DOI : 10.5958/2349-4433.2024.00005.9

J Krishi Vigyan 2024, 12(1) : 25-32

Compatible Bioagents to Enhance Efficacy Against *Sclerotinia sclerotiorum*

Munmi Bora, B C Das , Tasvina R Borah , Dorodi Priyom Duarah and Rizwanul Helim
Krishi Vigyan Kendra, Kamrup, AAU, Kahikuchi Campus, Guwahati-781017

ABSTRACT

Sustainable crop production systems require phytopathogens to be managed effectively with the use of microorganisms. In recent years major thrust is given on other alternative methods including biological control with the inclusion of ecologically well-adopted, bio agents, which is environment-friendly and also curtail the hazards of intensive use of toxic materials and add as a major component of modern integrated disease management strategy. The concept of developing microbial consortia for bio-control relies on the fact that bioagents under natural habitats live in communities with some benefits for plants. Attempts were made to evaluate the relative compatibility of *Trichoderma harzianum* with a few other commonly used soil antagonists *viz*. *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma viride*, *Aspergillus terreus*, *Aspergillus flavus* and *Gliocladium virens* by dual culture technique. The most compatible antagonist was found to be *G. virens* with *T. harzianum* followed by *T. koningii*, *T. viride* and *A. flavus*. The radial growth of *T. harzianum* was more as compared to other soil antagonists on dual culture assay except in case of *T. harzianum* and *A. terreus* where the *A. terreus* was seen to suppress the growth of *T. harzianum.* The best pair of compatible antagonists was found to be *T. harzianum - G. virens.* Under pot condition, dual application of bioagents- *T.harzianum* and *G.virens* showed significant reduction in percent disease incidence and greater efficacy in increasing plant height, dry weight of root, shoot and crop yield as compared to the control.

Key Words: Bioagents, *Trichoderma harzianum*, *Gliocladium virens*, white mold, French bean

INTRODUCTION

French bean is susceptible to a wide range of disease causing pathogens. White mold or Stem rot of French bean caused by *Sclerotinia sclerotiorum* (Lib) de Bary is the most important disease which has caused serious and unpredictable yield losses as high as 100 per cent (Tu, 1989). It affect plant in all stages *i.e.* seedling, matured, harvested stages of crops. The disease has been found to be one of the most destructive in French bean as reported by several workers (Bag, 2000). The fungus attacks practically all vegetable crops and has a host range of 64 plant families, 225 genera and 361 species and 22 other cultivars from a total of 385 species and considered to be the most non-specific phytopathogen. The pathogen is worldwide in distribution and is pathogenic to more than 500 species of higher plants (Willetts and Wong, 1980) and causes numerous soft rots of horticultural and agricultural crops. It was originally believed to occur only in cool, moist areas, but is now known to occur in hot, dry areas as well. The fungus can survive on infected tissues, in the soil, and on living plants. White mold can spread quickly in the field from plant to plant.

It can also spread in a storage facility throughout the harvested crop. It survived by production of sclerotia upto 4-5 years. White mold epidemics of beans are produced by sclerotia of *S. sclerotiorum* (Abawi and Grogan, 1979). Soil pH, nutrient status or inorganic supplement to the soil generally do not reduce the survivability, hence, there is every possibility of reappearance of the disease in the subsequent crop season resulting from either myceliogenic or apothecial germination of the sclerotia (Huang and Hoes, 1985). But it has been found that the biological component of soil affect the survival of sclerotia in soil most significantly (Adams and Ayers, 1979). The different conventional and non-conventional methods like crop rotation, field sanitation, clean cultivation, reduced irrigation, growing of resistant varieties etc. do not curb the activity of the organism completely .Frequent and indiscriminate use of fungicides often leads to atmospheric pollution and development of fungicide-resistance in pathogen for which use of chemicals needs to be restricted. Therefore, in recent years major thrust is given on other alternative methods including

Corresponding Author's Email:munmi4u@gmail.com

biological control with the inclusion of ecologically well-adopted, biocontrol agents, which is environment-friendly and curtail the hazards of intensive use of toxic materials apart from having suitability for inclusion as a major component of modern integrated disease management strategy .Integration of one or more bioagents can therefore, constitutes a very promising way of controlling various soil- borne pathogens. Therefore, an integrated approach of using combination of two or more biocontrol agent may hold a promising way for an effective control of *Sclerotinia sclerotiorum* on French bean.

MATERIALS AND METHODS

The experiments were carried out in the Department of Plant Pathology, Assam Agricultural University, Jorhat situated at 26°47'N latitude, 94°12'E longitude and at an altitude of 86.60 meters above mean sea level. Infected French bean (*Phaseolus vulgaris*) plants showing typical symptoms of white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary were collected from the Experimental Farm. The target pathogen was isolated on Potato Dextrose Agar (PDA) media and incubated at 25±2°C. Pure culture of the pathogen was obtained through hyphal tip culture. Five widely accepted saprophytic antagonists of the plant pathogens, viz., *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers, *Trichoderma koningii* Oudem, *Gliocladium virens* Millers, *Aspergillus flavus* Link and *Aspergillus terreus* Thom were obtained from the culture collections of the Department of Plant Pathology, AAU, Jorhat. Pure culture of *Trichoderma koningii* was collected from Indian Type Culture Collection (ITCC No. 7112), Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi.

Compatibilty of *T. harzianum* with commonly used soil antagonists *viz*., *T. koningii*, *T. viride*, *A. terreus*, *A. flavus* and *G. virens* was tested by dual culture technique (Das, 1992). Mass multiplication of the highly virulent isolate of the pathogen (*S. sclerotiorum*) was done on maize-meal-sand medium. Mass multiplication and soil application of bioagents was done on wheat bran medium. Fifteen day- old culture of *S. sclerotiorum*, *T. harzianum* and *G. virens* multiplied on wheat bran medium were applied @ 5g and @ 10 g/pot respectively for the pot experiment with French bean cultivar (cv. Contender) susceptible to white mold. Different treatment combinations with 6 replications as T_1 – Soil application of *T. harzianum* + fungicidal spray; T_2 – Soil application of compatible antagonist + fungicidal spray; T_3 - *T. harzianum* + compatible antagonist; T_4 – Fungicidal spray at recommended dose; T_5 - *T. harzianum* (alone); T_6 - Compatible antagonists (alone); T_7 - *S. sclerotiorum* (control); T_8 - Absolute control was carried out. Laboratory and pot experiment data were subjected to Statistical analysis. Completely Randomised Design (CRD) and Fischer's method of analysis of variance (ANOVA) was employed for statistical analysis of the collected data.

RESULTS AND DISCUSSION

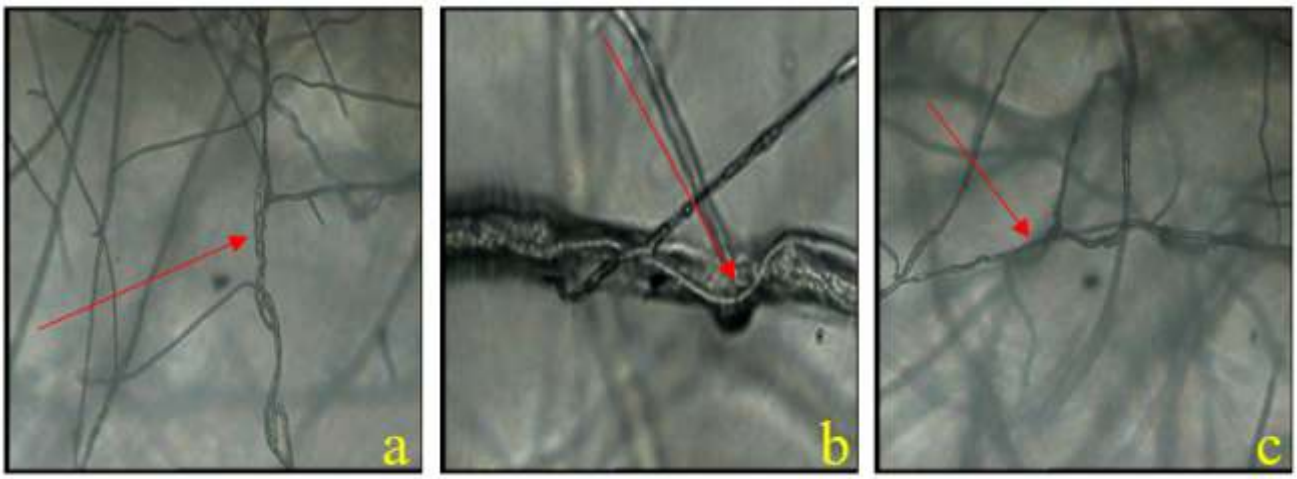
The symptoms produced by *Sclerotinia sclerotiorum* in French bean were first seen just above the soil surface on germination of sclerotia to mycelia at 15 days after emergence of french bean plants. The biocontrol agents *viz*., *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma viride*, *Aspergillus terreus*, *Aspergillus flavus* and *Gliocladium virens* isolates showed significant inhibition of *S. sclerotiorum*.

Relative compatibility of *T. harzianum* with soil antagonists

Microscopic observation showed that the hyphae of *S. sclerotiorum* were directly parasitized by the bioagents. It was observed that the radial growth of *T. harzianum* was more as compared to other soil antagonists on dual culture assay except in case of *T. harzianum* and *A. terreus* where the *A. terreus* was seen to suppress the growth of *T. harzianum* (Table 1; Fig.1). Based on percent inhibition of mycelial growth of *T. harzianum* and other soil antagonists, it was observed that *T. harzianum* was slightly antagonistic to *T. koningii*, *T. viride* and *A. flavus* whereas the *A. terreus* was moderately antagonistic to *T. harzianum*. Compatible pair of fungal isolates either showed very small degree of inhibition zone. The best pair of compatible antagonists was found to be *T. harzianum* - *G. virens* (Table 2). Lysis of *T. harzianum* hyphae was observed by *A. terreus* thus depicting moderately compatible reaction (Fig. 2; Fig. 3).

Table 1. Radial growth of *T. harzianum* with other soil antagonists in dual culture at 72 h of incubation *in vitro*

Treatment	Radial growth (cm) of antagonists over control after 72 hours of incubation					
	T.h	**T.k**	**T.v**	**A.t**	**A.f**	**G.v**
T_1 = *Trichoderma harzianum* (T.h) + *Trichodermakoningii* (T.k)	5.34	5.19	-	-	-	-
T_2= *Trichoderma harzianum* (T.h)+ *Trichoderma viride* (T.v)	6.80	-	6.51	-	-	-
T_3= *Trichoderma harzianum* (T.h) + *Aspergillus terreus* (A.t)	3.12	-	-	5.01	-	-
T_4= *Trichoderma harzianum* (T.h) + *Aspergillus flavus* (A.f)	5.26	-	-	-	4.84	-
T_5= *Trichoderma harzianum* (T.h) + *Gliocladium virens* (G.v)	6.75	-	-	-	-	6.71
T_6= *Trichoderma harzianum* (T.h) (Control)	9	-	-	-	-	-
T_7= *Trichoderma koningii* (T.k) (Control)	-	9	-	-	-	-
T_8= *Trichoderma viride* (T.v) (Control)	-	-	9	-	-	-
T_9= *Aspergillus terreus* (A.t) (Control)	-	-	-	9	-	-
T_{10}= *Aspergillus flavus* (A.f) (Control)	-	-	-	-	9	-
T_{11}= *Gliocladium virens* (G.v) (Control)	-	-	-	-	-	9



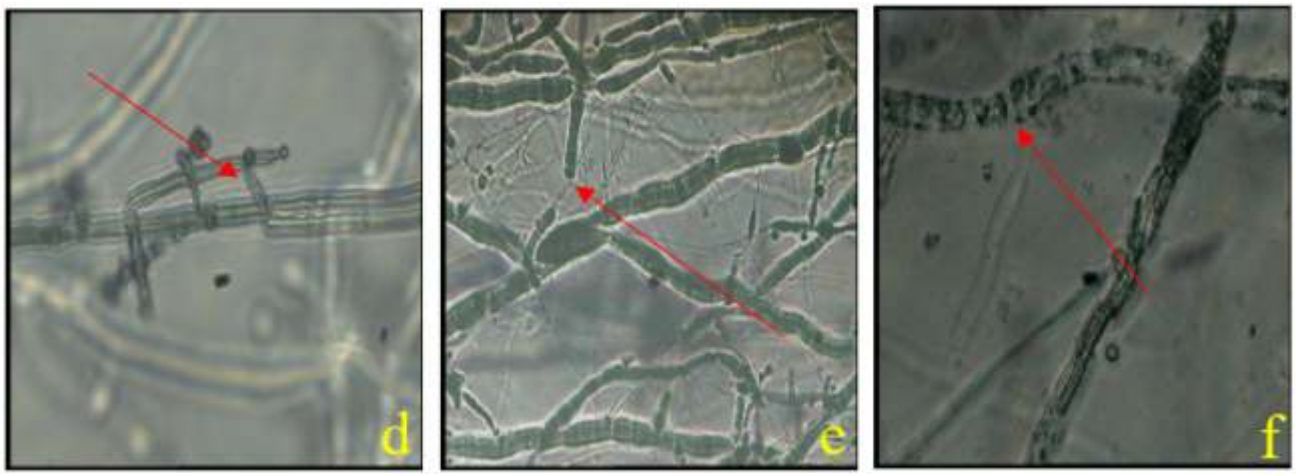


Fig.1 (a-f). Mycoparasitism and lysis of biocontrol agents against *S. sclerotiorum* a. Hyphal coiling of *S. sclerotiorum* by *T. harzianum* b. Hyphal coiling of *S. sclerotiorum* by *G. virens* c. Hyphal coiling of *S. sclerotiorum* by *T. koningii* d. Formation of hook like structure on *S. sclerotiorum* hypha by *T. viride* e. Lysis of *S. sclerotiorum* hypha by *A. terreus* f. Lysis of *S. sclerotiorum* hypha by *A. flavus*

Table 2. Percent inhibition of mycelial growth of *T. harzianum* and other soil antagonists at 72 h of incubation *in vitro*

Treatment	Per cent Inhibition over control						Per cent inhibition of counterpart antagonists	Class
	T.h	T.k	T.v	A.t	A.f	G.v		
T.h + T.k	40.66	42.33	-	-	-	-	1.67	2
T.h + T.v	24.44	-	27.66	-	-	-	3.22	2
T.h + A.t	64.33	-	-	44.33	-	-	20	3
T.h + A.f	41.55	-	-	-	46.22	-	4.67	2
T.h + G.v	25	-	-	-	-	25.44	0.44	1

Class 1 = Less than 1%-No antagonistic effect
Class 2 = 1-5%-Slightly antagonistic effect
Class 3 = 5.1-20%-Moderately antagonistic effect
Class 4 = 20.1-50%-Highly antagonistic effect
Class 5 = More than 50%-Extremely antagonistic effect
(Modified Bell's Scale- Bell *et al.*, 1982)

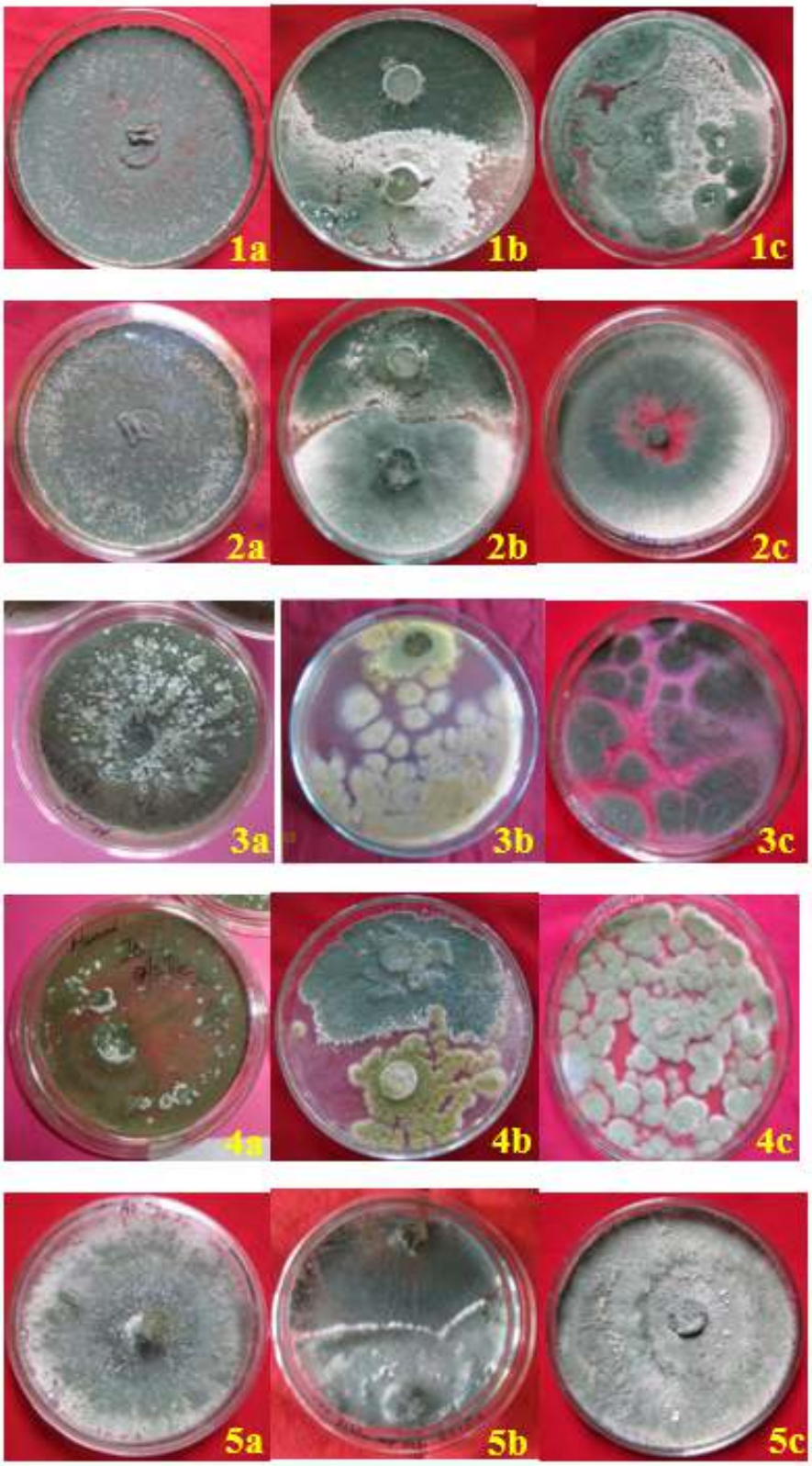


Fig. 2(1a-5c). Compatibility test of *T. harzianum* with soil antagonists (1a-5a) Normal growth of *T. harzianum* (1b-5b) Dual culture of *T. harzianum* with other antagonists 1b. *T. harzianum* + *T. koningii* 2 b . *T. harzianum* + *T. viride* 3b. *T. harzianum* + *A. terreus* 4b. *T. harzianum* + *A. Flavus* 5b. *T. harzianum* + *G. virens* (1c-5c). Normal growth of other antagonists 1c. *T. koningii* 2c. *T. viride* 3c. *A. terreus* 4c. *A. flavus* 5c. *G. virens*

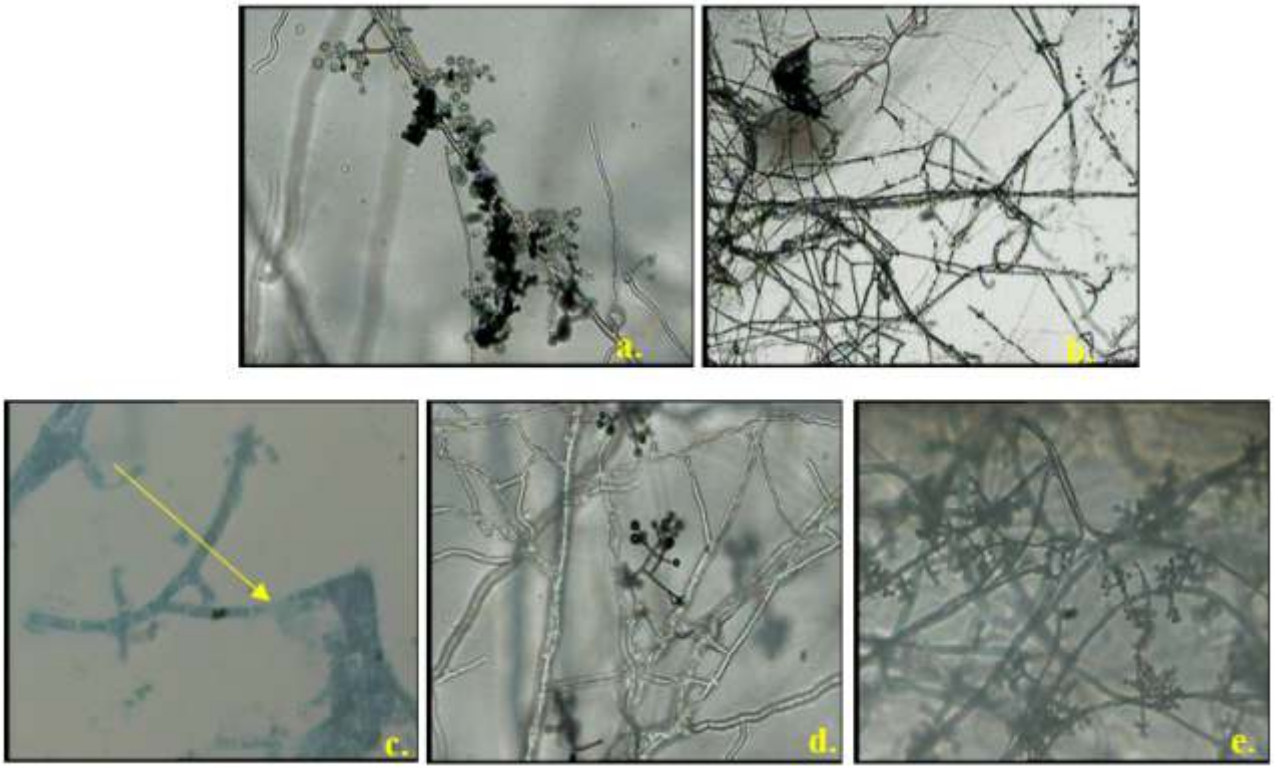


Fig. 3(a-e). Antagonism of *T. harzianum* and other soil antagonists a. *T. harzianum* + *T. koningii* b. *T. harzianum* + *T. viride* c. *T. harzianum* + *A. terreus* d. *T. harzianum* + *A. flavus* e. *T. harzianum* + *G. virens*

Management of white mold disease of French bean with integration of bioagents

Percent disease incidence of white mold in French bean infected by *S. sclerotiorum* was reduced significantly in all the treatments. However, the percent disease incidence was greater when *S. sclerotiorum* was added along with *T. harzianum* and fungicidal spray. Maximum control (13.5%) was achieved with the integration of *T. harzianum*, *G. virens* altogether followed by fungicidal treatment inoculated with *S. sclerotiorum* (Table 3). Effect of the treatments on yield attributing characters in Table 3 showed that maximum increase in height was achieved with integration of *T. harzianum*, and *G. virens* which was followed by only application of fungicide. Both the treatments were at par with each other. The maximum dry weight of root was observed with application of *T. harzianum* and *G. virens* which was at par with all the other treatments including the control. The maximum dry weight of shoot was observed with application of *T. harzianum* and *G. virens* which was at par with fungicidal treatment. The yield per pot increased significantly in all the treatment combinations (Table 3). The maximum yield per pot was recorded with inoculation of *T. harzianum* + *G. virens* which was at par with *G. virens* + fungicidal spray

In the present study the antagonists *T. harzianum*, *T. koningii*, *T. viride*, *Aspergillus terreus*, *A. flavus* and *Gliocladium virens* could significantly inhibit the mycelial growth of *S. sclerotiorum* in dual culture. The mycelial growth of the pathogen was restricted as evidenced by formation of zone of inhibition. The similar results was observed by Das (2001). The mycoparasitic behaviour of *T. harzianum*, *T. koningii*, *T. viride, A. terreus*, *A. flavus* and *G. virens* against *S. sclerotiorum* on 1% water agar revealed that hyphae of *T. harzianum*, *T. koningii*, *T. viride* and *G. virens* ran parallel and adpressed to the hyphae of *S. sclerotiorum*. Then they started to coil and finally cause lysis. *A. flavus* and *A. terreus* showed gradual lysis of the hyphae of the pathogen without mycoparasitism.

T. harzianum showed different reaction to different soil antagonists in dual culture technique. It showed varying degrees of compatibility with *T. koningii*, *T. viride*, *A. flavus* and *G. virens* while

Table 3. Effect of integration of compatible bioagents on white mold disease and growth parameters of French bean

Treatment	Percent disease incidence			Growth parameters			
	30 DAS	60 DAS	90 DAS	Height of the plant (cm)	Root dry weight (g)	Shoot dry weight (g)	Yield (g/pot)
T_1= Soil application of *T. harzianum* + Fungicidal spray + *S. sclerotiorum*	7.58 (15.98)^{c}	10.86 (19.21)^{c}	23.50 (28.99)^{b}	20.17^{d}	0.83^{a}	3.80^{b}	204.00^{c}
T_2= Soil application of *G. virens* + Fungicidal spray + *S. sclerotiorum*	8.48 (16.93)^{b}	11.00 (19.34)^{c}	22.6 (28.41)^{bc}	23.17^{c}	0.74^{a}	2.65^{b}	229.99^{a}
T_3= *T. harzianum* + *G. virens* + *S. sclerotiorum*	6.16 (14.37)^{d}	8.96 (17.34)^{d}	13.5 (21.55)^{e}	30.33^{a}	0.99^{a}	5.36^{a}	230.37^{a}
T_4= Seed and foliar spray of fungicide + *S. sclerotiorum*	7.35 (15.72)^{c}	13.58 (21.61)^{bc}	19.25 (26.02)^{d}	29.17^{a}	0.36^{a}	5.09^{a}	212.31^{b}
T_5= *T. harzianum* + *S. sclerotiorum*	7.75 (16.16)^{c}	12.19 (20.42)^{c}	21 (27.26)^{d}	26.00^{b}	0.55^{ab}	2.48^{bc}	196.08^{e}
T_6= *G. virens* + *S. sclerotiorum*	8.58 (17.03)^{b}	14.38 (22.25)^{b}	22.23 (28.13)^{d}	25.60^{b}	0.42^{a}	2.65^{b}	195.01^{e}
T_7= *S. sclerotiorum* (control)	18.16 (25.21)^{a}	22.83 (28.52)^{a}	37.66 (37.85)^{a}	18.00e	0.31^{a}	1.03^{d}	181.00^{f}
T_8= Absolute control	2.45 (8.98)^{e}	3.84 (11.28)^{e}	5.48 (13.53)^{f}	24.00^{bc}	0.48^{a}	1.25^{c}	200.16^{d}
S.Ed ±	0.25	0.65	0.38	0.78	0.15	0.57	1.27
$CD_{0.05}$	0.57	1.52	1.34	1.77	0.33	1.29	2.86

moderate antagonistic behavior by *A. terreus* against *T. harzianum*. The most compatible antagonists with *T. harzianum* was found to be *G. virens* followed by *T. koningii*, *T. viride* and *A. flavus*. There is not much information on the compatibility between different fungal biocontrol agents. Variation in compatibility of *T. harzianum* with *G. virens*, *T. koningii*, *T. viride* and *A. flavus* may be due to the competition for food and space. Although lysis of *T. harzianum* by *A. terreus* was observed in the interaction with the presence of inhibition zone which indicated that there might be either some secretion of non-volatile compounds or might be due to mycoparasitism. Rayner and Webber (1984) reported that fungi utilizing the same resource may interact in at least three broadly defined ways – mutualistically and neutralistically. Baiswar and Chandra (2007) reported that *T. harzianum* and *T. viride* were found compatible with each other for the management of corm rot of Gladiolus which give support to the present findings. Biocontrol attributes also are more in consortia in comparison to using single isolates (Thakkar and Saraf *et al.,* 2015).

The results on integration of the bioagents in different treatment combinations revealed that maximum disease control was recorded in the plants treated with both *T. harzianum* and *G. virens* indicating that integration of more than one bioagents has high potential of success. Integration of *T. harzianum* and *G. virens* proved much effective against white mold of French bean under pot condition, which also improved in growth parameter and yield significantly over individual components. This might be due to either early establishment of antagonist in rhizosphere and release of antibiotic substance or direct competition between three organisms *viz., T. harzianum*, *G. virens*

and *S. sclerotiorum*, thereby reducing the disease incidence which is similar to the findings of Jhumishree *et al*. (2018) and Patole *et al.,* (2017). Application of *G. virens* with fungicidal spray did not exhibit much difference in yield and infact these were at par with each other.

CONCLUSION

Integration of more than one biocontrol agents constitutes a very promising way of managing plant pathogens due to its minimal interference with the biological equilibrium in nature. It may be concluded that integration of *T. harzianum* and *G. virens* for combating white mold of French bean caused by *S. sclerotiorum* with correspondence increased in yield and plant biomass. Although, foliar application of the fungicide (0.2%) was also effective in reducing the incidence of the disease, its application alone should preferably be avoided because chemical control measures are not only uneconomical, but these are also not eco-friendly for their potentially hazardous toxic effects.

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Received on 8/1/2024 Accepted on 10/2/2024