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Screening of Biocontrol Agents against Pathogens causing Diseases of Brinjal

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A B S T R A C T

Brinjal growers of Assam completely depend on the chemocentric cultivation practices to combat the crop diseases, which increases the cost of cultivation and affect the consumers with pesticides residues. Moreover, excessive use of a wide range of fungicides has resulted harmful effect to the environment and increased the resistant pathogen populations. Development of an alternative eco-friendly disease management module for sustainable crop production is the need of the hour. Present study was conducted to find out the effective biocontrol agents against important pathogens of brinjal showed that out of six (6) indigenous biocontrol agents viz. *Beauveria bassiana, Metarhizium anisopliae, Trichoderma asperellum, T. harzinum, Paecilomyces lilacinus* and *Gliocladium virens, T. harzianum* showed maximum inhibition of *Rhizoctonia solani* (74.44%), *Fusarium solani* (70.68%), *Alternaria melongenae* (72.48%), *Sclerotinia sclerotiorum* (69.15%) and *Phomopsis vexans* (77.82). *T. asperellum* and *G. virens* also showed significantly better inhibition against these pathogens causing diseases in brinjal over *B. bassiana, M. anisopliae* and *P. lilacinus*. The present study showed that *T. harzianum, T. asperellum* and *G. virens* can be used for preparation of bioformulation for management of diseases of brinjal.

Introduction

Brinjal (*Solanum melongena*) is one of the most important vegetables in South and South-East Asia. In India, it is grown on over 669,000 ha with a production of 12400 thousand MT, while in Assam brinjal is grown over 17670 ha with a production of 286.43 Thousand MT (Ministry of Agriculture& Farmers' Welfare, GOI, 2017). The productivity of brinjal in Assam is 16.20 MT/ha which is less as compared to the national productivity (18.50 MT/ha). The lower productivity of this vegetable in Assam may be due to attack of diseases. The major diseases of brinjal observed during the year 2014-15 and 2015-16 in Barpeta and Jorhat district of Assam were damping off (*Rhizoctonia* solani), leaf spot (*Alternaria melongenae), Phomopsis* blight and fruit rot *(Phomopsis vexans), Sclerotinia* blight (*Sclerotinia sclerotiorum)* and wilt (*Fusarium*

soani). Farmers generally depend on the fungicides like Bavistin (Carbendazim 50WP), Indofil M-45 (Mancozeb), Ridomil MZ (Metalaxyl 8% + Mancozeb 64%) for management of these diseases. However, intensified utilization of fungicides has resulted in harmful effect on non-target organisms, the development of resistance races of the pathogens, and the possible carcinogenicity (Vinale *et al.,* 2008; Doley and Jite, 2012). Indiscriminate use of synthetic fungicides leads to environmental pollution, development of resistance in pathogens and adverse effects on beneficial insects, wild life, human beings, and ultimately lead to ecological imbalance. In this context, biological control is considered as an alternative and ecofriendly way to control plant diseases and reduce the chemical in agriculture. Biological control assumes major importance for suppressing the crop diseases and pests with bio control agents like *Bacillus subtilis*, *Gliocladium* spp, *Trichoderma* spp, *Pseudomonas fluorescens*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinus* etc. Few success stories have been reported by earlier worker (Abdullah *et al*., 2008; Najar *et al.*, 2011; Kamala and Indira, 2014) for the management of wilt, *Pythium* and *Phytophthora* damping off etc. by biocontrol agents like *Trichoderma* sp, *Pseudomonas fluorescens*, *Bacillus* sp etc. Several fungi are known to antagonize numerous fungal pathogens and among them, *Trichoderma* spp are found effective in reducing major soil borne disease of vegetable crops particularly the solanaceous vegetables. The current study was conducted *in vitro* to assess the antagonistic activity of indigenous bio control agents viz. *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma asperellum*, *T. harzinum*, *Paecilomyces lilacinus* and *Gliocladium virens* against the major pathogens of brinjal found in Barpeta and Jorhat district of Assam.

Materials and Methods

The important diseases of brinjal observed in Experimental Farm, Department of Horticulture, AAU, Jorhat and Demonstration Farm of Krishi Vigyan Kendra, Barpeta were damping off of seedling caused by *Rhizoctonia solani*, *Alternaria* leaf spot by *Alternaria melongenae*, *Phomopsis* blight and fruit rot by *Phomopsis vexans*, *Sclerotinia* blight by *Sclerotinia sclerotiorum* and *Fusarium* wilt caused by *Fusarium solani*. Pure culture of the pathogens causing diseases of brinjal were obtained in potato dextrose agar (PDA) media using the methods followed by the earlier worker viz. *Alternaria melongenae*(Johnston and Booth, 1983), *Rhizoctonia solani* (Jiskani *et al*., 2007), *Sclerotiniasclerotiorum* (Stevens, 1974), *Phomopsis vexans* (Namo Das *et al.*, 2014), *Fusarium solani* (Sahar *et al.*, 2013). Biocontrol agents like *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma asperellum*, *T. harzianum*, *Paecilomyces lilacinus* and *Gliocladium virens* were collected from the culture bank maintained in Nanolab of Department of Plant Pathology, AAU, Jorhat. *In vitro* antagonistic behavior of bio control agents against counterpart pathogen of brinjal *viz.*, *R.*solani, *A. melongenae*, *P.vexans*, *S. sclerotiorum* and *F.solani* were studied using standard dual culture method (Dennis and Webster, 1971) with three replicates. Percentage growth inhibition was calculated by using the following formula.

$$\text{Inhibition (\%)} = \left(\frac{\text{C-T}}{\text{C}}\right) \times 100$$

Whereas, C = Diameter of fungus colony (cm) in control plate, T = Diameter of fungus colony (cm) in treated plate.

The pathogen and bio control agent combination studied were as follows:

Combination-1	**Combination-2**
***Rhizoctonia solani* alone**	*Fusarium solani*alone
R. solani+ Beauveria bassiana	*F. solani+ B. bassiana*
R. solani+ Metarhizium anisopliae	*F. solani+ M. anisopliae*
R. solani+ Trichoderma asperellum	*F. solani+ T. asperellum*
R. solani+ T. harzinum	*F. solani+ T. harzianum*
R. solani+ Paecilomyces lilacinus	*F. solani+ P. lilacinus*
R. solani+ Gliocladium virens	*F. solani+ G. virens*
Combination-3	**Combination-4**
***Alternaria melongenae*alone**	*Sclerotiniasclerotiorum*alone
A. melongenae+ B. bassiana	*S. sclerotiorum+ B. bassiana*
A. melongenae+ M. anisopliae	*S. sclerotiorum+ M. anisopliae*
A. melongenae+ T. asperellum	*S. sclerotiorum+ T. asperellum*
A. melongenae+ T. harzinum	*S. sclerotiorum+ T. harzinum*
A. melongenae+ P. lilacinus	*S. sclerotiorum+ P. lilacinus*
A. melongenae+ G. virens	*S. sclerotiorum+ G. virens*
Combination-5	
***Phomopsis vexans* alone**	
P. vexans + B. bassiana	
P. vexans + M. anisopliae	
P. vexans + T. asperellum	
P. vexans + T. harzinum	
P. vexans + P. lilacinus	
P. vexans + G. virens	

Results and Discussion

The data presented in the Table 1 revealed that all the biocontrol agents inhibited the growth of pathogens. *B. bassiana*, *M. anisopliae* and *P. lilacinus* were found to be less efficient in inhibiting the growth of pathogens of brinjal. Out of two entomopathogens, *B.bassiana* was found to be the superior in inhibiting the growth and found to inhibit 56.67 per cent radial growth of *P.vexans* and 56.32 per cent of *F.solani*. *M. anisopliae* exhibited 37.77 per cent inhibition of radial growth of *R. solani* which was the lowest among all the biocontrol agents tested in the experiment. The nematopathogenic fungi *P. lilacinus* exhibited the radial growth of 34.30 mm with 61.66 per cent growth inhibition against *A.melongenae* which was statistically superior over *B. bassiana* (49.83%), *M. anisopliae* (44.51%) and control. Mycopathogens *T. harzianum*, *T. asperellum* and *G. virens* showed better inhibition of growth of brinjal pathogens over *B. bassiana*, *M. anisopliae* and *P. lilacinus*. *T. harzianum* showed the highest inhibition of radial growth of *R. solani* (74.44%) followed by *T. asperellum* (65.56%) and *G. virens* (65.55%) (Fig. 1&2). The highest inhibition of radial growth of *F. solani* was noticed with *T. harzianum* (70.68%) followed by *T. asperellum* (68.71%) and *G. virens* (61.41%). However, there was no significant difference in the radial growth of *F. solani* with *B. bassaina*, *G. virens* and *P. lilacinus*. Lowest radial growth of *A. melongenae* was observed in dual culture with *T. harzianum* (24.30 mm) and showed 72.48% inhibition in the growth of the pathogen followed by *T. asperellum* (71.00%) and *G. virens* (71.00%). In the dual

culture experiment, *T. harzianum* (69.15%),*T. asperellum*(69.99%) and *G. virens*(63.63%), showed statistically similar efficiency in inhibiting the radial growth of *Sclerotinia sclerotiorum* and found superior over other biocontrol agents. *T. harzianum* showed marked inhibition (77.82%) against *P. vexans* followed by *T. asperellum* (72.41%) and *G. virens* (71.26%). It was observed from the entire dual culture experiment that *T.harzianum* was the most efficient in inhibiting the radial growth of all the pathogens of brinjal followed by *T. asperellum*and *G. virens*. The mycopathogen, *T. harzianum* was found to be the best among all the BCA tested. The entomopathogen, *B. bassiana* was found to be better than *M. anisopliae* in inhibiting the pathogen in dual culture. Similarly, nematopathogen, *P. lilacinus* was also recorded better inhibition potential than *M.anisopliae* against brinjal pathogen in dual culture. Ghosh and Chakraborty (2012) found the isolate BB-1 of *B.bassiana* as class I antagonist i.e. completely overgrowth the pathogen *Colletotrichum gloeosporioides*, the causal organism of anthracnose of *Roulvolfia serpentina* in dual culture experiment. The efficiency of *B. bassiana* and *P. lilacinus* was also reported against *Macrophomatheicola* by Mareeswaran *et al*., (2016) in dual culture. Antibiosis and mycoparasitism may be the reason behind the inhibition of radial growth of the pathogens by *B. bassiana* in dual culture. The highest percentage of growth inhibition of *Rhizoctonia solani* by *T. harzianum* was observed and was followed by *T. asperellum* in our study. Similar findings of interaction of *Trichoderma*sp (*T. hamatum* T 614, *T. hamatum* T 612, *T.harzianum* T 447, *T. harzianum* T 969, *T. virens* T 523 and *Trichoderma*sp T) with *R. solani* were recorded by Hajieghrari *et al.*, 2008. The inhibition of radial growth of two interacting organisms in dual culture has been attributed to secretion of extracellular hydrolytic enzymes (Scirmbock *et al.*, 1994), production of antibiotics (Howel, 1998) as well as some cell wall degrading enzymes such as chitinase, glucanase which destroy cell wall integrity (Elad, 2000). *T. harzianum* and *T. asperellum* were found to be the superior in inhibiting the radial growth of *Fusarium solani* with inhibition of 70.68 per cent and 68.71 per cent respectively in dual culture. Similar results were also obtained by Sarker *et al*., 2013 they found 75.75 per cent inhibition of *F. oxysporum* f.sp *lycopersici* with *T. harzianum*. Sundar *et al.,* 1995 and Deshmukh and Raut, 1992 reported that *T. harzianum* grew over the colonies of *F. oxysporum.* Chabbi and Matrod (2002) reported 77 per cent growth inhibition of *F. oxysporum* with *T. harzianum*. Marked inhibition of mycelium of *Alternaria melongeane* was recorded with *T. harzianum* (72.48%) followed by *T. asperellum* (71%) in the present investigation. Ambuse (2012) also observed 76.66% inhibition of *A. tenuissima* with *T. harzianum* in dual culture.

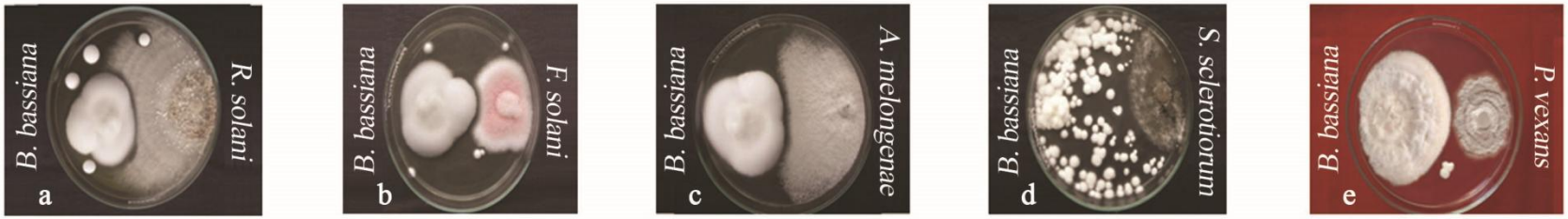
The result was almost in conformity with Ibrahim El Gali (2015) who reported 83.3 per cent inhibition of *A. alternate* with *T. harzianum* at 7 days of inoculation. However, decreased growth inhibition of *A. solani* was noticed with *T. viride* (34.76%) in dual culture by Tapwal *et al.,* (2011). The inhibition of *A. alternata* in dual culture with *T. harzianum* was reported as 72.2 per cent which was similar to our observation (Tagaram *et al*., 2015). Significant differences ($p<0.05$) among the mycopathogens, nematopathogen and entomopathogens were observed in respect of percentage of radial growth inhibition of *S.sclerotiorum* in present dual culture experiment. The best biocontrol agent against *S. sclerotiorum* was *T. asperellum* (69.99%) and was followed by *T. harzianum* (69.15%) and *G.virens* (63.63%).

Table.1 Radial growth inhibition (%) of pathogens of brinjal by different bio control agents

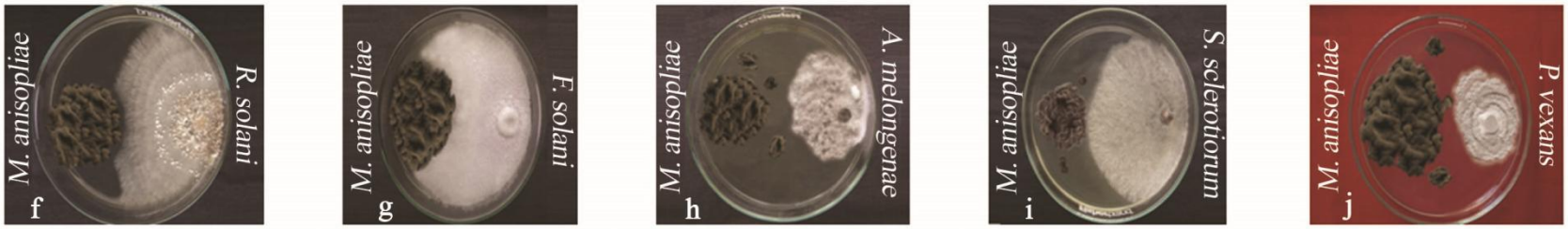
BCA	*Rhizoctonia solani*		*Fusarium solani*		*Alternaria melongenae*		*Sclerotinia sclerotiorum*		*Phomopsis vexans*	
	Radial growth (mm)	**Growth inhibition (%)**	**Radial growth (mm)**	**Growth inhibition (%)**	**Radial growth (mm)**	**Growth inhibition (%)**	**Radial growth (mm)**	**Growth inhibition (%)**	**Radial growth (mm)**	**Growth inhibition (%)**
Control	90.00 ^{a}	-	86.30 ^{a}	-	88.30^{a}	-	83.30^{a}	-	87.00 ^{a}	-
B. bassiana	40.00 ^{c}	55.55	37.70 ^{c}	56.32	44.30^{b}	49.83	37.30^{b}	55.22	37.00 ^{b}	56.67
M. anisopliae	56.00 ^{b}	37.77	49.70 ^{b}	42.41	49.00 ^{b}	44.51	39.00 ^{b}	53.18	40.00 ^{b}	54.02
T. asperellum	31.00 ^{cd}	65.56	27.00 ^{de}	68.71	25.60^{d}	71.00	25.00^{d}	69.99	24.00^{cd}	72.41
T. harzianum	23.00 ^{d}	74.44	25.30 ^{e}	70.68	24.30^{d}	72.48	25.70^{d}	69.15	19.30^{d}	77.82
P. lilacinus	38.00^{d}	57.78	40.70 ^{c}	52.83	34.30^{c}	61.16	34.30^{bc}	58.82	35.70^{b}	58.97
G. virens	31.00 ^{cd}	65.55	33.30^{cd}	61.41	25.60^{e}	71.00	30.30^{cd}	63.63	25.00^{c}	71.26
SEd(±)	4.40		3.60		2.90		2.70		2.30	
CD at 0.05	9.10		7.40		6.01		5.60		4.70	

Data are mean of three replications

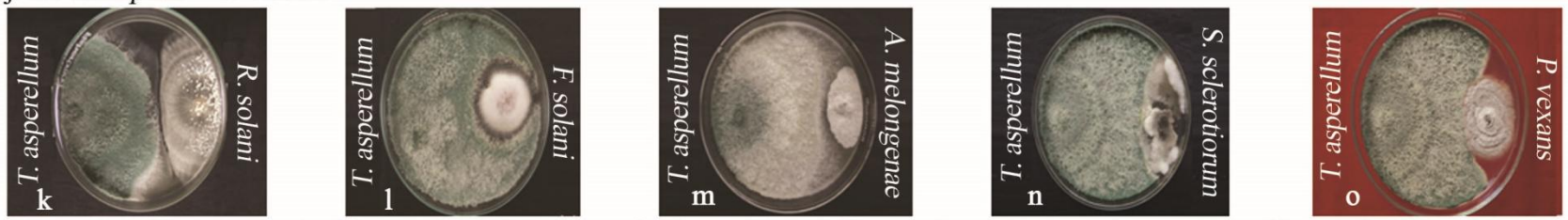
Fig.1 Radial growth inhibition of pathogens causing diseases of brinjal by different biocontrol agents



a. *B. bassiana* + *R. solani*, b. *B. bassiana* + *F. solani*, c. *B. bassiana* + *A. melongenae*, d. *B. bassiana* +*S. sclerotiorum*, e. *B. bassiana* + *P. vexans*

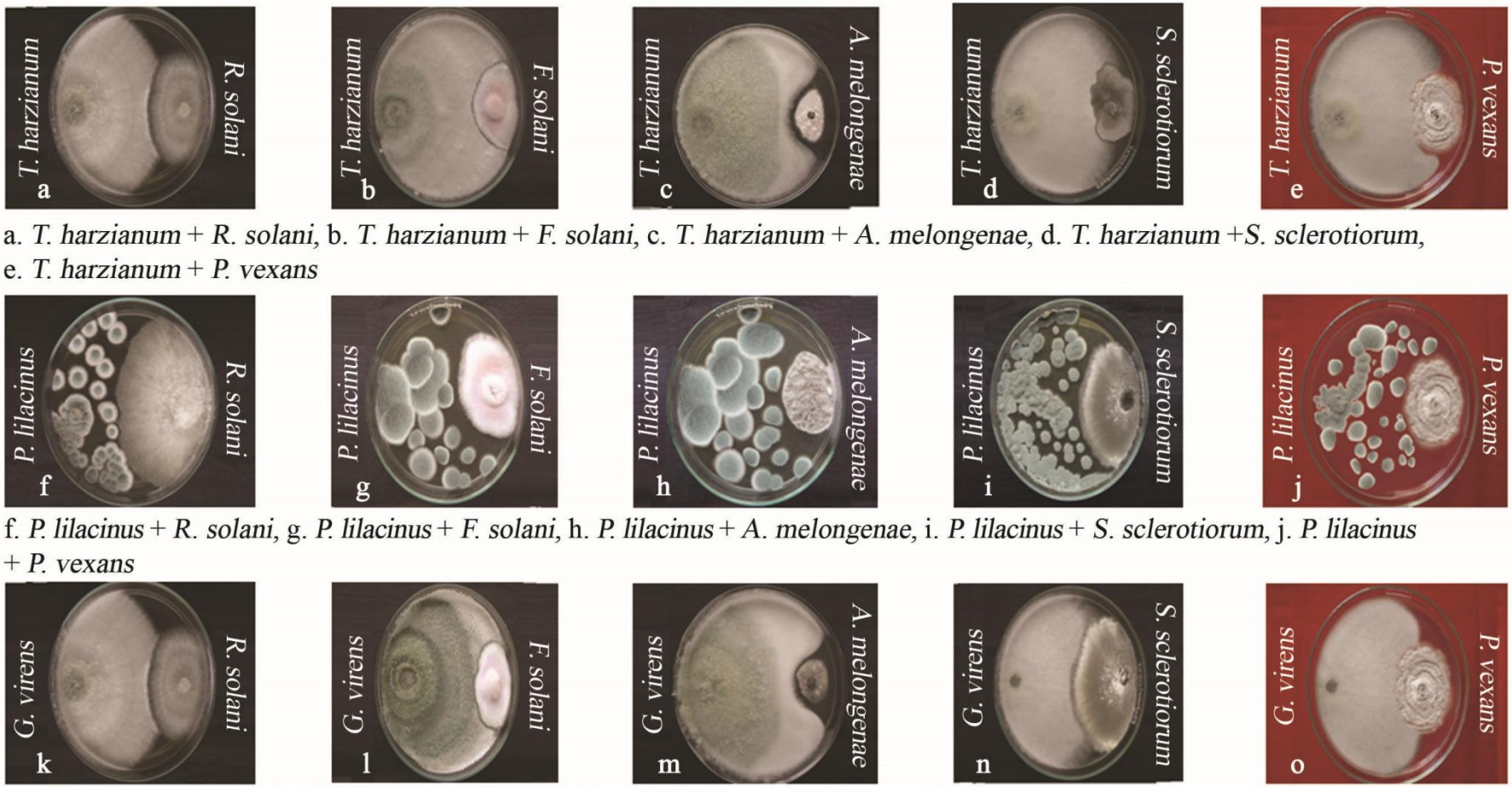


f. *M. anisopliae* + *R. solani*, g. *M. anisopliae* + *F. solani*, h. *M. anisopliae* + *A. melongenae*, i. *M. anisopliae* + *S. sclerotiorum*, j. *M. anisopliae* + *P. vexans*



k. *T. asperellum* + *R. solani*, l. *T. asperellum* + *F. solani*, m. *T. asperellum* + *A. melongenae*, n. *T. asperellum* + *S. sclerotiorum*, o. *T. asperellum* + *P. vexans*

Fig.2 Radial growth inhibition of pathogens causing diseases of brinjal by different biocontrol agents



a. *T. harzianum* + *R. solani*, b. *T. harzianum* + *F. solani*, c. *T. harzianum* + *A. melongenae*, d. *T. harzianum* +*S. sclerotiorum*, e. *T. harzianum* + *P. vexans*

f. *P. lilacinus* + *R. solani*, g. *P. lilacinus* + *F. solani*, h. *P. lilacinus* + *A. melongenae*, i. *P. lilacinus* + *S. sclerotiorum*, j. *P. lilacinus* + *P. vexans*

k. *G. virens* + *R. solani*, l. *G. virens* + *F. solani*, m. *G. virens* + *A. melongenae*, n. *G. virens* + *S. sclerotiorum*, o. *G. virens* + *P. vexans*

The similar trends of results were obtained by Castillo *et al.,* (2011) against *S. sclerotiorum* with *T. harzianum* strain T_4 and *T. asperellum* strain $T_1$with 60.3 per cent and 57.6 per cent inhibition respectively in dual culture. Shaigan *et al.,* (2008) also observed better inhibition of *T. viride* against *Sclerotium rolfsii* than *T. harzianum, T. hamatum, T. longibrachiatum* and *T. paraseramosum*. The higher inhibition of the pathogen by biocontrol agents in dual culture might be due to antagonistic competition for space and nutrients (Shaigan *et al*., 2008). *T. harzianum* was found to be the best among all BCA in inhibiting the mycelial growth of *P.vexans* (77.82%). However, *T. asperellum* (72.41%) was also at par with *T. harzianum* in inhibiting the growth of *P. vexans*. The present findings were found in agreement with the report of Namo Das *et al.,* (2014) who reported 84.00 per cent inhibition of *P. vexans* with *T. viride* and 78.22 per cent with *T. harzianum*.

The present study concludes that *T. harzianum*is the best biocontrol agent against the pathogens causing diseases of brinjal followed by *T. asperellum, Gliocladium virens*. The data obtained from this experiment could provide a way for biological management of brinjal diseases by developing a bioformulation using the best biocontrol agents.

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