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Effect of Biofumigation on *Fusarium* wilt of Eggplant caused by *Fusarium oxysporum f. sp. melongenae*

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Authors' contributions

This work was carried out in collaboration among all authors. Author VGR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DND, KDN and KTA managed the analyses of the study. Author CVA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Eggplant is the most traditional vegetable crop in India and susceptible to a number of diseases, among which *Fusarium* wilt caused by *Fusarium oxysporum* f.sp. *melongenae* (FOM) that reduce yield and quality. The present study on effect of biofumigation on *Fusarium* wilt of eggplant caused by *Fusarium oxysporum* f.sp*. melongenae* under natural field conditions showed that the biocidal volatiles released by Brassica tissue incorporation decreased the wilt incidence greatly from 14.57(Radish) to 50.88(Mustard) per cent reduction over control and significantly enhanced the Yield 30.43 (Radish) to 51.95 (Mustard) per cent increase over control and also enhanced the yield parameters *viz*., leaf area (10.15 to 35.87), plant height(13.01 to 26.39), root length(23.10 to 49.29) per cent increase over control respective.

Keywords: Fusarium wilt; eggplant; Fusarium oxysporum; biofumigation.

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1. INTRODUCTION

Interest in non-chemical approaches for managing soil borne diseases has been rekindled with the recent concept of sustainable agriculture. 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 [1]. 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 [2]. 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 [3]. The adverse effects of crucifer residues on soil borne diseases are reported to be positively correlated with the amount of GSL in the crop [4].

Biofumigation may be one promising management method. Biofumigation refer to the suppression of soil borne pests and pathogens by biocidal compounds released in soil when glucosinolates, thioglucoside compounds in brassica crops, are hydrolyzed [5]. *Fusarium* spp. are reported to be sensitive to glucosinolate breakdown products [4-8]. Results from field studies, however, have been inconsistent on population reduction and disease management. Inconsistencies in reducing microbial populations with brassica amendments may be attributed to the use of different brassica species, physical environments and target organisms [9], soil temperature [10] and amount of crop residue incorporated [11]. Smolinska [7] reported significant reductions of numbers of chlamydospores with Brassica juncea cv. Malopolska and variable reduction with *B. napus* cv. Bolko. Takehara et al. [12] reported significant reductions indensities of *F. oxysporum* f. sp. *spinacia* and *F. oxysporum* f. sp. *lycopersici* in soils amended with *B. juncea*. Amending soil with broccoli (*B. oleracea*) did not significantly reduce inocula of *F. oxysporum* f.sp. *asparagi* and *Fusarium* spp. [9,11].

The potential of using brassica crops for managing soil borne pathogens has been evaluated in the laboratory [4,6,13] and in the field [7,9] to assess fungistatic or fungicidal effects of glucosinolate breakdown products, i.e., isothiocyanates, on different fungi. In vitro tests are important, but field studies such as that by Gardiner et al., [14] suggest that the concentration of isothiocyanates in soil after brassica incorporation might be too low for effective pathogen control. Additional field studies are needed to relate isothiocyanate concentration in soil to population reductions in naturally infested soil and to disease management. Soil disinfestation with biofumigation may be improved by increasing the time of isothiocyanates are present in soils. Isothiocyanates are volatile [6,14] and preventing their escape from soil by laying polyethylene mulch, immediately after brassica incorporation, could possibly enhance pathogen management. The objective of the present study was to evaluate the activity of some cruciferous plants against fusarial wilt of egg plants under natural epiphytotics condition.

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2. MATERIALS AND METHODS

The experiment has conducted in wilt prone area for two consecutive years during *kharif* 2018-19 and *kharif* 2019-20 for the management of *Fusarium* wilt of eggplant under natural field conditions at the Horticulture Research Scheme (Vegetable), VNMKV,Parbhani. The soils of the farm are deep black in nature with good moisture retention capacity. Plot size of 2.40 x 3.15 m^2 was prepared following randomized block design with three replications.The fresh mass of selected brassica species collected from Horticulture Research Scheme (Vegetable) brought in to the plot and local variety of mustard grown in situ was incorporated for biofumigation treatments covering six treatments and control plot was kept untreated. The field was well prepared and irrigated until the saturation of soil before the incoporation. The test crop brinjal was transplanted after 30 days of biofumigation and managed well throughout the period of experiment by following recommended agronomic practices. The experiment has designed with Randomized Block Design (R B D) and employed the test Cultivar Arka shirish cultivated following the spacing 60 x 45 cm^2 .Treatment details are T1 to T6 with incorporation of Cabbage, Cauliflower, Knol khol, Mustard, Radish, and Broccoli respectively.

Incorporation of brassica species: During *Kharif* 2018 and *Kharif* 2019, the fresh mass of brassica species were collected from Horticulture Research Scheme (Vegetable) brought in to the plot and local variety of mustard grown in situ was incorporated for biofumigation treatments covering six treatments and control plot was kept untreated. Selected brassica species plant parts at 50 % flowering stage were distributed across the plots uniformly and chopped in to small pieces along with leaves, stem, flowers and roots. In line with biofumigation recommendations by Reddy [15], the crushed residues were immediately incorporated into the soil, with atractor operated rotary tiller, to a depth of about 15 cm. GSL hydrolysis was favored by irrigating all plots before the incorporation. The quantity of freshly harvested brassica residues incorporated into the soil was adjusted equivalent to 4–8 t of fresh weight per hectare. The bio mass of brassica species were incorporated @ 10 kg per plot for all biofumigant treatments. The plots were covered with black polythene sheats for 30 days to conserve the volatiles released during brassica tissue degradation. The control plots were left untreated. Later the test crop brinjal (*Fusarium* wilt susceptible eggplant cultivar Arka Shirish) was transplanted in the pulverized soil after 30 days of biofumigation and managed well throughout the period of experiment by following recommended agronomic practices.

3. RESULTS AND DISCUSSION

In the present investigations on the effect of biocidal volatiles released by *Brassica* tissue soil incorporation on the *Fusarium* wilt incidence of eggplant and yield showed the prominent effect during *Kharif* 2018-19 and *Kharif* 2019-20 under natural filed conditions.

3.1 Effect of Biofumigation on *Fusarium* wilt Incidence under Natural Field Conditions during *Kharif* 2018-19

Effect on wilt incidence: All the treatments were highly significant in reducing the disease incidence of *Fusarium* wilt of eggplant during *kharif* 2018-19 and the results are presented in Table I .Soil incorporation of Mustard tissue resulted the lowest wilt incidence (25.08%) followed by Broccoli tissue incorporation (31.63%) with an admirable disease reduction per cent over control (47.66 and 33.99 %) respectively. However, the next best performance was recorded with treatments of Cabbage tissue (32.00 %), Cauliflower tissue (35.17%), Knol khol tissue (38.63%) and Radish tissue (39.03%) respectively which were considerably better in disease reduction of 33.22, 26.60, 19.38 and 18.55 per cent respectively when compared with control.

Effect on yield: In perusal of data (Table I) all the treatments were highly significant in increasing the yield of eggplant during *kharif* 2018-19. Soil incorporation of Mustard tissue recorded the highest yield (196.00 q/ha) followed by Broccoli tissue (186.60 q/ha) with an appreciable per cent increase over control (107.85 % and 97.88%) respectively. However, the next impressive performance was recorded with treatments of Cabbage tissue incorporation (156.50q/ha), Cauliflower tissue incorporation (146.60 q/ha, Knol khol tissue incorporation (146.70) and Radish tissue incorporation (135.60). which were considerably better in yield increase (65.96, 55.46, 55.57, and 43.80 %) respectively when compared with control.

Effect on leaf area: All the treatments of data scrutinized in Table I was highly significant to increase the leaf area of eggplant during *kharif* 2018-19. Soil incorporation of Mustard tissue recorded the highest leaf area (55.25 cm^2) followed by Broccoli tissue (53.27 cm^2) with an appreciable percent increase over control (46.28 and 41.04 %) respectively. However, the next effective leaf area performance was recorded with treatments Cabbage tissue (51.18 cm^2), Cauliflower tissue (45.78 cm^2), Knol khol tissue (45.15cm^2) and Radish tissue (43.54 cm^2) respectively which were considerably better per cent increase in leaf area (35.50, 21.21, 19.54 and 15.28 %) respectively when compared with control.

Effect on plant height: In the review of data (Table I) all the treatments were highly significant in increasing the plant height of eggplant during *kharif* 2018-19. Soil incorporation of Mustard tissue recorded the highest plant height (121.33 cm) followed by Broccoli tissue (114.66 cm) with an appreciable per cent increase over control (32.37 and 25.09 %) respectively. However, the next awesome plant height performance was recorded with treatments Cabbage tissue incorporation (112.00 cm), Cauliflower tissue (110.66 cm), Knol khol tissue (106.33cm) and Radish tissue (101.33cm). Which were considerably better per cent in leaf area (22.19, 20.73, 16.00 and 10.55 %) respectively when compared with control .

Table I. Effect of biofumigation on *Fusarium* wilt incidence under natural field conditions during *Kharif* 2018-19

Tr. No.	Treatments	Wilt incidence (%)	Per cent reduction over control	Yield (q/ha)	Per cent increase over control	Leaf area (cm)	Per cent increase over control	Plant height (cm)	Per cent increase over control	Root length (cm)	Per cent increase over control
T_1	Cabbage	32.00 (34.43)	33.22	156.50	65.96	51.18	35.50	112.00	22.19	24.88	46.35
T_2	Cauliflower	35.17 (34.20)	26.6	146.60	55.46	45.78	21.21	110.66	20.73	23.29	37.00
T_3	Knolkhol	38.63 (38.41)	19.38	146.70	55.57	45.15	19.54	106.33	16.00	22.92	34.82
T_4	Mustard	25.08 (30.03)	47.66	196.00	107.85	55.25	46.28	121.33	32.37	30.11	77.12
T_5	Radish	39.03 (38.64)	18.55	135.60	43.80	43.54	15.28	101.33	10.55	22.71	33.59
T_6	Broccoli	31.63 (36.35)	33.99	186.60	97.88	53.27	41.04	114.66	25.09	27.78	63.41
T_7	Control	47.92 (43.78)	0.00	94.30	0	37.77	0	91.66	0	17.00	0
	SE(m)±	1.01		1.84		1.28		1.81		1.28	
	C.D(P=0.05)	3.16		5.74		4.01		5.64		4.01	

Table II. Effect of biofumigations on *Fusarium* wilt incidence under natural field conditions during *Kharif* 2019-20

Tr. No.	Treatments	Wilt incidence (%)	Per cent reduction over control	Yield (q/ha)	Per cent increase over control	Leaf area (cm)	Per cent increase over control	Plant height (cm)	Per cent increase over control	Root length (cm)	Per cent increase over control
T_1	Cabbage	29.81 (33.07)	30.72	167.48	82.98	46.31	43.64	109.62	27.11	22.85	73.50
T_2	Cauliflower	32.59 (34.79)	24.26	148.31	62.03	42.09	30.55	108.76	26.11	20.86	58.39
T_3	Knolkhol	35.73 (36.68)	16.96	135.63	48.18	39.64	22.95	101.33	17.50	18.74	42.29
T_4	Mustard	19.59 (26.25)	54.47	190.77	108.42	53.93	67.28	120.39	39.60	29.37	123.01
T_5	Radish	38.66 (38.29)	10.15	130.37	42.43	33.59	4.19	99.76	15.68	16.29	23.69
T_6	Broccoli	25.51 (30.32)	40.71	180.50	97.20	48.05	49.04	113.47	31.57	26.72	102.89
T_7	Control	43.03 (40.97)	0.00	91.53	0	32.24	0	86.24	0	13.17	0
	SE(m)±	0.59		4.37		1.60		1.15		1.17	
	CD(P=0.05)	1.86		13.63		4.99		3.60		3.64	

Table III. Effect of biofumigations on *Fusarium* wilt incidence under natural field conditions during *Kharif* 2018-19 and 2019-20(Pooled mean)

Treatments		Wilt incidence (%)			Per cent reduction over control	Yield (q/ha)			Per cent increase over control	Leaf area (cm^2)	Per cent increase over control	Plant height (cm)	Per cent increase over control	Root length (cm)	Per cent increase over control
		2018-19	2019-20	Mean		2018-19	2019-20	Mean		Pooled mean		Pooled mean		Pooled mean	
T_1	Cabbage	32.00 (34.43)	29.81 (33.07)	30.90	32.44 (31.74)	156.50	167.48	161.99	74.34	48.745	39.25	110.81	24.58	23.87	58.29
T_2	Cauliflower	35.17 (34.20)	32.59 (34.79)	33.88	25.49 (32.40)	146.60	148.31	147.46	58.70	43.935	25.51	109.71	23.34	22.08	46.42
T_3	Knolkhol	38.63 (38.41)	35.73 (36.68)	37.18	18.24 (24.87)	146.70	135.63	141.17	51.93	42.39	21.10	103.83	16.73	20.83	38.13
T_4	Mustard	25.08 (30.03)	19.59 (26.25)	22.33	50.88 (45.21)	196.00	190.77	193.39	108.14	54.59	55.95	120.86	35.87	29.74	97.21
T_5	Radish	39.03 (38.64)	38.66 (38.29)	38.84	14.57 (21.18)	135.60	130.37	132.99	43.13	38.56	10.16	100.53	13.02	19.51	29.38
T_6	Broccoli	31.63 (36.35)	25.51 (30.32)	28.57	36.76 (37.01)	186.60	180.50	183.55	97.55	50.66	44.72	114.07	28.24	27.25	80.70
T_7	Control	47.92 (43.78)	43.03 (40.97)	45.47	0.00 (0.00)	94.30	91.53	92.915	0	35.005	0	88.95	0	15.08	0
	SE(m)+	1.01	0.59	1.09	1.48	1.84	4.37	2.68	0.65	0.86	1.24	0.86	0.58	0.92	2.27
	CD(P=0.05)	3.16	1.86	3.41	4.63	5.74	13.63	8.36	2.04	2.68	3.89	2.68	1.80	2.87	7.09

Effect on root length: All the treatments Scrutinized of data in Table I has highly significant in increasing the root length of eggplant during *kharif* 2018-19. Soil incorporation of Mustard tissue recorded the highest root length (30.11cm) followed by (T6) Broccoli tissue (27.78 cm) with an appreciable per cent increase over control (77.12 and 63.41 %) respectively. However, the further promising performance observed in root length was recorded with treatments Cabbage tissue incorporation (24.88cm), Cauliflower tissue (23.29 cm), Knol khol tissue (22.92cm) and Radish tissue (22.71cm). which were considerably better in per cent increase of root length (46.35 , 37.00 , 34.82 and 33.59 %) respectively when compared with control .

3.2 Effect of Biofumigation on *Fusarium* Wilt Incidence under Natural Field Conditions during *Kharif* 2019-20

Effect on wilt incidence: All the treatments were highly significant in reducing the disease incidence of *Fusarium* wilt of eggplant during *kharif* 2019-20 also and the results are presented in Table II. Soil incorporation of Mustard tissue recorded the lowest wilt incidence (19.59 %) followed by Broccoli tissue (25.51%) with a noticeable disease reduction per cent over control (54.47 and 40.71 %) respectively. However, the further better performance was recorded with treatments Cabbage tissue incorporation (29.81%), Cauliflower tissue incorporation (32.59%), Knol khol tissue incorporation (35.73%) and Radish tissue incorporation (38.66%).Which were performed considerably better in disease reduction (30.72,24.26,16.96 and 10.15%) respectively when compared with control.

Effect on yield: In perusal of data in Table II all the treatments were highly significant in increasing the yield of eggplant during *kharif* 2019-20. Soil incorporation of Mustard tissue recorded the highest yield (190.77 q/ha) followed by Broccoli tissue (180.50 q/ha) with an appreciable per cent increase over control (108.42 and 97.20 %) respectively. However, the next impressive performance was recorded with treatments Cabbage tissue (167.48q/ha), Cauliflower tissue (148.31 q/ha, Knol khol tissue (135.63 q/ha) and Radish tissue (130.37 q/ha) respectively which were delivered considerably better yield increase (82.98, 62.03,48.18 and 42.43 %) respectively when compared with control .

Effect on leaf area: Scrutiny of data (Table II) all the treatments were highly significant in increasing the leaf area of eggplant during *kharif* 2019-20. Soil incorporation of Mustard tissue recorded the highest leaf area (53.93 cm^2) followed by Broccoli tissue (48.05 cm^2) with an appreciable per cent increase over control (67.28 and 49.04 %) respectively. However, the next noticeable performance was recorded with treatments (T1) Cabbage tissue incorporation (46.31 cm^2), Cauliflower tissue incorporation(42.09 cm^2), Knol khol tissue incorporation (39.64 cm^2) and Radish tissue incorporation(33.59 cm^2). Which were noted relatively better per cent increase in leaf area (43.64,30.55,22.95 and 4.19 %) respectively when compared with control .

Effect on plant height: In the review of data in Table II all the treatments were highly significant in increasing the plant height of eggplant during *kharif* 2019-20. Soil incorporation of Mustard tissue recorded the highest plant height (120.39 cm) followed by Broccoli tissue (113.47 cm) with an appreciable percent increase over control (39.60 and 31.57 %) respectively. However, the next superior performance in plant height was recorded with treatments Cabbage tissue (109.62 cm), Cauliflower tissue (108.76 cm), Knol khol tissue (101.33cm) and Radish tissue (99.76cm). Which were considerably better in per cent increase over control (27.11,26.11,17.50 and 15.68%).

Effect on root length: Investigation of data in Table II all the treatments were highly significant in increasing the root length of eggplant during *kharif* 2019-20. Soil incorporation of Mustard tissue recorded the highest root length (29.37cm) followed by Broccoli tissue (26.72 cm) with an appreciable per cent increase over control (123.01 and 102.89 %) respectively. However, the next impressive performance in root length was recorded with Cabbage tissue incorporation (22.85cm), Cauliflower tissue (20.86 cm), Knol khol tissue (18.74cm) and Radish tissue (16.29cm). Which were comparatively better per cent increase in leaf area (73.50,58.39,42.29 and 23.69 %) respectively when compared with control .

3.3 Effect of biofumigations on *Fusarium* wilt Incidence Under Natural Field Conditions (Pooled)

The cumulative results in Table III revealed that all the treatments significantly influenced the

incidence of *Fusarium* wilt and other attributes of eggplant during *kharif* 2018-19 and 2019-20.

Effect on *Fusarium* wilt incidence: The pooled mean per cent *Fusarium* wilt incidence recorded with all the treatments were ranged from 22.33 to 38.84 per cent, as against 45.47 per cent in untreated control. However, the treatment found most effective with significant least incidence was Mustard tissue (22.33 %) followed by Broccoli tissue (28.57%) with a magnificent mean reduction over control (50.88 and 36.76%). However, the seriale results were observed with Cabbage tissue (30.90%) followed by Cauliflower tissue (33.88%), Knol khol tissue (37.18%) and lastly Radish tissue (38.84%) with a corresponding mean per cent reduction over control (32.44, 25.49, 18.24 and 14.57%) respectively.

Effect on yield: The pooled results (Table III) indicated that the eggplant yield (q/ha) was significantly influenced by various imposed treatments to manage *Fusarium* wilt disease. The yield with various treatments was ranged from 132.99 to 193.39 q/ha, as against 92.91 q/ha. Among the treatments, significantly highest yield (193.39 q/ha) was recorded with the treatment Mustard tissue incorporation followed by Broccoli tissue (183.55 q/ha) with an impressive mean per cent increase over control (108.14 and 97.55%) respectively. However, the effective yield improvement sequence was observed with Cabbage tissue (161.99 q/ha) followed by Cauliflower tissue (147.46 q/ha), Knol khol tissue (141.17 q/ha) and leastly Radish tissue (132.99 q/ha) with an amicable per cent increase over control (74.34 ,58.70,51.93 and 43.13 %).

Effect on leaf area: Pooled mean data in Table III on all the treatments were highly significant in increasing the leaf area of eggplant. Soil incorporation of Mustard tissue recorded the highest leaf area (54.59cm^2) followed by Broccoli tissue (50.66 cm^2) with an appreciable per cent increase over control (55.95 and 44.72 %) respectively. However, the next succeeding performance was recorded with treatments Cabbage tissue incorporation (48.74 cm^2) followed by Cauliflower tissue incorporation (43.93 cm^2), Knol khol tissue incorporation (42.39 cm^2) and Radish tissue incorporation (38.56 cm^2),Which were noted relatively better per cent increase in leaf area (39.25,25.51,21.10 and 10.16 %) respectively when compared with control .

Effect on Plant height: Review of pooled mean data in Table III showed that all the treatments were highly significant in increasing the plant height of eggplant during consecutive two years. Soil incorporation of Mustard tissue recorded the highest plant height (120.86 cm) followed by Broccoli tissue (114.07 cm) with an appreciable percent increase over control (35.87% and 28.24 %) respectively. However, the next efficient performance in plant height was recorded with Cabbage tissue (110.81 cm),Cauliflower tissue (109.71 cm), Knol khol tissue (103.83cm) and Radish tissue (100.53cm),Which were considerably better per cent increase over control recorded (24.58,23.34,16.73 and 13.02%).

Effect on root length: Investigation of pooled data in Table III revealed that all the treatments were highly significant in increasing the root length of eggplant. Soil incorporation of Mustard tissue recorded the highest root length (29.74cm) followed by Broccoli tissue (27.25 cm) with an appreciable per cent increase over control (97.21 and 80.70 %) respectively. However, the next superior performance in root length was recorded with treatments Cabbage tissue (23.87cm), Cauliflower tissue (22.08 cm), Knol khol tissue (20.83cm) and Radish tissue (19.51cm) , which were comparatively better per cent increase in leaf area (58.29,46.42,38.13 and 29.38 %) respectively when compared with control.

Present results were in agreement with the previous workers research findings [1,16-30]. 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 [16]. 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 suppress by biofumigation with *Brassica* species containing glucosinolates that release high levels of propenyl isothiocyanate [7].

Many researchers previously described alternative and ecofriendly strategies for the management of *Fusarium oxysorum* by adding *Brassica juncea* which had a similar effect like Dazomet and Metham Sodium in greenhouse [25]. *Brassica* species contains high Glucosinolates (GSL) and enzyme hydrolysis leads to the production for some antimicrobial activity of different sulphur compounds during incorporation thereby reduce the population of soil pathogens [28]. Among several bioactive molecules, myrosinase as a biofumigant, stand out as a promising alternative for the management of a variety of soilborne pathogens [30]. However; *T.harzianum* was less sensitive in terms of glucanase and chitinase gene expression relative to the pathogens studied. More specifically, upregulated expression of chitinase and glucanase genes in various *Trichoderma* isolates may enhance *T.harzianum* biocontrol function [28,30].

4. CONCLUSIONS

The present study was designed to evaluate the effectiveness of Brassica crops for the management of *Fusarium* wilt of eggplant. The pooled mean data from two years (kharif 2018-19 and 2019-20) field studies showed that the biocidal volatiles released by Brassica tissue incorporation decreased the wilt incidence greatly from 14.57(Radish) to 50.88(Mustard) per cent reduction over control and significantly enhanced the Yield 30.43 (Radish) to 51.95 (Mustard) per cent increase over control and also enhanced the yield parameters *viz*., leaf area (10.15 to 35.87), plant height(13.01 to 26.39), root length(23.10 to 49.29) per cent increase over control respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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