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Induction of Enzyme Activities in Tuberose Plants Treated with Antagonists and Organic Fungicide under Artificial Inoculation of *Sclerotium rolfsii* Sacc.

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A B S T R A C T

A pot experiment was conducted to study the antioxidant responses of tuberose treated with biocontrol agents and organic fungicide in combating wilt disease caused by *Sclerotium rolfsii* Sacc. The antagonists, *Trichoderma viride*4 (Tv4), *Bacillus subtilis*1 (Bs1) and *Pseudomonas fluoresence*3 (Pf3) were used alone and in combinations. Mahua cake was used as the source of organic fungicide. The treatment with the commonly used chemical fungicide, hexaconazole was taken as check. Among the fifteen treatments, soil application of Tv4 @2.5 kg ha^{-1} + Pf3 @2.5 kg ha^{-1} + Bs1 @ 2.5 kg ha^{-1} + Mahua cake @ 250 kg ha^{-1} recorded the highest enzymatic activities of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase on the fourth day after challenge inoculation with *Sclerotium rolfsii* Sacc. This treatment also recorded the highest phenol content (1.179 µg g^{-1} FW) on the fourth day after challenge inoculation with *S. rolfsii*. This treatment recorded the lowest wilt incidence (7.56 %) which indicated 90.41 percent disease reduction over control.

Introduction

Tuberose (*Polianthes tuberosa* L.) is a commercially important ornamental bulbulous plant cultivated in India for cut and loose flower trade and also for the extraction of its highly valued natural flower oil which is one of the most expensive raw materials of high-grade perfumes. It is native of Mexico, from where it has spread to different parts of world and is now one of the most important ornamentals of tropical and sub-tropical areas. It is used for artistic garland, floral ornaments, bouquets and buttonholes. The long flower spikes are excellent as cut flowers for table decoration (Padagunur *et al.*, 2005).

Tuberose is often attacked by *Sclerotium rolfsii* Sacc. Which is a ubiquitous endemic soil borne plant pathogen. The initial symptom of the disease is flaccidity and drooping of leaves. The leaves become yellow and dry up. The fungus mainly affects the roots and the infection gradually spreads upward through the tuber and collar portion of the stem. Both tubers and roots show rotting

symptoms. Thick cottony growth of the fungus is visible on the rotten stem and on petioles at the soil level. Hexaconazole is a systemic fungicide used for the control of *Sclerotium rolfsii* (Virupakshaprabhu and Hiremath, 2003). But, chemical control is not always effective and there is concern about the side effects of these fungicides on the environmental safety. Natural plant products and biological control, using antagonistic microorganisms, is an alternate approach to control the pathogenic attack. Biological control of soil borne pathogens offers environmentally safe alternative to chemicals. Different species of bacteria *Bacillus subtilis*, *Pseudomonas fluoresence* and fungi *Trichoderma viride* are reported to be effective biocontrol agents against soil borne plant pathogens (Sivasakthi *et al.,* 2014; Zape *et al.,* 2014).

Plants are known to produce various stress compounds when they are exposed to the pathogens (Lavania *et al.,* 2006; Kim *et al.,* 2008). Studies have shown that plants possess an effective antioxidant machinery to combat disease causing pathogen attack (Demidchik, 2012). Activation of a wide array of defense responses slows down or halts infection at certain stages of the host-pathogen interaction. An increase in the activities of phenolics related enzymes and the accumulation of phenolics has been correlated to plant resistance to biotic stresses (Anjum *et al.,* 2012). This study was conducted to explore the changes in some enzyme activities and phenol content in tuberose plants treated with bio-control and organic fungicide after inoculation with *Sclerotium rolfsii*.

Materials and Methods

Pathogen source

Six pathogenic isolates of *Sclerotium rolfsii* Sacc. were isolated from the diseased tuberose plants collected from the six different tuberose growing areas of Madurai, Dindigul, Dharmapuri and Sivagangai districts of Tamil Nadu. These isolates produced the typical wilt symptoms on the artificially inoculated tuberose plants in pot culture. The degree of virulence varied among the isolates of *S. rolfsii* to cause wilt of tuberose. The most virulent isolate was selected and mass multiplied in sand maize medium and used for this study.

Plant materials and treatment

The present investigation was carried out during November 2014 at Agricultural College and Research Institute, Madurai. Three months old tuberose plants were used for this experiment. Fifteen treatments were set up with soil application of antagonists (Tv4, Bs1, Pf3), Mahua cake and hexaconazole (Table 1). Inoculation with *S. rolfsii* was done and the leaves were collected from the plants on the 0, 2, 4, 6 and 10 days after inoculation. Leaves were washed several times with sterile distilled water and used for enzyme assays and phenol estimation.

Enzyme assay

Peroxidase activity was estimated by the method of Hammerschmidt *et al.,* (1982). One gram of fresh leaf tissue was homogenisedwith5 ml of 0.1M phosphate buffer pH 7.0 in a pre-cooled pestle and mortar. The homogenate was centrifuged at 15,000 rpm at 4ºC for 15 minutes.

The supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05M pyrogallol, 0.1 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The change in absorbance of the reaction mixture was recorded at 420 nm at 30 seconds interval for three minutes at room temperature. The boiled enzyme preparation served as blank. The

enzyme activity was expressed as change in absorbance $min^{-1}g^{-1}FW$.

Polyphenol oxidase was assayed by the method adopted by Mayer *et al.,* (1965). One g of fresh leaf sample was homogenised in 1 ml of 0.1 M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 15,000 rpm for 15 minutes at 4ºC and the supernatant was used as the enzyme source. The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer pH 6.5 and 0.1 ml of the enzyme extract. The reaction was initiated by the addition of 0.2 ml of catechol (0.01M). The absorbance at 495 nm at 30 sec intervals for three minutes was recorded. The enzyme activity was expressed as change in absorbance $min^{-1}g^{-1}FW$.

The activity of phenylalanine ammonia lyase was estimated by the method of Dickerson *et al.,* (1984). Five hundred mg of leaf was homogenized in 5 ml of cold 25mM borate HCl buffer (pH 8.8) containing 5mM mercaptoethanol. The homogenate was centrifuged at 15,000 rpm for 15 minutes at 4ºC and the supernatant was used as enzyme source. The assay mixture consisted of 0.2 ml of enzyme extract, 1.3 ml water and 0.5 ml borate buffer. The reaction was initiated by the addition of 1 ml of 12mM L-Phenylalanine. The reaction mixture was incubated for one hour at 32ºC. The reaction was stopped by the addition of 0.5 ml of 2N HCl. A blank was run in which phenylalanine was added after adding 2N HCl. The absorbance was measured at 290 nm. The enzyme activity was expressed as mol trans cinnamic acid min^{-1} $g^{-1}FW$.

Estimation of total phenols

One g of the leaf sample was ground well in a pestle and mortar after adding 10 ml of 80% methanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was collected and evaporated to dryness and the residue was dissolved in 5 ml of distilled water. From this, 0.2 ml was taken and the volume was made up to 3 ml with distilled water and to that 0.25 ml of Folin-Ciocalteau reagent (1N) was added. After three minutes, one ml of 20% sodium carbonate was added and placed in boiling water bath for one min and cooled. The absorbance was measured at 725 nm against a reagent blank. The total phenol content was expressed as µg catechol $g^{-1}FW$ (Zieslin and Ben Zaken, 1993).

Results and Discussion

Enzyme activities

Peroxidases (PO) has been implicated in a number of diverse phenomena observed in plants such as lignification, suberization, cell elongation, growth and regulation of cell wall biosynthesis and plasticity, which diversified during disease period (Chanda and Singh, 1997). This enzyme is also known to produce a toxic environment for the pathogens through the production of oxidative burst (Passardi *et al.,* 2005).

The activity of peroxidase was induced in the plants treated with biocontrol agents, organic amendments and challenge inoculation with the pathogen *S. rolfsii*. The results revealed that the activity of PO was significantly higher in tuberose plants treated with consortial formulation of Tv4 @ 2.5 kg ha^{-1} + Pf3 @ 2.5 kg ha^{-1} + Bs1 @ 2.5 kg ha^{-1} + Mahua cake @ 250 kg ha^{-1} (0.849 change in absorbance min^{-1} g^{-1} FW) at four days after challenge inoculation with *S. rolfsii* (Table 1). Similar result was reported by Karthikeyan *et al.,* (2008).

The accumulation of polyphenol oxidase (PPO) plays an important role in plants defense mechanism for inhibition of pathogens by the mechanism of cell wall reinforcements (Ngadze *et al.,* 2012). PPO is a nuclear encoded enzyme that catalyzes the

oxygen dependent oxidation of phenols to quinones. PPOs are considered to have broad antimicrobial properties. In the present study, an increasing trend of PPO activity was seen up to four days after inoculation with *S. rolfsii* in all treatments and then decreased (Table 2). The treatment Tv4 @ 2.5 kg ha^{-1} + Pf3 @ 2.5 kg ha^{-1} + Bs1 @ 2.5 kg ha^{-1} + Mahua cake @ 250 kg ha^{-1} recorded the maximum (1.289 change in absorbance min^{-1} g^{-1} FW) PPO activity as compared to the other treatments in tuberose plants.

Table.1 Changes in peroxidase (PO) activity in tuberose plants inoculated with *S. rolfsii* and treated with soil application of antagonists and organic fungicide

T. No.	Treatment details		PO activity (Change in absorbance $min^{-1}g^{-1}$ FW)*				
			Days after inoculation				
			0	2	4	6	10
T_1	Tv_4 (2.5 kg ha^{-1})	Inoculated	0.372	0.589	0.693	0.659	0.485
		Healthy	0.201	0.331	0.289	0.221	0.223
T_2	Pf_3 (2.5 kg ha^{-1})	Inoculated	0.361	0.487	0.678	0.653	0.466
		Healthy	0.187	0.329	0.388	0.344	0.234
T_3	Bs_1 (2.5 kg ha^{-1})	Inoculated	0.296	0.478	0.589	0.538	0.418
		Healthy	0.129	0.278	0.372	0.279	0.179
T_4	Mahua cake (250 kg ha^{-1})	Inoculated	0.384	0.546	0.741	0.696	0.482
		Healthy	0.198	0.352	0.456	0.424	0.346
T_5	Tv_4 (2.5 kg ha^{-1})+ Pf_3 (2.5 kg ha^{-1})	Inoculated	0.351	0.523	0.710	0.672	0.559
		Healthy	0.232	0.364	0.453	0.371	0.262
T_6	Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.378	0.586	0.724	0.702	0.495
		Healthy	0.182	0.229	0.347	0.253	0.197
T_7	Tv_4 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.389	0.568	0.733	0.706	0.563
		Healthy	0.238	0.252	0.327	0.289	0.154
T_8	Tv_4 (2.5 kg ha^{-1}) + Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.331	0.521	0.702	0.684	0.423
		Healthy	0.288	0.381	0.456	0.327	0.232
T_9	Tv_4 (2.5 kg ha^{-1})+ Pf_3 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.322	0.467	0.798	0.665	0.479
		Healthy	0.203	0.284	0.347	0.264	0.197
T_{10}	Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.357	0.427	0.678	0.636	0.346
		Healthy	0.212	0.288	0.393	0.343	0.212
T_{11}	Tv_4 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.322	0.418	0.587	0.539	0.398
		Healthy	0.232	0.257	0.293	0.221	0.122
T_{12}	Tv_4 (2.5 kg ha^{-1}) + Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.418	0.647	0.849	0.789	0.523
		Healthy	0.245	0.319	0.452	0.363	0.245
T_{13}	Hexaconazole (0.1%)	Inoculated	0.373	0.591	0.826	0.796	0.528
		Healthy	0.219	0.322	0.668	0.443	0.294
T_{14}	Control (Inoculated)		0.329	0.366	0.501	0.479	0.276
T_{15}	Healthy control		0.336	0.387	0.519	0.491	0.288
	CD (p=0.05) Treatments 0.007 Days 0.004 Treatment x Days 0.017						

*All values are means of three replications

Table.2 Changes in polyphenol oxidase (PPO) activity in tuberose plants inoculated with *S.rolfsii* and treated with soil application of antagonists and organic fungicide

T.No.	Treatment details		PPO activity (Change in absorbance $min^{-1}g^{-1}$ FW)* Days after inoculation				
			0	2	4	6	10
T_1	Tv_4 (2.5 kg ha^{-1})	Inoculated	0.443	0.589	0.788	0.643	0.603
		Healthy	0.418	0.478	0.701	0.598	0.475
T_2	Pf_3 (2.5 kg ha^{-1})	Inoculated	0.438	0.583	0.788	0.649	0.607
		Healthy	0.366	0.487	0.701	0.608	0.575
T_3	Bs_1 (2.5 kg ha^{-1})	Inoculated	0.419	0.432	0.674	0.610	0.573
		Healthy	0.389	0.407	0.546	0.512	0.464
T_4	Mahua cake (250 kg ha^{-1})	Inoculated	0.464	0.632	0.806	0.735	0.536
		Healthy	0.412	0.578	0.739	0.662	0.484
T_5	Tv_4 (2.5 kg ha^{-1})+ Pf_3 (2.5 kg ha^{-1})	Inoculated	0.467	0.532	0.798	0.694	0.634
		Healthy	0.398	0.486	0.679	0.601	0.559
T_6	Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.474	0.989	1.018	0.752	0.549
		Healthy	0.423	0.910	0.932	0.634	0.488
T_7	Tv_4 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.446	0.785	0.943	0.715	0.517
		Healthy	0.412	0.702	0.821	0.669	0.436
T_8	Tv_4 (2.5 kg ha^{-1}) + Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.423	0.656	0.832	0.698	0.626
		Healthy	0.388	0.553	0.797	0.558	0.516
T_9	Tv_4 (2.5 kg ha^{-1})+ Pf_3 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.476	0.772	0.859	0.683	0.615
		Healthy	0.422	0.537	0.798	0.542	0.521
T_{10}	Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.467	0.679	0.812	0.648	0.632
		Healthy	0.416	0.523	0.735	0.463	0.412
T_{11}	Tv_4 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.425	0.498	0.791	0.647	0.472
		Healthy	0.386	0.399	0.637	0.598	0.313
T_{12}	Tv_4 (2.5 kg ha^{-1}) + Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.994	1.177	1.289	0.920	0.618
		Healthy	0.883	0.948	1.022	0.757	0.485
T_{13}	Hexaconazole(0.1%)	Inoculated	0.493	0.735	0.957	0.884	0.816
		Healthy	0.423	0.676	0.848	0.788	0.456
T_{14}	Control (Inoculated)		0.402	0.425	0.605	0.479	0.308
T_{15}	Healthy control		0.414	0.423	0.658	0.506	0.347
	CD (p=0.05) Treatments 0.011 Days 0.006 Treatment x Days 0.026						

*All values are means of three replications

Table.3 Changes in phenylalanine ammonia lyase (PAL) activity in tuberose plants inoculated with *S. rolfsii* and treated with soil application of antagonists and organic fungicide

T.No	Treatment details		PAL activity (µmol *trans*-cinnamic acid $min^{-1}g^{-1}FW$)* Days after inoculation				
			0	2	4	6	10
T_1	Tv_4 (2.5 kg ha^{-1})	Inoculated	0.462	0.588	0.697	0.686	0.604
		Healthy	0.323	0.546	0.589	0.538	0.424
T_2	Pf_3 (2.5 kg ha^{-1})	Inoculated	0.412	0.517	0.663	0.645	0.593
		Healthy	0.378	0.486	0.545	0.328	0.234
T_3	Bs_1 (2.5 kg ha^{-1})	Inoculated	0.436	0.486	0.583	0.541	0.487
		Healthy	0.369	0.384	0.589	0.532	0.424
T_4	Mahua cake (250 kg ha^{-1})	Inoculated	0.478	0.523	0.721	0.702	0.676
		Healthy	0.388	0.472	0.681	0.568	0.538
T_5	Tv_4 (2.5 kg ha^{-1})+ Pf_3 (2.5 kg ha^{-1})	Inoculated	0.463	0.498	0.707	0.626	0.578
		Healthy	0.425	0.436	0.688	0.596	0.482
T_6	Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.481	0.578	0.745	0.686	0.648
		Healthy	0.416	0.526	0.692	0.578	0.498
T_7	Tv_4 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.462	0.524	0.737	0.678	0.536
		Healthy	0.428	0.469	0.688	0.567	0.436
T_8	Tv_4 (2.5 kg ha^{-1}) + Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.434	0.512	0.726	0.632	0.512
		Healthy	0.386	0.464	0.679	0.588	0.448
T_9	Tv_4 (2.5 kg ha^{-1})+ Pf_3 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.416	0.507	0.714	0.608	0.538
		Healthy	0.389	0.462	0.684	0.546	0.462
T_{10}	Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.407	0.473	0.711	0.567	0.523
		Healthy	0.326	0.387	0.674	0.558	0.477
T_{11}	Tv_4 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.392	0.442	0.708	0.519	0.473
		Healthy	0.323	0.344	0.689	0.483	0.425
T_{12}	Tv_4 (2.5 kg ha^{-1}) + Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.507	0.686	0.872	0.732	0.687
		Healthy	0.428	0.567	0.736	0.687	0.632
T_{13}	Hexaconazole (0.1%)	Inoculated	0.491	0.533	0.732	0.712	0.646
		Healthy	0.367	0.437	0.658	0.646	0.548
T_{14}	Control (Inoculated)		0.398	0.516	0.704	0.579	0.426
T_{15}	Healthy control		0.384	0.426	0.628	0.433	0.403
	CD (p=0.05) Treatments 0.010 Days 0.005 Treatment x Days 0.022						

*All values are means of three replications

Table.4 Changes in total phenolic content in tuberose plants inoculated with *S. rolfsii* and treated with soil application of antagonists and organic fungicide

T.No.	Treatment details		Total phenols (µg catechol g^{-1} FW)* Days after inoculation				
			0	2	4	6	10
T_1	Tv_4 (2.5 kg ha^{-1})	Inoculated	0.453	0.588	0.789	0.646	0.606
		Healthy	0.412	0.482	0.711	0.596	0.473
T_2	Pf_3 (2.5 kg ha^{-1})	Inoculated	0.435	0.582	0.786	0.648	0.602
		Healthy	0.368	0.486	0.703	0.608	0.576
T_3	Bs_1 (2.5 kg ha^{-1})	Inoculated	0.418	0.431	0.676	0.615	0.573
		Healthy	0.389	0.408	0.544	0.516	0.466
T_4	Mahua cake (250 kg ha^{-1})	Inoculated	0.476	0.636	0.804	0.738	0.536
		Healthy	0.412	0.578	0.739	0.666	0.482
T_5	Tv_4 (2.5 kg ha^{-1})+ Pf_3 (2.5 kg ha^{-1})	Inoculated	0.463	0.536	0.796	0.694	0.644
		Healthy	0.398	0.486	0.689	0.621	0.569
T_6	Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.486	0.689	0.818	0.752	0.546
		Healthy	0.423	0.536	0.732	0.635	0.484
T_7	Tv_4 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.442	0.783	0.643	0.718	0.516
		Healthy	0.426	0.702	0.826	0.669	0.436
T_8	Tv_4 (2.5 kg ha^{-1}) + Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1})	Inoculated	0.438	0.662	0.834	0.696	0.628
		Healthy	0.386	0.552	0.794	0.568	0.521
T_9	Tv_4 (2.5 kg ha^{-1})+ Pf_3 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.476	0.772	0.859	0.683	0.615
		Healthy	0.432	0.548	0.785	0.552	0.526
T_{10}	Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.468	0.683	0.822	0.658	0.622
		Healthy	0.414	0.538	0.757	0.473	0.432
T_{11}	Tv_4 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.425	0.498	0.791	0.647	0.472
		Healthy	0.383	0.389	0.635	0.588	0.323
T_{12}	Tv_4 (2.5 kg ha^{-1}) + Pf_3 (2.5 kg ha^{-1}) + Bs_1 (2.5 kg ha^{-1}) + Mahua cake (250 kg ha^{-1})	Inoculated	0.984	0.998	1.179	0.890	0.756
		Healthy	0.873	0.858	1.012	0.798	0.685
T_{13}	Hexaconazole (0.1%)	Inoculated	0.495	0.734	0.967	0.882	0.815
		Healthy	0.423	0.676	0.846	0.786	0.589
T_{14}	Control (Inoculated)		0.403	0.425	0.606	0.478	0.310
T_{15}	Healthy control		0.416	0.434	0.668	0.536	0.378
	CD (p=0.05) Treatments 0.011 Days 0.006 Treatment x Days 0.025						

*All values are means of three replications

Phenylalanine ammonia lyase (PAL) is the primary enzyme in the phenylpropanoid pathway, which leads to the conversion of L-phenylalanine to trans-cinnamic acid with the elimination of ammonia. It is the key enzyme in the synthesis of several defense related secondary compounds such as phenols and lignin (Anjum, 2012). Activity of phenylalanine ammonia lyase was induced in tuberose plants treated with effective biocontrol agents, organic fungicide and challenge inoculation with the pathogen *S. rolfsii*. The enzyme activity reached the maximum at four days after inoculation and maintained at higher level up to six days after challenge inoculation with respective pathogen and declined thereafter in all the treatments. Whereas, in healthy and pathogen inoculated control plants, the activity was less than that in the other treatments. Consortial

formulation of biocontrol agentsTv4 @ 2.5 kg ha^{-1} + Pf3 @ 2.5 kg ha^{-1} + Bs1 @ 2.5 kg ha^{-1} + Mahua cake @250 kg ha^{-1} recorded higher activity of PAL enzyme in plants. The maximum PAL activity (0.872 µ mol of transcinamic acid min^{-1} g^{-1} FW) was recorded at four days after challenge inoculation. Similar results were reported by Kannan and Karthik (2009) and Nandhini *et al.*, (2010) (Table 3).

Total phenols

Phenolics are known to be involved in plant-pathogen interactions. Some of the oxidized products of phenols are toxic to microorganisms. Phenols also act as antioxidants in scavenging reactive oxygen species (Demidchik, 2012).

The present study showed that total phenol content had an increasing trend up to fourth day. Consortial formulation of biocontrol agents Tv4 @ 2.5 kg ha^{-1} + Pf3 @ 2.5 kg ha^{-1} + Bs1 @ 2.5 kg ha^{-1} + Mahua cake @ 250 kg ha^{-1} recorded higher phenol content in plants. The maximum amount of phenol (1.179 µg of catechol g^{-1} FW) was recorded at four days after challenge inoculation and then declined slowly and reached 0.756 µg of catechol g^{-1} FW at ten days after inoculation (Table 4). Previous studies also reported similar trends (Angayarkanni, 2014).

It is concluded that defense related enzyme activity and phenol content increased when subjected to pathogen attack along with treatment with antagonists and fungicides.

The activity of defense related enzymes was higher in the treatment combination of Tv4 @ 2.5 kg ha^{-1} + Pf3 @ 2.5 kg ha^{-1} + Bs1 @ 2.5 kg ha^{-1} + Mahua cake @250 kg ha^{-1} which in turn resisted the growth of pathogen (*S. rolfsii* Sacc.), obviously controlling the wilt in tuberose.

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