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Talc Based Bioconsortia for the Management of *Sclerotium* Root Rot

R. Thilagavathi*

Department of Plant Pathology, Dr. M. S. Swaminathan Agricultural College and Research Institute, Tamil Nadu Agricultural University, Eachangkottai, Thanajvur – 614 902, India

**Corresponding author*

Abstract

The objective of present study was to assess the efficacy of individual and mixture of talc based bioformulations of Plant Growth Promoting Rhizobacteria and *Trichoderma* in the management of sugarbeet root rot caused by *Sclerotium rolfsii*. The result from field experiments revealed that next to the chemical treatment, significantly lower root rot incidence of (18.6%) was recorded in the combination of *Pseudomonas fluorescens* (Pf1)and *Trichoderma asperellum* (TTH1) followed by the combination of *P. fluorescens* (Pf1) and *Bacillus subtilis* (EPCO16) with 20.5% as against 28.5% in untreated control. However, the combination effect was not observed while combining TTH1 and EPCO16 as they are incompatible with each other. In the same way, increase in improvement of yield parameters was recorded in Pf1+TTH1 followed by Pf1+EPCO16 under field conditions. The results suggest that the approach of combined application of compatible biocontrol agents may provide improved biocontrol efficiency in controlling the crop diseases.

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Biocontrol agents, Consortia, root rot, *Sclerotium rolfsii*, Sugarbeet.

Introduction

Sugarbeet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Doll. family Chenopodiaceae) is abiennial sugar producing tuber crop with white roots of conical shape, growing deep into the soil with only the crown exposed. Two sugarbeet crops can be grown in a year as compared to 12 months in the case of sugarcane (Chatin *et al.,* 2004).

As it can produce 60 to 80 tonnes of beet per ha, it shares about 45% of the world's total sugar production and provides valuable by-products like beet tops as cattle feed and molasses for the production of vitamin-B complex through fermentation (Shewate *et al.,* 2009; Lal *et al.,* 2004). Sugarbeet is affected by number of soil borne pathogens such as *Sclerotium*, *Rhizoctonia*, *Phoma*, *Pythium*, *Fusarium* and *Rhizopus,* of which root rot caused by *Sclerotium rolfsii* considered as a serious problem (Eweis *et al.,* 2006; Khattabi *et al.,* 2001). The occurrence of root rot resulted in reducing yield and sugar content (Harveson and Rush, 2002).

As the pathogen could survive in the soil for long period in the form of sclerotia, it is very difficult to control solely by the application of fungicides. In this context, biocontrol is advocated in the place of chemical pesticides.

An innovative approach for improving soil-borne disease control could be the development of cocktails containing strains that communicate with each other to maximize

biocontrol efficacy (Saravanakumar *et al.,* 2009. Mixtures of biocontrol agents (BCA) can overcome the limitations with single BCA and may have advantages of broad spectrum activity, enhancing the efficacy and reliability of the biological control and it allows the combination of various traits without employing genetic engineering. Palaiah *et al.,* (2007) suggested for using more than one type of BCA for the management *S. rolfsii,* as their isolates varied in their sensitivity to the different BCAs, mainly due to inherent variability existing among them. Jetiyanon and Kloepper (2002) proposed a combinational use of different BCAs for improved and stable biocontrol activity against a complex of diseases.

Application of compatible mixture of fungal and bacterial biocontrol agents possessing various mechanisms of pathogen suppression is suggested as a reliable and potential means of disease suppression (Rajendran *et al.,* 2011). With this background, the current study was carried out to assess the efficacy of individual and mixture of BCAs for the management of sugarbeet root rot caused by *S. rolfsii*.

Materials and Methods

Biocontrol agents and their compatibility

The bacterial BCAs such as *P. fluorescens* (Pf1) and *B. subtilis* (EPCO16) were tested for their compatibility with each other following the method of Fukui *et al.,* (1994). The compatibility of *T. asperellum* (TTH1) with Pf1 or EPCO16 was tested by dual culture technique (Dennis and Webster, 1971). They were observed for the overgrowth among them without any inhibition zone for compatible strains or for the separation among them with inhibition zone for incompatible strains.

Preparation of bioformulations

The bioformulations were prepared for *P.fluorescens* (Pf1), *B. subtilis* (EPCO16) and *T. asperellum* (TTH1) separately. A loopful of Pf1 and EPCO16 strains were inoculated into the King's B and nutrient broth respectively and incubated on a rotary shaker for three days at 150 rpm at 28±2°C.

To 400 ml of bacterial suspensions containing 9 x 10^8 cfu/ml, 1 kg of the carrier material (talc powder), 15 g calcium carbonate (to adjust the pH to neutral) and 5 g Carboxy methyl cellulose (CMC) (adhesive) were added and mixed under sterile conditions to get an inoculum density of $1x10^8$cfu g^{-1} (Vidhyasekaran and Muthamilan, 1995).

An actively growing mycelial disc of *T. asperellum* TTH1 was inoculated into yeast molasses broth (30 ml molasses; 5 g yeast; made up to 1000 ml), and incubated for 15 days at 28±2°C.

The fungal biomass (containing $3x10^6$ cfu/ml) along with the spent broth was incorporated into the sterilized talc powder carrier material at the rate of 50 ml suspension per 100g and thoroughly mixed with addition of 500 mg CMC to get an inoculum density of 2 $x10^6$cfu g^{-1} (Ramakrishnan *et al.,* 1994).

Field study

A field experiment was conducted to assess the efficacy of BCAs against root rot disease in sugarbeet at hot spot locations. The field trial was laid out with seven treatments T_1 (*P. fluorescens* Pf1), T_2 (*B.subtilis* EPCO16), T_3 (*T. asperellum* TTH1), T_4 (T_1 + T_2), T_5 (T_1 + T_3), T_6 (Difenoconazole, 0.2% soil drenching), T_7 (Control) and replicated thrice using a plot size of 4x3 m in a randomized block design.

A spacing of 45x15 cm was adopted. A total of 525 plants were maintained per treatment. The bioformualtion was applied individually at the rate of 2.5 kg/ha and in combination at the rate of 2.5 kg of each to soil at 0, 30, 60 and 90 days after sowing. Growth parameters such as number of leaves, leaf length, leaf area, top weight, root weight and sugar content per plant were observed. Top and tuber yield were measured at 150 DAS. Sugar content was measured at 150 DAS using brix meter. Disease incidence (DI) was assessed upto 150 DAS.

Statistical Analysis

The data obtained were subjected to statistical analysis and were tested at five per cent level of significance to interpret the treatment differences following (Gomez and Gomez, 1984).

Results and Discussion

Compatibility

The biocontrol agents *P. fluorescens* (Pf1) and *T. asperellum* (TTH) were compatible with each other, however *B. subtilis* (EPCO 16) and *T. asperellum* (TTH)

were incompatible with each other as they showed inhibition with each other.

Field study

The field experiments recorded the lower root rot incidence in the combined application of *P. fluorescens* Pf1+ *T. asperellum* TTH1 (18.6%) followed by Pf1+ EPCO16 (20.5%) as against 28.5% in untreated control (Table 1b). However, the application of fungicide, Difenoconazole showed the greater reduction in root rot incidence (5.6%) under field conditions.

The observations on yield parameters revealed that next to the chemical treatment, enhanced top (6.21 t ha^{-1}) and tuber (58.85 t ha^{-1}) yield were recorded in the combination of Pf1+TTH1 application followed by Pf1+EPCO16 treatment with the top and tuber yield of 5.75 t ha^{-1} and 55.27 t ha^{-1} respectively.

Other yield attributing parameters such as number of leaves, leaf length, leaf area, top and tuber yield and sugar content $plant^{-1}$ were extensively increased in Pf1+TTH1 followed by Pf1+EPCO16 than other treatments. Control treatment observed with reduced top and tuber yield of 4.13 t ha^{-1} and 40.80 t ha^{-1} respectively (Table 1a).

A microbial consortium is a group of different species of microorganisms that act together as a community. The organisms with different modes of actions and survivability can perform better in the environment than single microorganisms (Davelos *et al.,* 2004).

A combinatory approach has the potential to overcome problems that occur with individual BCA (Meyer and Roberts, 2002). Mixed inoculants that interact synergistically are currently being devised for better disease control. In the present study, mixed application of Pf1+TTH1 showed significant increase in sugarbeet yield followed by Pf1+EPCO16 than they were applied alone.

Similarly, *Trichoderma* spp. in combination with *P. fluorescens* improved seedling growth in tomato (Rajendran *et al.,* 2017), chilli (Manoranjitham *et al.,* 2000), black gram (Babu and Seetharaman, 2002), green gram (Thilagavathi *et al.,* 2007; 2012) and Vanilla (Senthilraja *et al.,* 2013). Plant growth promoting ability of fluorescent pseudomonads and *Bacillus* were observed in tomato and hot pepper (Ramamoorthy *et al.,* 2002; Cakmakci *et al.,* 2006). In the combined application, certain growth promoting substances and secondary metabolites produced by both fungal and bacterial BCA might be responsible for the better plant growth as reported by Shanmugaiah *et al.,* (2009).

In the present study, the combination of Pf1+TTH1, Pf1+EPCO16 performed better in controlling sugarbeet root rot when compared to individual BCA and control treatments under field conditions. Similarly, combined inoculation of *T. harzianum* with *P. fluorescens* recorded maximum wilt suppression on Vanilla (Sandheep *et al.,* 2013).

T.viride with *P. fluorescens* recorded improved biocontrol activity against pre and post emergence damping off (*Pythium debaryanum*) and wilt (*Ralstonia solanacearum*) in tomato (Rajendraprasad *et al.,* 2017), stem rot (*Sclerotium rolfsii*) in groundnut (Manjula *et al.,* 2004) and root rot (*Macrophomina phaseolina*) in green gram (Thilagavathi *et al.,* 2007).

Trichoderma virens in combination with *Burkholderia cepacia* or *B. ambifaria* significantly improved suppression of cucumber damping off (*R. solani*) over individual applications (Roberts *et al.,* 2005). A combination of BCA with different mechanisms of disease control will have an additive effect and results in enhanced disease control compared to their individual application (Guetsky *et al.,* 2002).

Toxic exoproducts such as HCN, pyrrolnitrin, phenazine, pyoleuterin and 2,4-diacetyl phloroglucinol (Phl), exoproteases and lytic enzymes produced by *P. fluorescens* reported for their deleterious effect against fungal pathogens (Jousset *et al.,* 2008; Has and Keel, 2003; Ramamoorthy and Samiyappan, 2001).

An array of antifungal compounds including iturinproduced by the *Bacillus* responsible for the inhibitory effect on plant pathogens (Gumede, 2008; Bernal *et al.,* 2002).

Antifungal antibiotics and hydrolytic enzymes of *Trichoderma* strains (Monte, 2001; Vizcaíno *et al.,* 2005) reported to reduce the growth of fungal pathogens. Therefore, the results of the current study suggested that diverse groups of antimicrobial compounds and multiple mechanisms offered by combination of BCAs could attribute for better disease control than the individual BCA. Therefore, application of microbial consortia might be a useful and potential approach for the management of soil borne diseases.

Table.1a Efficacy of talc based bio-formulations on growth parameters of sugarbeet under field conditions

Treatments	No. of Leaves			Leaf length (cm plant^{-1})			Leaf area (cm whole plant^{-1})			Top weight (g plant^{-1})				
	DAS			DAS			DAS			DAS				
	30	60	90	30	60	90	30	60	90	30	60	90	120	150
Pf1	9.8	18.4	22.6	28.18	37.03	40.50	738	2309	2913	22.6	244	332	245	164
EPCO16	9.3	17.6	24.2	23.71	34.46	37.72	701	2175	2661	20.30	227	318	232	147
TTH1	11.2	19.8	24.9	27.33	38.00	39.8	763	2413	2847	24.7	253	340	257	150
Pf1+EPCO 16	11.2	20.5	26.5	27.52	41.23	42.61	801	2746	3005	28.5	259	349	266	168
Pf1+TTH1	11.5	21.7	27.6	28.75	43.35	45.34	836	2912	3202	32.6	265	357	271	176
Difenoconazole	9.3	17.8	23.3	22.56	32.6	36.43	657	2391	2829	19.3	207	280	215	147
Control	9.0	17.6	22.0	21.66	31.47	34.52	549	2105	2515	18.5	186	270	220	136
CD (0.05)	0.35	0.47	0.62	0.90	1.18	1.30	63.46	67.41	59.98	4.32	9.77	8.81	8.03	12.82
SEd	0.17	0.22	0.30	0.43	0.56	0.62	30.21	32.09	28.55	2.06	4.65	1.19	3.82	6.10

*DAS – Days after sowing; Values are mean of two experiments

Table.1b Efficacy of talc based bio-formulations on yield parameters and disease incidence of sugarbeet under field conditions

Treatments	Root weight (g plant^{-1})					Sugar content (%)	Top yield at 150 DAS (t ha^{-1})	Tuber yield at 150 DAS (t ha^{-1})	Percent disease incidence (PDI)
	DAS								
	30	60	90	120	150				
Pf1	3.0	148	423	535	591	18.2	5.38	48.60	21.1
EPCO16	2.7	137	450	505	596	17.4	5.55	48.27	22.5
TTH1	3.6	156	415	493	643	17.5	4.87	44.06	25.5
Pf1+EPCO16	4.3	164	461	574	708	18.6	5.75	55.27	20.5
Pf1+TTH1	5.0	170	518	588	712	20.8	6. 21	58.85	18.6
Difenoconazole	2.4	141	363	475	543	15.5	6.74	63.15	05.6
Control	2.1	132	360	462	535	14.7	4.13	40.80	28.5
CD (0.05)	0.45	8.93	8.62	11.57	10.76	0.70	12.82	11.18	1.90
SEd	0.22	4.25	4.10	5.51	5.12	0.33	6.10	5.32	0.91

*DAS – Days after sowing; Values are mean of two experiments

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