Fusarium* wilt of eggplant under glass house conditions (Pot culture).

Table 2: Efficacy of *Brassica* residues on *Fusarium* wilt of eggplant under glass house conditions (Pot culture).

Tr. No.	Treatment	Germination (%)	Percent increase over control	Pre emergence Seedling Mortality (PREM)	Post emergence Seedling Mortality (POSM)	Mean	Percent reduction over control		
							PREM	POSM	Mean
T_1	Cabbage	83.00 (65.64)*	62.74	17.00 (24.71)	17.50 (24.83)	17.25	65.30	60.89	63.06
T_2	Cauliflower	77.00 (61.40)	50.98	23.00 (24.07)	16.66 (22.69)	19.83	53.06	62.68	57.87
T_3	Knolkhol	64.66 (53.70)	26.79	35.33 (30.40)	25.63 (30.13)	30.49	27.89	42.60	35.24
T_4	Mustard	93.00 (76.15)	82.35	7.00 (13.56)	5.53 (12.30)	6.27	85.71	87.61	86.66
T_5	Radish	61.66 (51.73)	20.91	38.33 (34.69)	32.44 (34.74)	35.39	21.76	27.36	24.56
T_6	Broccoli	86.00 (68.08)	68.62	14.00 (21.48)	13.44 (20.36)	13.72	71.42	69.89	70.66
T_7	Control	51.00 (45.55)	0.00	49.00 (41.89)	44.66 (40.16)	46.83	0.00	0.00	0.00
	SE(m)+	**2.68**	-	**1.30**	**0.71**	-	-	-	-
	C.D.(P=0.01)	**8.23**	-	**3.99**	**2.2**	-	-	-	-

Reduction in mortality. All the treatments were found to reduce both pre-emergence seed rot and post-emergence seedling mortality over the untreated control (Table 2, Fig. 2 and Plate 2). The reductions in both PREM and POSM ranged from 21.76 to 85.71 percent and 27.36 to 87.61 percent, respectively. However, the highest reductions in PREM and POSM were recorded with mustard leaf tissue incorporation at 85.71 and

87.61 percent, respectively, with a mean of 86.66 percent, followed by broccoli leaf tissue at 71.42 and 69.89 percent, cabbage leaf tissue at 65.30 and 60.89 percent, cauliflower leaf tissue at 53.06 and 62.68 percent, Knol Khol leaf tissue at 27.89 and 42.60 percent, and finally radish leaf tissue at 21.76 and 27.36 percent, respectively. Similar trends in percent reduction over control were observed with 70.66, 63.06, 57.87, 35.24, and 24.56 percent, respectively. Our findings were in agreement with previous studies (Hassan *et al.* 2016; Bharat and Jitender 2015; Fayzalla *et al.*, 2009).

Comparison of Sinigrin (Glucosinolate) concentrations in *Brassica* species

Comparison of Sinigrin concentration among leaf tissues. The sinigrin concentration in the leaf tissues of *Brassica* species showed different quantities among the six species tested (Table 3, Fig. 3). The highest sinigrin concentration was recorded in mustard leaf tissue (512.00 mg/100 g dry weight). while the next best was broccoli leaf tissue (36.40 mg), followed by cabbage leaf tissue (28.40 mg), cauliflower leaf tissue (17.20 mg), and Knol khol tissue (2.60 mg). However, the lowest sinigrin concentration, i.e., less than 0.1 mg, was recorded in radish leaf tissue.

Table 3: Sinigrin concentration in different tissues of *Brassica* species.

Tr. No.	Treatments	Sinigrin concentration (mg/100 gm Dry weight)	
		Leaf	Root
T_1	Cabbage	28.40	<0.1
T_2	Cauliflower	17.20	<0.1
T_3	Knolkhol	2.60	<0.1
T_4	Mustard	512.00	38.20
T_5	Radish	<0.1	<0.1
T_6	Broccoli	36.40	2.80
T_7	Control	0.00	0.00

Comparison of Sinigrin concentration among root tissues: The sinigrin concentration in the root tissues of *Brassica* species showed different amounts among the six *Brassica* species tested (Table 3 and Fig. 3). The highest sinigrin concentration was recorded with treated mustard root tissue (38.20 mg/100 g dry weight). The next was broccoli root tissue (2.80 mg). However, the remaining treatments, *viz*., cabbage root tissue, cauliflower root tissue, Knol khol tissue, and radish root tissue, showed less than 0.1 mg of sinigrin concentration.

The results were in agreement with Bhandari *et al.* (2015), who determined the profiles and concentrations of glucosinolate (Sinigrin) in different tissues across nine species of *Brassica*, including cauliflower, cabbage, broccoli, radish, baemuchae, pakchoi, Chinese cabbage, leaf mustard, and kale. In most crops, seeds had the highest total concentrations of glucosinolate, while shoots had the lowest. The compositions and concentrations of individual glucosinolates were recorded to differ among the crops, tissues, and stages of development. Of the nine crops tested, broccoli had the highest total concentration of glucosinolate in seeds (110.76 µmol/g) and sprouts (162.19 µmol/g1), while mustard had the highest total concentration of glucosinolate in shoots (61.76 µmol/g) and roots (73.61 µmol/g). However, among all tissues tested, the radish had the lowest glucosinolate concentrations.

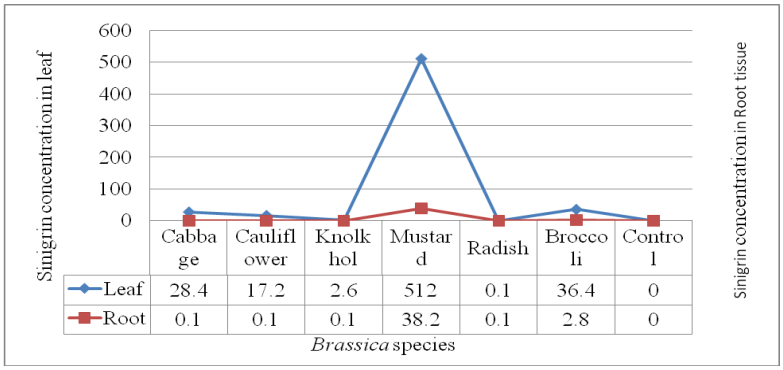


Fig. 3. Sinigrin concentration in different tissues of *Brassica* species.

Effect of biocidal volatile compounds on population of *Fusarium oxysporum* f.sp. *melongenae* under greenhouse conditions (pot culture). The populations of *Fusarium oxysporum* f.sp. *melongenae* was significantly reduced by the incorporation of *Brassica* tissues in soil at the rate of 100 gm per pot (Table 4, Fig. 4).

Among the six *Brassica* species tested, incorporation with Mustard tissue recorded lowest population (3.77 × 10^6). Where, the next was observed with (T6) Broccoli tissue (6.56×10^6) followed by Cabbage tissue (7.73×10^6), Cauliflower tissue (10.33×10^6) and Knol khol tissue (12.66×10^6). However, the highest population was observed with treatment with Radish tissue (12.83×10^6).

These findings concurred with those of the earlier researchers' studies (Villapudua and Munnecke 1988; Mawar and Lodha 2002; Sintayehu *et al.*, 2011; Bharat

and Jitender 2015; Gilardi *et al.*, 2016; Prasad and Kumar, 2017). Prasad and Kumar 2017). All the treatments has shown the significant effect over wilt incidence and other attributes due to the fungistatic effects of volatile compounds released from brassica root, shoot and seed meal tissues on the mycelial growth of *Fusarium.* The root and shoot tissues of *Brassica* species were more effective at flowering than at maturity. The degree of fungal suppression by the different tissues of *Brassica* was linked to the concentration and form of isothiocyanates produced, which varied with the species of Brassica, tissue age and type of tissue (Kirkegaard *et al.*,1996; Insam and Seewald 2010).

Table 4: Effect of biofumigation on population of *F. oxysporum* .sp. *melongenae* at 100 DAS in pot culture.

Population of FOM at 100 DAS as colony forming units(x $10^6 g^-$ of soil)				
Treatments		**Before Brassica incorporation (A)**	**After Brassica incorporation (B)**	**Difference(A-B)**
T_1	Cabbage	13.50	7.73 (2.94)	5.77
T_2	Cauliflower	13.50	10.33 (3.36)	3.17
T_3	Knolkhol	13.50	12.66 (3.69)	0.84
T_4	Mustard	13.50	3.77 (2.16)	9.73
T_5	Radish	13.50	12.83 (3.71)	0.67
T_6	Broccoli	13.50	6.56 (2.74)	6.94
T_7	Control	13.50	17.33 (4.28)	-3.83
SE(m) ±			**0.69**	
C.D.(P=0.01)			**2.13**	

Bioassays done by many researches revealed that Phenyl isothiocyanate could volatilize and were found to be most fungistatic. Likewise, propenyl, benzyl, and ethyl isothiocyanates that were inhibit mycelial growth, suppress conidial and chlamydospore germination of pathogen. *F. oxysporum* isolates may be suppressed by biofumigation with *Brassica* species containing glucosinolates that release high levels of propenyl isothiocyanate (Smolinska *et al.* 2003; Schroeder and MacGuidwin 2010).

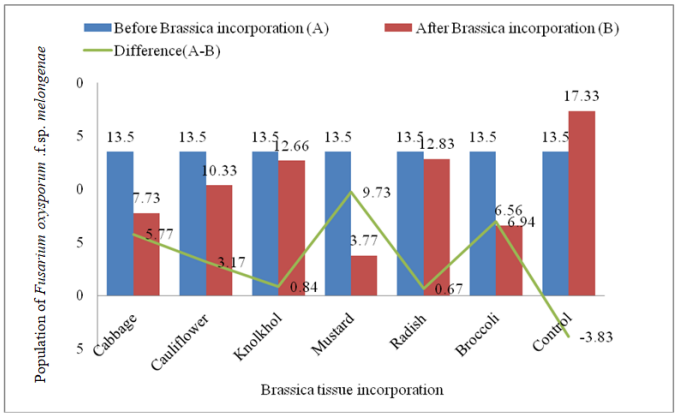


Fig. 4. Effect of biofumigation on population of *Fusarium oxysporum melongenae* under glass house conditions (Pot culture).

CONCLUSIONS

Soil borne plant pathogens are among the major factors limiting the productivity of agro-ecosystems. They are often difficult to control with conventional strategies such as the use of resistant host cultivars and synthetic fungicides. The lack of reliable chemicals, the occurrence of fungicide resistance in pathogens and associated environmental risks, and the breakdown or circumvention of host resistance by pathogen populations are some of the reasons underlying efforts to develop new disease control measures. Over the last decade, the attention for bioactive natural molecules is strongly increased because public opinion considers them as a mild, safe and reliable option to prevent or to fight not only several diseases in humans, but even different plant pathogens, thus limiting the use of synthetic pesticides in agriculture. In the present study among the six *Brassica* spp. tested, Biocidal volatiles released by Mustard leaf tissue exhibited maximum mycelial growth inhibition (90.15% and 77.65%) followed by Broccoli leaf tissue (65.50% and 73.86%) at 5 DAI and 7 DAI respectively. Similarly, *in vitro* population of test pathogen was inhibited (3.77×10^6 to 12.83×10^6) because of Sinigrin concentrations (512.00 mg and 38.20 mg in leaf and root tissue of mustard) present.

FUTURE SCOPE

Biofumigant properties of various plants especially of Brassicaceae family have been heavily investigated against various soil borne pathogens, weeds etc, but practical applications under field conditions are currently limited. However, some members of the Brassicaceae family are also susceptible to few soil borne pathogens like *R. solani.* The effectiveness of a wide variety of biofumigant crops from other families is unknown; at the same time, agronomic factors (such as

seed rate, time of sowing, and optimal incorporation time with or without solarization), environmental factors (soil temperature and moisture), and soil or growing medium factors (soil type, organic matter, and fertilizer contents) determine the efficacy of biofumigant crops and need testing and validation against various soil borne pathogens, which opens wide avenues of opportunities in the field of innovative research.

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Conflict of Interests. None.

REFERENCES

Bhandari, S. R., Jo, J. S. and Lee, J. G. (2015). Comparison of glucosinolate profiles in different tissues of nine Brassica crops. *Molecules*, *20*(9), 15827-15841.

Bharat, N. K., and Jitender, S. (2015). Management of Fusarium wilt of tomato under protected conditions using Brassica crop residues, AM fungi and biocontrol agents. *Indian Phytopathology*, *68*(3), 287-292.

Clarke, D. B. (2010). Glucosinolates, structures and analysis in food. *Analytical Methods*, *2*, 310–325.

Dangi, S. R., Tirado-Corbalá, R., Gerik, J. and Hanson, B. D. (2017). Effect of long-term continuous fumigation on soil microbial communities. *Agronomy*, *7*(2), 37.

Dennis, K. L. and Webster, J. (1971). Antagonistic properties of species group of *Trichoderma* and hyphal interaction. Trans. *British Mycol. Soc*., *57*, 363-396.

Effmert, U., Kalderás, J., Warnke, R. and Piechulla, B. (2012). Volatile mediated interactions between bacteria and fungi in the soil. *Journal of chemical ecology*, *38*, 665-703.

Fayzalla, E. A., El-Barougy, E., and El-Rayes, M. M. (2009). Control of soil-borne pathogenic fungi of soybean by biofumigation with mustard seed meal. *Journal of Applied Sciences*, *9*(12), 2272-2279.

Gilardi, G., Pugliese, M., Gullino, M. L. and Garibaldi, A. (2016). Effect of different organic amendments on lettuce fusarium wilt and on selected soilborne microorganisms. *Plant Pathology*, *65*(5), 704-712.

Halkier, B. A. and Gershenzon. J. (2006). Biology and Biochemistry of Glucosinolates. *Annu Rev Plant Bio.*, *57*, 303-333.

Insam, H. and Seewald, M. S. (2010). Volatile organic compounds (VOCs) in soils. *Biology and fertility of soils*, *46*, 199-213.

Kirkegaard, J. A., Wong, P. T. W. and Desmarchelier, J. M. (1996). In vitro suppression of fungal root pathogens of cereals by Brassica tissues. *Plant pathology*, *45*(3), 593-603.

Kruger, D. H. M., Fourie, J. C. and Malan, A. P. (2013). Cover crops with biofumigation properties for the suppression of plant-parasitic nematodes: a review. *South African Journal of Enology and Viticulture*, *34*(2), 287-295.

Mawar, R. and Lodha, S. (2002). Brassica Amendments and Summer Irrigation for the Control of *Macrophomina phaseolina* and *Fusarium oxysporum* f.sp. *Cumini* in Hot Arid Region. *Phytopathologia Mediterranea*, *41*(1), 231-234.

Mayee, C. D. and Datar, V. V. (1986). Phytopathometry, Technical Buletien-1 (Special Buletein-3), Marathwada Agric. *Univ. Parbhani*, *India*, *218.*

Mayton, H. S., Olivier, C., Vaughn, S. F. and Loria, R. (1996). Correlation of fungicidal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology*, *86*(3), 267-271.

Mazzola, M., Hewavitharana, S. S. and Strauss, S. L. (2015). Brassica seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation. *Phytopathology*, *105*(4), 460-469.

Prasad, P. and Kumar, J. (2017). Management of Fusarium wilt of chickpea using brassicas as biofumigants. *Legume Research-An International Journal*, *40*(1), 178-182.

Prasad, P., Kumar, J., and Pandey, S. (2015). Biofumigation: Success and prospects in soilborne plant disease management. *International Journal of Applied and Pure Science and Agriculture*, *1*(6), 47-59.

Rahmanpour, S., Backhouse, D., and Nonhebel, H. M. (2009). Induced tolerance of *Sclerotinia sclerotiorum* to isothiocyanates and toxic volatiles from Brassica species. *Plant Pathology*, *58*(3), 479-486.

Relevante, C. A. and Cumagun, C. J. R. (2013). Control of Fusarium wilt in bittergourd and bottlegourd by biofumigation using mustard var. Monteverde. *Archives of Phytopathology and Plant Protection*, *46*(6), 747-753.

Sarwar, M. and Kirkegaard, J. A. (1998). Biofumigation potential of brassicas: II. Effect of environment and ontogeny on glucosinolate production and implications for screening. *Plant and Soil*, 91-101.

Schroeder, N. E. and MacGuidwin, A. E. (2010). Mortality and behavior in *Heterodera glycines* juveniles following exposure to isothiocyanate compounds. *Journal of Nematology*, *42*(3), 194.

Schulz-Bohm, K. Martín-Sánchez, L. and Garbeva, P. (2017). Microbial volatiles: small molecules with an important role in intra-and inter-kingdom interactions. *Frontiers in microbiology*, *8*, 2484.

Sintayehu, A., Sakhuja, P. K., Fininsa, C. and Ahmed, S. (2011). Management of fusarium basal rot (Fusarium oxysporum f.sp. cepae) on shallot through fungicidal bulb treatment. *Crop Protection*, *30*(5), 560-565.

Smolinska, U., Morra, M. J., Knudsen, G. R. and James, R. L. (2003). Isothiocyanates produced by Brassicaceae species as inhibitors of Fusarium oxysporum. *Plant disease*, *87*(4), 407-412.

Villapudua, R. J. and Munnecke, D. E. (1988). Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f.sp. *conglutinans* and other organisms. *Phytopathology*, *78*, 289-295.

Vivaldo, G., Masi, E., Taiti, C., Caldarelli, G. and Mancuso, S. (2017). The network of plants volatile organic compounds. *Scientific Reports*, *7*, 1–18.

Watson, T. T., Nelson, L. M., Neilsen, D., Neilsen, G. H. and Forge, T. A. (2017). Soil amendments influence Pratylenchus penetrans populations, beneficial rhizosphere microorganisms, and growth of newly planted sweet cherry. *Applied Soil Ecology*, *117*, 212-220.

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