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Effect of different varieties of *Raphanus sativus* as bio-fumigants and microbial biocontrol agents for the management of *Pythium aphanidermatum* causing damping off in tomato

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ABSTRACT: The study was conducted to evaluate the effect of root and shoots of different varieties of *Raphanus sativus* as biofumigant alone and in combination with PBAT-3 (*Trichoderma* and *Pseudomonas*) against *Pythium aphanidermatum* causing pre and post emergence damping off of tomato under glasshouse and in the field conditions. Volatiles released from three varieties of *R. sativus viz*,. Japanese white, Pusa Chetki, and MAHY 22 significantly reduced the mycelial growth of *P. aphanidermatum*. Roots of MAHY 22 was found to be most effective as resulted into maximum radial growth inhibition 82.06 per cent followed by Japanese white shoot. Under glasshouse, minimum pre and post emergence damping off was observed in combination of MAHY 22 root +PBAT-3 (11.1%) and Japanese white +PBAT-3 (5.2%) respectively over control. Whereas, in field, minimum pre and post emergence damping off was recorded in MAHY 22 root +PBAT-3 i.e., 15.16 per cent and 4.20 per cent respectively over control. Similarly, the combination of biofumigation and biocontrol agents resulted in higher seedling emergence. The results showed *R. sativus* as biofumigant alone and in combination with PBAT-3 in suppressing the growth of *P. aphanidermatum* both *in vitro* and in vivo conditions.

Key words: Biofumigation, damping off, *Pythium aphanidermatum*, tomato

Tomato is an herbaceous plant which belongs to the family *Solanaceae*. It is considered as the second greatest significant vegetable crops in the world next to potato (Mohamed *et al*., 2010). Damping off caused by *Pythium* in nurseries is a major limitation in tomato production accounts for 62% mortality of seedlings (Ramamoorthy *et al.,* 2002). Almost all the *Pythium* species are known to affect the seedlings and ultimately causes severe damage (Kucharek and Mitchell, 2000). Damping-off occurs in two phases, pre-emergence and post emergence phase. Pre-emergence phase is characterized by death of seedlings just before they reach the soil surface. The young radical and plumule are killed and complete rotting of the seedlings takes place. In post-emergence phase young tissues of the collar region are infected. The infected tissues become soft, water soaked and the seedlings topple down or collapse on the ground (Chaudhary, 2012).

Management of soil-borne diseases have always been a challenge because it is difficult to predict the severity of epidemics from year to year and to target accurately the niches in which the pathogens are found. In the early 90s, methyl bromide because of its broad-spectrum activity and better efficacy has long been considered as one of the premier soil fumigant in agriculture for managing plant parasitic nematode, pathogens, insects and weeds (Yates *et al.,* 1996). Methyl bromide was effectively used in tomato crops and accounts for about 30% of its use in the word. But later methyl bromide was found responsible for the depletion of ozone layer in stratosphere which leads to its worldwide phase-out in 1997 by means of the Montreal Protocol (Stapleton *et al.*, 2000). Soil borne pathogens were effectively controlled by using fungicides, but the misuse of these compounds has led to enormous problems to environment and human health as well as resistant strains of pathogen were developed making the use of these chemicals ineffective. These problems have stimulated the research of alternative measures. In this context, biofumigation with cruciferous plants has emerged as an alternative for chemicals being used as fumigants for managing soil-borne diseases.

Biofumigation refers to the practice of growing and incorporating certain *Brassica spp.* into the soil, leading to the release of isothiocynate compounds (ITCs) through the hydrolysis of Glucosinolate (GSL) compound present in the plant tissues (Kirkegaard *et al*., 1993). Many *Brassica* species produce significant amount of glucosinolates which are contained in plant cells separately from the enzyme myrosinase and are themselves not fungitoxic (Manici *et al.,* 1997). However, upon disruption of plant cell the glucosinolate are hydrolysed by

myrosinase in the presence of water in order to release several hydrolysis compounds including isothiocynate (Van *et al.,* 2009). Isothiocynate have a wide range of biocidal properties and are toxic to a variety of pest and pathogens (Chew, 1987). *R. sativus* has potential to produce higher concentration of glucosinolates mainly 4-methylthio-3-butenyl glucosinolate (MTB-GSL) which are converted to corresponding isothiocynate by enzyme myrosinase. MTB-ITC is known to possess biocidal properties against fungi bacteria and nematodes (Hassan *et al*., 2016). The aim of this study is to evaluate the efficacy of biofumigation with radish shoots and roots for managing Pythium *aphanidermatum* infecting tomato seedlings under glasshouse and field conditions. Due to rising concern for the use of agro chemicals globally biological control provides an alternative to the use of synthetic pesticide with the benefit of reduced environmental impact and greater public acceptance (Reino *et al*., 2008).

MATERIALS AND METHODS

In vitro toxicity of volatiles released from *Raphanus sativus* against *P. aphanidermatum*

The *in vitro* test was carried out in the Biocontrol laboratory, Department of Plant Pathology in order to find out the efficacy of three varieties of radish a sbio-fumigants (*viz.* Japanese white, Pusa Chetki, and MAHY 22), The radish plants were uprooted 60 days after sowing and immediately taken to the laboratory in autoclaved polypropylene bags. After that the plants were cleaned with sterilized distilled water and separated into root and shoot and stored at -20°C till use. The 5 mm disc of fungal mycelial plug was taken from the margin of 7 days old actively growing culture of *Pythium aphanidermatum* and placed in the centre of PDA plate amended with chloramphenicol at 100µg ml^{-1}. Crushed radish shoot and roots were placed (100mg/ plate) onto the upturned lid of the plates with the inverted bottom containing the fungal plug. Sterile distilled water at a rate 1:1 (w /v) was added to the radish material to induce releasing the isothiocyanates (ITCs) and the plate was immediately sealed with parafilm. The plates were incubated at 25±2ÚC for 8 days.The inhibition percentage was calculated by the formula I= C-T /C ×100,where I= percent of growth inhibition, C= radial growth in control and T= radial growth treatment (Prasad *et al.,* 2016).

Treatments for glasshouse and field experiments

T1	:	Shoots of Japanese white
T2	:	Roots of Japanese white
T3	:	Shoots of Pusa Chetki
T4	:	Roots of Pusa Chetki
T5	:	Shoots of MAHY-22
T6	:	Roots of MAHY-22
T7	:	T1 + Seed treatment with Pant Biocontrol Agent-3
T8	:	T2 + Seed treatment with Pant Biocontrol Agent-3
T9	:	T3+ Seed treatment with Pant Biocontrol Agent-3
T10	:	T4+ Seed treatment with Pant Biocontrol Agent-3
T11	:	T5+ Seed treatment with Pant Biocontrol Agent-3
T12	:	T6+ Seed treatment with Pant Biocontrol Agent-3
T13	:	Seed treatment with Pant Biocontrol Agent-3
T14	:	Control

(Pant Biocontrol Agent-3 is a consortium of *Trichoderma asperellium*[PBA1] and *Pseudomonus fluoroscens* [PBA2] developed at Biocontrol Laboratory,Department of Plant

Biofumigation with radish under glasshouse conditions

Each pot with capacity of 10 kg were inoculated with 200g of *Pythium* colonized sorghum grains three days prior to the incorporation of *Raphanus sativus*. Sixty days old plants of three varieties of radish viz; Japanese white, Pusa Chetki and MAHY-22 grown at Vegetable Research Center were uprooted and washed thoroughly with tap water to remove soil adhering to them. The green shoots and roots of each variety of radish were separately chopped into small pieces. These chopped plant parts were mixed into sterilized soil @ 500g pot^{-1} as per treatments. In control there was no incorporation of *R. sativus* in the soil. After filling the pots with desired treatments water was added to the pots in order to hydrolyse the glucosinolate present in the plant parts of *R. sativus.* Thereafter each pot was tightly covered with a transparent polythene sheet for two weeks. After removing polythene sheet, soil was raked. Each treatment was replicated thrice. Tomato seeds were used for experimental purpose. The seeds were bio primed with PBAT-3 @ 10 g per kg seeds 48 hr prior to sowing. PBAT-3 treated FYM was also added to the soil. Two days after removing the polythene sheet from the pots, treated seeds of tomato were sown in each pot @ 15 seeds per pot. Proper moisture was maintained by regular watering in the pots.

Biofumigation with radish in field conditions

Freshly harvested roots and shoots were chopped seperately with the help of shovel into small parts. Chopped shoots and roots of the three different varieties of radish being used as biofumigant were incorporated at a depth of approximately 15 cm in the soil according to the desired treatments. The plots were then watered to hydrolyse the glucosinolate present in the radish tissues. After addition of water each plot was covered with the help of polythene sheet and air tightened. These plots were

kept covered for 2 weeks in order to conserve the volatiles released from the degradation of radish tissues. Observation on seed and seedling mortality were recorded at 15, 30 and 45 days after sowing. Tomato seeds were sown in each plot @1000seeds per plot (100 seeds per row). The seeds were bio-primed with Pant Bio-agent -3 @ 10 g per kg seeds 48 hours prior to sowing for each treatment except control.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

$$\text{Pre emergence mortality (\%)} = \frac{\text{No. of seeds damped off before emergence}}{\text{Total No. of seeds sown}} \times 100$$

$$\text{Post emergence mortality (\%)} = \frac{\text{No. of seeds damped off before emergence}}{\text{Total No. of seeds sown}} \times 100$$

Vigour Index = Seedling Length × Germination per cent

RESULTS AND DISCUSSION

In vitro toxicity of volatiles against *Pythium aphanidermatum*

Results of the experiments revealed that volatiles from all the three varieties of the radish significantly reduce the radial mycelial growth of the *Pythium*. At 100 mg/ plate dose the maximum inhibition of mycelial growth was recorded in MAHY 22 roots (82.06%) followed by Japanese white shoot (78.00%) and MAHY shoots (75.83%). While shoots of Pusa Chetki was found least effective with 40.98% inhibition followed by PusaChetki roots (43.75%). All the treatments are found significant in comparison to control.

Evaluation of different varieties of *R. sativus* as biofumigant for the management of damping off in tomato under glasshouse conditions.

Table1: *In vitro* toxicity of volatiles against *Pythium aphanidermatum*

Treatments	*Pythium aphanidermatum*	
	Radial growth (mm)	Percent Inhibition
Japanese White Shoot	17.33	78.00
Japanese White Root	22.20	72.25
Pusa Chetki shoot	47.31	40.98
Pusa Chetki Root	45.00	43.75
MAHY 22 Shoot	19.34	75.83
MAHY 22 Root	14.35	82.06
Control	80.00	0
CD (0.05)	0.16	-
SEm+	.054	-
CV (%)	2.67	-

The observation on the effects of different treatments on incidence of pre-emergence damping off was recorded at 15 days after sowing was taken. Minimum pre-emergence damping off incidence of tomato under glasshouse condition were found in the treatment MAHY 22 root+PBAT-3 and Japanese white shoot+PBAT-3 (11.11%) followed by MAHY 22shoot +PBAT-3 (13.33%) and maximum incidence of damping off among treatments were recorded in pots amended with Pusa Chetki roots (28.89%) followed by Pusa Chetki shoot amended pots (26.67%) which differ from the control (37.78%).

Effect on post emergence damping-off

The observation on the effect of different treatments on incidence of post emergence damping off at 45 days after sowing was taken. The results presented showed minimum post emergence mortality were found in treatment Japanese white root+PBAT-3 (5.26 %) followed by MAHY Shoot +PBAT-3 (5.38%) and MAHY 22 root +PBAT-3 (5.5%) which differed from control (25%) while maximum post emergence mortality among treatments was reported in Pusa Chetki shoot and roots incorporated pots which was 12.7 per cent and 12.8 per cent respectively.

Effect of biofumigation and its combination with biocontrol agents on incidence of damping off in tomato under field conditions

Effect on pre-emergence damping-off in field conditions

The observation on the effect of different treatments on incidence of pre-emergence damping off was taken 15 DAS. The results presented Table 3 showed that minimum pre-emergence mortality was found in treatment MAHY 22 root +PBAT-3 (15.16%) followed by Japanese white shoot+PBAT-3 (16.60%) and MAHY 22 Shoot +PBAT-3 (17.00%) which were found significant as compared with the control (26.33%).Maximum pre-emergence mortality among treatments were reported in Pusa Chetki root and shoot incorporated plots which was 21.90% and 21.33% respectively.

Effect on post emergence damping-off in field

The data on the influence of different treatments on pre and post emergence damping off revealed that minimum post emergence mortality was observed in MAHY 22 root +PBAT-3 (4.20 %) followed by Japanese white shoot+PBAT-3 (4.63 %) and Japanese white root +PBAT-3 (4.97%) as compare with the control (17.78 %). Maximum post emergence mortality among treatments was reported in Pusa chetki shoot and root incorporated plots which were 10.59 per cent and 10.37 per cent respectively. All the treatments were found significant in comparison

Table 2: Effect of biofumigation and its combination with biocontrol agents on incidence of damping off in tomato under glasshouse conditions

Treatments	Plant Stand (15DAS) No.	Germination (%)	Pre-emergence damping off (%)	Plant stand (30 DAS) No.	Plant stand (45DAS) No.	Post emergence damping off (%)
Japanese White shoot	12	80.00	20.00	11.33	11	8.33
Japanese White root	11.67	77.78	22.22	11	10.6	9.14
PusaChetkishoot	11	73.33	26.67	10.33	9.6	12.7
Pusa Chetki root	10.67	71.11	28.89	10	9.3	12.8
MAHY 22 Shoot	11.67	77.78	22.22	10.66	10.6	9.14
MAHY 22 root	12	80.00	20	11.67	11	8.33
Japanese White shoot+ PBAT-3	13.33	88.89	11.11	13	12.6	5.50
Japanese White root+ PBAT-3	12.66	84.44	15.55	12	12	5.26
Pusa Chetki shoot+ PBAT-3	12.33	82.22	17.78	11.67	11.3	8.37
Pusa Chetki root+ PBAT-3	12.33	82.22	17.77	11.6	11.3	8.37
MAHY 22 shoot+ PBAT-3	13	86.67	13.33	12.67	12.3	5.38
MAHY 22 root +PBAT-3	13.33	88.89	11.11	13	12.6	5.5
PBAT-3	12	80.00	20	11.33	11	8.33
Control	9.33	62.22	37.78	8.00	7	25
CD (.05)	1.26	-	-	1.31	1.43	-
CV	6.32	-	-	6.9	7.8	

Table 3:Effect of biofumigation and its combination with biocontrol agents on incidence of damping off in tomato under field conditions

Treatments	Plant Stand (15DAS) No.	Germination (%)	Pre-emergence damping off (%)	Plant stand (30 DAS) No.	Plant stand (45DAS) No.	Post emergence damping off (%)
Japanese White shoot	801.67	80.16	19.83	766.00	737.00	8.06
Japanese White root	795.00	79.50	20.50	753.00	720.67	9.35
Pusa Chetki shoot	786.66	78.66	21.33	747.33	703.33	10.59
Pusa Chetki root	781.00	78.10	21.90	734.67	700.00	10.37
MAHY 22 Shoot	798.33	79.83	20.16	755.67	729.00	8.68
MAHY 22 root	807.33	80.73	19.26	770.66	752.00	6.85
Japanese White shoot+ PBAT-3	834.00	83.40	16.60	815.33	795.33	4.63
Japanese White root+ PBAT-3	824.33	82.43	17.56	804.67	783.33	4.97
Pusa Chetki shoot+ PBAT-3	817.67	81.76	18.23	783.00	769.67	5.87
Pusa Chetki root+ PBAT-3	810.66	81.06	18.93	780.33	760.33	6.20
MAHY 22 shoot+ PBAT-3	830.00	83.00	17.00	804.00	786.33	5.26
MAHY 22 root +PBAT-3	848.33	84.83	15.16	819.33	812.66	4.20
PBAT-3	792.00	79.20	20.80	772.33	729.67	7.87
Control	736.67	73.66	26.33	672.66	605.66	17.78
CD (.05)	51.37	-	-	46.93	41.75	-
CV	3.80	-	-	3.63	3.80	-

to control.

Among three varieties of radish tested for their biofumigation potential MAHY 22 and Japanese white were found most effective against *Pythium* while a lower biofumigation activity was observed in Pusa Chetki. Dunne *et al*. (2003) reported the findings of different species of *Brassica* as bio-fumigants and types of isothiocynate produced during their decomposition that were differ in their toxicity under *in vitro* against soil borne pathogenic fungi. The results are in support with the findings of Kishor and Mishra (2016) who also studied the efficacy of radish as biofumigant against *Pythium aphanidermatum* and found significant reduction in disease over control. Numerous studies have reported lower incidence of soil borne pathogen in various crops after the application of *Trichoderma* and *Pseudomonas* (Akrami *et al*., 2012).

The reduced incidence of damping off in combination of radish and PBAT-3 are in agreement with the findings of earlier workers who suggested that disease control can be improved by this integrated approach as they reported that

Trichoderma is less sensitive to the volatiles released by *brassica* residue in comparison to other pathogen (Galletti *et al.,* 2008; Salvador, 2014). Roots and shoots of radish contains significant amount of glucosinolate content which can be utilized for the suppression of soil borne pathogens. It can be an alternative to soil solarization.

CONCLUSION

The study demonstrated that roots and shoots of radish contain significant amount of glucosinolate, which can be utilized for the suppression of *Pythium aphanidermatum* a pathogen of tomato causing damping off disease. The combination of biofumigation with MAHY 22 roots and seed bio priming with PBAT-3 can effectively manage both pre and post-emergence damping off of tomato and promote seedling emergence and increase seedling vigour in soil infested by damping off disease. The recommendations can be applied in the management of damping off disease in tomato nurseries.

REFERENCES

Akrami M.O., Khiavi H.K., Shikhlinski H. andKhoshvaghtei H.(2012). Bio controlling two pathogens of chickpea *Fusarium solani* and *Fusarium oxysporum* by different combinations of *Trichoderma harzianum, Trichoderma asperellum and Trichoderma virens* under field condition. *International Journal of Agricultural Science Research*, 1(3):41-45.

Chaudhary V., Prasanna R., Nain L., Dubey S.C., Gupta V., Singh R., Jaggi S. and Bhatnagar A. K.(2012). Bioefficacy of novel cyanobacteria-amended formulations in suppressing damping off disease in tomato seedlings. *World Journal of Microbiology and Biotechnology*, 1;28(12):3301-10.

Chew, F. S. (1987) Biologically active natural products-potential use in agriculture. In:Comstock Society. MJ, ed. ACS Symposium Series. USA: American Chemical

Dunne C.P., Dell B. and Hardy G.S. (2003). Effect of biofumigants on the vegetative growth of five *Phytophthora* species *in vitro*. *Acta horticulturae*,602: 45-51

Galletti S., Sala E., Leoni O., Burzi P.L. andCerato C. (2008). *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. *Biological Control,*45(3):319-27.

Hassan A.K., Kareem T.A. and Matar S.S.(2016). Effect of biofumigation with radish *(Raphanus sativus)* leaves fresh and seed meals to Control root knot nematode and *Fusarium* wilt disease complex infecting eggplant. *Journal of Biology, Agriculture and Healthcare,* 6 (4): 21-25.

Kirkegaard J.A., Gardner P.A., Desmarchelier J.M. and Angus J. F. (1993). Biofumigation—using *Brassica* species to control pests and diseases in horticulture and agriculture. InProc. 9th Australian Research Assembly on *Brassicas*, Wagga Wagga, Pp. 77-82.

Kishor R. and Mishra R. S. (2016). Effect of different concentrations of bio-fumigants against *Pythium aphanidermatum* causing soft rot disease of ginger. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 8(1):56-9.

Kucharek T. and Mitchell D. (2000). Diseases of agronomic and vegetable crops caused by *Pythium*. Plant pathology fact sheet PP-53. University of Florida, Gainesville, FL.

Manici L.M., Lazzeri L. and Palmieri S. (1997). *In vitro* fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. *Journal of Agricultural and Food Chemistry*, 45(7):2768-73.

Mohamed, A. A. N., Ismail, M. R. and Rahman, M. H. (2010) *In vitro* response from cotyledon and hypocotyls explants in tomato by inducing 6-benzylaminopurine. *African Journal of Biotechnology*, 9(30), pp.4802-4807.

Ramamoorthy, V., Raguchander T. and Samiyappan, R. (2002) Enhancing resistance of tomato and hot pepper to Pythium disease by seed treatment with fluorescent 429. pseudomonads. *European Journal of Plant Pathology*, 108(5): 429

Reino J.L., Guerrero R. F, Hernández-Galán R. and Collado I.G. (2008). Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochemistry Reviews*,7(1):89-123.

Salvador P., Staltari S., Chorzempa S.E., Astiz G. and Molina M. D. (2014). Biological control of *Fusarium graminearum*: use of *Trichoderma* spp. and biofumigation with aerial part of *brassica juncea*. Revista de la Facultad de CienciasAgrarias, *Universidad Nacional de Cuyo*,46(2):45-56.

Stapleton J., Elmore C. andDeVay J. (2000). Solarization and biofumigation help disinfest soil. *California Agriculture,* 54(6):42-5.

Van Dam, N. M., Tytgat,T. O. G. and Kirkegaard, J. A. (2009) Root and shoot glucosinolates: A comparison of their diversity, function and

interactions in natural and managed ecosystems. *Phytochemistry Reviews,* 8(1): 171-186.

Yates S.R., Gan J., Ernst F.F., Mutziger A. and Yates M.V. (1996). Methyl bromide emissions from a covered field: I. Experimental conditions and degradation in soil. *Journal of Environmental Quality*, 25(1):184-92.

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