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Research Article

Associated changes in metabolic activities of host –pathogen system in some infected plants by bio-inducer

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Abstract

Sclerotia of the three pathogens namely; *Sclerotium rolfsii, Rhizoctonia solani* and *Sclerotinia sclerotiorum* actually negative affected by biocontrol inducer in some plants namely; *T. harzianum* and *T. viride*. The percent of inhibition ranged between 53.8 to 83.1%. On the other hand, the inhibition of conidia formation of Trichoderma by occurring pathogens was ranged between 5.3 to 29.8%. Conidia germination of *T. viride* was retarded by 56% also. S. *sclerotiorum* affected negatively on surviving of *T. harzianum and T. viride* by 30.3 and 14.4% respectively. There were a mutual effects between pathogens and biocontrols. *T. harzianum and T. viride* showed different modes in acting as biological control depended on the nature of hosts and pathogens. These modes may be explained the significant changes in metabolites of pathogen-host system and pathogen-inducer-host system. Contrast in changes especially in phenols and dynamic changes in individual amino acids may be taken into account to explain the capability to control disease according to nature of host and virulence of pathogen in vivo. The comparison between the disease symptoms percent and metabolic changes in systems was studied. *S. rolfsii* recorded high disease index in bean caused 74.2% infection. *T. harzianum and T. viride* significantly caused decrease the disease symptoms by 45 and 30% in bean respectively.

Keywords: Sclerotia, biocontrol inducer , *T. harzianum* and *T. viride*, pathogen-host system

Introduction

Many phytopathogenic fungi produce dark-pigmented Sclerotia. *Sclerotium rolfsii*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* are of these fungi caused several plant diseases. Such diseases include root rot, stem rot, web blight and damping off of economic vegetable plants which cause great loss in yield (Babu and Paramageetham, 2013; Bagwan, 2010; Amin *et al.*, 2010; Abdollahzableh *et al.*, 2008 and Keyser and Ferreira,1988). Sclerotia which are important in the life cycle of these fungi, longevity of sclerotia in soil has been reported to be as long as 8 years or more. These species has good adaptation in environment (Amany, 2004; Cooke, 1971). Sclerotia, in general, endure surprising long periods in natural environment, even when exposed to large, diverse and active microbial communities. Most sclerotia-producer fungi are polyphagous soil borne pathogen e.g. *Sclerotium rolfsii* has an ability to infect over 500 plant species worldwide causing huge losses (Hedge *et al.,* 2010 and Demirci *et al.,* 2011). For the soil borne pathogens, use fungicides is not practical due to exorbitant cost and environmental hazard involved. For many years ago, chemicals have been used to control plant diseases. There is now considerable evidence that the fungi which were originally affected by fungicides are developing resistance towards such chemicals. Furthermore accumulation of chemical residues may be harmfull to human and other animal (Helena and Ferreira,1988). Hence, integrated management of the disease using bio-control agents is

the best biological control methods aimed to improve the resistance of the host or favoring micro-organisms to antagonist the pathogen such as bacteria and fungi (Harman *et al.*, 2004).

Biological control of plant pathogen has been considered as a potential control strategy in recent years and search for these biological agents is increasing. *Trichoderma* is the most commonly used fungal biological control agent and have long been known as affective antagonists against plant pathogenic fungi (Chet *et al.,* 1981; Windham *et al.,* 1986 and Mukerji,1983). There are many reports of successful uses of biological control agent (Chet *et al.,* 1979; Hoch and Abawi, 1979; Rakh *et al.,* 2011 and Bagwan, 2011). Al-Twaijri (2008) studied the root rot of cucumber controlled by Rhizosphere isolates *Bacillus subtilis* and *T. viride*. *S. rolfsii* was controlled by *Pseudomonas monteilli* (Rakh et al., 2011) and by *P. aurigenosa* (Babu and Paramageetham,2013). While Deepthi (2013) applied some biological agents on *S. rolfsii* causing stem rot of groundnut. Al-Hinai *et al.,* (2010) studied the effect of *Pythium auriginosa* on *P.aphanidermateus.* Asha *et al.,* (2011) controlled *Fusarium oxysporum lycopersici* caused wilt of tomato by *P. Flourescence*. In nature, resistance is the rule rather than exception. This principle implies that every plant has some mechanisms to resist most of the micro-organisms in its environment which some of them are pathogenic to other species (Amany *et al.,* 1998).

The relationship between free amino acids and resistance or susceptibility to fungal plant has been discussed by many workers (Hancock, 1975; Prasad and Sinha 1987; EL.Deep *et al.,* 1987, Amany *et al.,* 1998 and Al-tuwaijri, 2009 Sundaramoorthy and Balabaskar ,2013). Dynamic of accumulation of free amino acids in wheat plant under influence of a disease resistance inducer were studied by Tyuterev *et al.,* (1994), they found an accumulation of free amino acids due to the treatment of the inducer and phenylalanine highly increased by 350-400 % in cucumber diseases with powdery mildew when treated with the bio-control agents. Amany *et al.,* (1998) found that the bio-inducers could rebalance most amino acids changes associated with infection with root rot pathogen. Also it was reported that the induced resistance in infected plant against pathogen may be presented by the changes of metabolic activities in system (Amany *et al.,* 1998 and Al-Tuwaijri 2009). The accumulation of certain phenolic compound were roughly correlated with the degree of host resistance (Amany *et al.,* 1998 and Cherif *et al.,* 2007 Al-Tuwaijri 2009). The mechanism of action of phenolic compounds may be proposed to function as phytoalexins which are involved in defense responses to fungal infection. Phytoalexins produced in plant act as toxins to the attacking organism. They may puncture the cell wall, delay maturation, disrupt metabolism or prevent reproduction of the pathogen in question. Their importance in plant defense is indicated by an increase in susceptibility of plant tissues to infection when phytoalexin bio-synthesis is inhibited (Favaron *et al.,* (2009) and Temperio *et al.,* (2012). In plants the positive correlation between level of phenol and polypholoxidase (PPO) and the resistance to pathogen and herbivores is frequently observed. Evidence for the induction of PPO in plants, particularly under condition of stress and pathogen attack is considered. A clear role of PPO in a least two bio-synthetic processes has been clearly demonstrated. In both case a very high degree of substrate specificity has been found. In fungi, the function of PPO is probably different from that in plants, but there is some evidence indicating that here too PPO has a role in defense against pathogen. PPO also may be a pathogenic factor during the attack of fungi on other organism (Chen *et al.,* 2000 and Temperio *et al.,* (2012) .

The use of biological control for controlling soil-borne pathogenic fungi produced sclerotia is an attractive possibility, the present work aimed to study the antagonistic effect of bio-inducers isolated from rhizosphere against soil-borne pathogenic fungi produced sclerotia which infected some economic vegetables. *In vitro*, successfully control of the better bio-inducers on most common vegetables under greenhouse condition will be studied. Also variation in metabolic activities in all systems will be studied, in a trial to establish the role of pathogen in changing the metabolic activities of host and of bio-inducers in disease control.

Materials and Methods

A. laboratory experiments

All fungi were grown on PDA medium in Petri dishes and incubated at 23oC for 7days . PDA medium was poured in sterilized Petri dishes and seeded on side

with 0.6 mm in diameter, discs of Trichoderma culture. Similar discs of the pathogenic fungi grown in the same manner were placed in the opposite side of the Petri dish. Each treatment was replicated 3 times .Culture were incubated at 23oc for 5 days.

The antagonist effect of *Trichoderma spp.* against the three pathogens after 5 days of incubation af23oC was measured as following:

1. Measuring the linear growth of both pathogen and antagonist.
2. Counting the total conidia of *Trichoderma spp.* (millions per ml) water which formed on 0.6 cm in diameter disc.
3. Determining the percent of germination conidia of *Trichoderma spp.* after 24 hrs of incubation at 23°C.
4. Counting the total sclerotic formed per plate of the three pathogens after 21 days incubation at 23oC.
5. Testing the surviving of all pathogens, and antagonists after 6 days growing on PDA medium 23°C.

B. Green house experiments:

The antagonistic effect of *T. harzianum* and *T. viride* against some pathogenic fungi on some vegetable crops was tested in the greenhouse using the mentioned pathogens on different vegetable host. The tested fungi were *R. solani, S. rolfsii* and *S. sclerotiorum* .

The host were tomato (*Lycopersicon esculentum* Mill) Super Marmand variety, pepper (Capsicum annuam L.) colifornia wonder var., cucumber (*cucumis sativus* L.) Beit – Alfa var., Bean (*Phaseolus vulgaris L.*) Giza. 3 var.

The fungal inoculums was prepared by growing each fungus in 500 ml sterilized bottles containing100 g. oat medium and was incubated for one month at 25°C.

The sandy clay soil (1 :1) by weight was autoclaved at 20 Lbs/1ns for 2 hours. The soil was packed in 15 cm formaldehyde sterilized pots. Each pot was inoculated by 2% oat culture. Eight seeds of cucumber, squash , bean were planted in each pot. Eight seedlings (30 day age) of tomato and pepper were transplanted in each pot. Three replicates were made for each fungus three pots filled with sterile soil were used as a cheek (control). The hosts were planted 4 days after soil inoculation . Relative plant infections caused by the fungi were measured after month from cultivation as percentage of post emergence damping off.

Tissue extraction and estimation

After one month from cultivation , the Tissue of healthy, infected or treated by bioinducers were extracted. One gram of froze tissue for each sample was blended in 10 ml distilled water for two minutes.

After blending, the mixture was squeezed through several layer of cheesecloth and purified by centrifuging at 7000 r. p. m for 20 minutes and kept at 5oC until assaying ,total amino acids was assayed by the Russell (1944) method using 1,2 naphtha quinone - 4- euphonic acid reagent .

Protein was spectrophotometrically determined according to Lowry et al. (1951). The light absorbance was recorded at 750 nm Data were expressed as mg/g fresh weight of tissues.

Phenolic compounds estimated according to Snell and Snell (1953) was used to determine free and total phenols compounds spec to photometrically at 540 nm. Conjugated phenols were calculated by subtracting the value of free phenols from that total.

The extraction procedure for free amino acids was modified Nicholas (1972) from that carried out earlier (Coclotelo et al., 1971) .Quantitative determination was carried out using a Bacmkanspicno Amino Acid Analyzer model 121.

Statistical Analysis

Data were analyzed with the statistical analysis system. All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using least significance difference (LSD) at P = 0.05 (Danlial , 1978).

Results

The possible use of *T. harzianum* and *T. viride* in biological control of three pathogenic fungi had been studied in laboratory. The results in table1 indicated the inhibitory effect of *Trichoderma spp.* on *R. solani , S. rolfsii* and *S.sclerotiorum. T. harzianum* recorded

a highest inhibitory effect on the linear growth of *S. sclerotiorum* (64.0 %) and a lowest effect on that of *S. rolfsii* (26.2%). At the same time the linear growth of *R. solani* and *S. rolfsii* inhibited with *T. viride* (43.0 and 54.1%respectively).The antagonistic effect of T. *harzianum* is more than the effect of *T. viride* on *S. sclerotiorum* (64.0 and 28.9% respectively).

Table 1. Evaluation antagonistic of *Trichoderma spp.* against soil borne fungal pathogens using dual culture after 5 days incubation at 23°C on PDA medium

Treament	Linear growth of test pathogens						
	Control		*T. harzianum*		*T. viride*		LSD
	L.g. (mm)	In %	L.g. (mm)	In %	L.g. (mm)	In %	
R. solani	89.0	0.0	57.2	35.7	45.7	43.0	9.251
S. rolfsii	68.2	0.0	50.3	26.2	31.1	54.1	13.o18
S. sclerotiorum	90.0	0.0	32.4	64.0	46.2	28.9	9.386

Lg. (mm) = Linear growth in mm
In % = percent of inhibition in

Data tabulated in table 2 indicated that Trichoderma growth was significantly inhibited by all pathogens. *T. viride* (40.7%) was more negatively affected by *R. solani* than *T. harzianum* (8.9%), while *S. rolfsii* has more effected on *T. harzianum* than on *T. viride* (66.3 and 54.7% respectively).

Table 2. Evaluation antagonistic of soil borne fungal pathogen isolates against *Trichoderma spp.* using dual culture after 5 days incubation at 23°C on PDA medium

Treatment	**Linear growth of Trichoderma**								**LSD**
	Control		***R.solani***		***S.rolfsii***		***S.sclerotiorum***		
	Lg(mm)	**In %**	**Lg(mm)**	**In%**	**Lg(mm)**	**In%**	**Lg(mm)**	**In%**	
T.harzianum	**60.5**	**0.0**	**35.1**	**8.9**	**20.4**	**66.3**	**43.3**	**28.4**	**11.612**
T.viride	**90.0**	**0.0**	**43.3**	**40.7**	**40.8**	**54.7**	**55.8**	**38**	**8.821**

Lg(mm)= Linear growth in mm
In%=Percent of inhabitation in linear growth
LSD at $p \leq 0.05$

It is worthy to mentioned, there was overlapping of *T. harzianum* mycelium on *R. solani* mycelium and vice versa occurred by the two isolates of *R. solani* and *S. sclerotiorum* on *T. viride* mycelium. Average number of conidia of *T. viride* is significant affected by the three pathogens, ranged from 0.56 to 0.80 (millions/ml) compared to the control (1.88), while that of *T. harzianum* is insignificant decrease in all cases (table 3). Percentage of germination conidia of *T. harzianum* not affected after 24 hrs incubation but highly negatively affected in case of *T. viride* recorded by *R. solani* (42%), *S. rolfsii* (52%) and *S. sclerotiorum* (70.2%).

Table 3. Average number of conidia of *Trichoderma* isolates affected by soil borne fungal pathogens (number of conidia/ml contained 0.6 cm in diameter disc and percentage of germinated conidia after 24 hrs incubation at 23°C

Treatment	*T. harzianum*		*T. viride*	
	Number of conidia (millions/ml)	% of germination	Number of conidia (millions/ml)	% of germination
R. solani	**1.90**	**98.5**	**0.80**	**42**
S. rolfsii	**2.13**	**99.5**	**1.06**	**52**
S. sclerotiorum	**1.94**	**98.4**	**0.56**	**70.2**
Control	**2.25**	**99.8**	**1.88**	**98.5**
L.S.D	1.093	0.933	1.142	9.331

LSD at $p \leq 0.05$

Average numbers of sclerotia formed per plate after 21 days incubation of all pathogens were significant affected by *T. harzianum* more than *T. viride* (table 4). While the most significant affecting of *Trichoderma spp*. was recorded in case of *S. sclerotiorum.*

Table 4. Average number of sclerotia formed per plate after 21 days incubation at 23°C of soil borne fungal pathogen affected *by Trichoderma* isolates

Treatment	control		*T.harzianum*		*T.viride*		LSD
	Sclerotia no	%	Sclerotia no	%	Sclerotia no	%	
R.solani	**110.4**	**100**	**30.5**	**72.4**	**18.7**	**83.1**	**7.972**
S.rolfsii	**121.1**	**100**	**30.4**	**74.9**	**25.3**	**79.1**	**10.310**
S.sclerotiorum	**33.1**	**100**	**15.3**	**53.8**	**12.4**	**62.5**	**4.674**

LSD at $p \leq 0.05$

From table 5, positively surviving occurred in *S. rolfsii* after exposure to antagonism by *T. harzianum* and *T. viride* while negatively surviving occurred in *R. solani* after exposure to antagonism *by T. harzianum* (-45.5%) and *S. sclerotiorum* after exposure to antagonism by *T. viride* (-16.6%).

Table 5. Surviving of pathogens after antagonistic effect of *Trichoderma spp* as linear growth on PDA medium in mm after 6 days of incubation at 23°C.

Treatment	Control LG(mm)	*T.harzianum*		*T.viride*		LSD
		LG(mm)	%	LG(mm)	%	
R.solani	**79.3**	**43.2**	**- 45.5**	**90.0**	**+13.5**	13.73
S.rolfsii	**78.2**	**90.0**	**+12.1**	**90.0**	**+15.1**	10.241
S.sclerotiorom	**90.0**	**90.0**	**0.0**	**75.3**	**-16.6**	11.252

LSD at $p \leq 0.05$

S. sclerotiorum the only one of the all could decrease the surviving percentage of *T. harzianum* and *T. viride* by 3.3 and 14.4 % respectively (table 6).

Table 6. Surviving of *Trichoderma* spp. after antagonistic effect of different pathogens as linear growth on PDA medium in mm after 6 days incubation at 23°C

Treatment	Control LG(mm)	*R.solani*		*S.rolfsii*		*S.sclerotiorum*		LSD
		L(mm)	%	L(mm)	%	LG (mm)	%	
T.harzianum	**90**	**90**	**100**	**90**	**100**	**87**	**3.3**	**11.412**
T.viride	**90**	**90**	**100**	**90**	**100**	**77**	**14.4**	**9.334**

LSD at $p \leq 0.05$

Greenhouse experiments

Biological control gained by *T. harzianum* or *T. viride* against some soil borne fungi produced sclerotia as *R. solani, S. rolfsii* and *S. sclerotiorum* carried out in greenhouse, to control disease on some vegetable crops .The experimental work was commenced on 10/11 2014 to 20/4/2015.

From table 7, average percent of disease symptoms was highly occurred in case of *R. solani* in order: Bean > pepper > tomato. *Trichoderma spp*., in general, had no significant effect on disease caused by *R. solani* in greenhouse experiments but *T. harzianum* reduced the infection percent of *R. solani* in tomato and bean also, *T. viride* in pepper.

Table 7. Average percent of disease symptoms occurred on vegetable plants caused *by R. solani* alone or combined with *Trichoderma spp* in greenhouse

Treatment	Tomato		Pepper		Bean	
	I%	IR	I%	IR	I%	IR
R.solani	**37.7**	**H**	**49.8**	**H**	**61.2**	**V.H**
R. solani* + *T. harzianum	**34.4**	**M**	**36.5**	**H**	**39.0**	**H**
R. solani* + *T. viride	**37.5**	**H**	**32.4***	**M**	**52.7**	**H**
L.S.D	**4.197**		**2.441**		**1.583**	

I%: average percent of infection

IR: infection range

L <15%,M=15-35%,H=35-55%,V.H >55%

LSD at $p \leq 0.05$

In case of *S. rolfsii*, the disease symptoms were recorded in bean (74.2%) more than in tomato and cucumber (40.1 and 20.8% respectively). *T. harzianum* decreased the disease symptoms by 45% in bean but in tomato and cucumber 13.9 and 6.7% respectively. *T. viride* got a highest reduce in disease symptoms by 30% in bean (table, 8)

Table 8. Average percent of disease symptoms occurred on vegetable plants caused by *S. rolfsii* alone or combined with *Trichoderma spp* in greenhouse

Treatment	Tomato		Bean		Cucumber	
	I%	IR	I%	IR	I%	IR
S. rolfsii	**40.1**	**H**	**74.2**	**H**	**20.8**	**M**
S. rolfsii* + *T. harzianum	**26.3**	**M**	**29.2**	**M**	**13.9**	**L**
S. rolfsii* + *T. viride	**31.6**	**M**	**44.2**	**H**	**14.9**	**L**
L.S.D	**1.586**		**2.244**		**2.321**	

L <15%,M=15-35%,H=35-55%,V.H >55% **IR: infection range,** **I%: average percent of infection**

LSD at $p \leq 0.05$

Disease symptoms of *S. sclerotiorum* were moderate in tomato (35.5%) and cucumber (23.5%) while low in bean (9.9%). Trichoderma, generally, had no significant reduction in symptoms disease of plants (table, 9).

Table 9.Average percent of disease symptoms occurred on vegetable plants caused by *S.sclerotiorum* alone or combined with *Trichoderma spp* in greenhouse

Treatment	Tomato		bean		Cucumber	
	I%	IR	I%	IR	I%	IR
S.sclerotiorum	**35.5**	**M**	**9.9**	**L**	**23.5**	**M**
S.sclerotiorum + ***T.harzianum***	**33.6**	**M**	**20.7**	**M**	**13.4**	**L**
S.sclerotiorum + ***T.viride***	**52.7**	**H**	**23.8**	**M**	**23.7**	**M**
L.S.D	**1.374**		**3.886**		**1.374**	

I%: average percent of infection
IR: infection range
L <15%, M=15-35%, H=35-55%,V. H >55%
LSD at $p \leq 0.05$

It is worthy to know that *T. harzianum* caused an increase of disease symptoms in bean by 10.8 % and *T. viride* also caused an increase in disease symptoms in tomato and bean by 17.2 and 13.9% respectively.

Table 10 reveals a significant increase from 25 and 50 to 30 and 80 mg/g fresh tissue in total amino acids and a significant decrease from 220 and 200 to 175 and 150 mg/g fresh tissue in protein content accompanied with infection by *S. rolfsii* in both tomato and bean systems respectively.

Table 10.Total free amino acids and protein (mg/g fresh tissue) in tomato and bean and infected with *S rolfsii* alone, pathogen – host or pathogen – host – inducer after 30 days from plantation under green house conditions at $25^{o}C \pm 2$.

Treatment	Amino acids (mg/g fresh tissue)		Protein (mg/ g fresh tissue)	
	Tomato	Bean	Tomato	Baan
Control	25	50	220	200
S. rolfsii	30	80	175	150
S .rolfsii + *T. harzianurm*	25	53	200	220
S. rolfsii + *T. viride*	37	50	200	175
L.S.D	8.658	11.616	13.689	11.415

LSD at $p \leq 0.05$

In contrary, In all biocontrol systems by Trichoderma total amino acids declined by 23 to 33.8% and protein content raised in the rate ranging from 14 to 46% in tomato and bean respectively in respect to infected ones.

Generally, phenolic compounds in healthy tomato were higher than that in bean (126 and 95 ug /g fresh tissue respectively). Free and conjugated phenols increased significantly in presence of *S. rolfsii* in both tomato and bean systems (table11). While by

introducing *T. harzianum*, all phenols retarded in both systems by 34 and 94 ug/g fresh tissue . *T. viride* also caused in lowering phenol contents in both systems, otherwise, total phenols in both biocontrolled system of bean were equal .

Table 11. Free, conjugated and total phenols (ug catechol /g fresh tissue) in Tomato and bean tissue infected with *S. rolfsii* alone or with either *T. harzianum* or *T. viride* after 30 days from plantation under green house conditions at 25°C± 2.

Treatment	Free phenols (ug/g fresh tissue)		Conjugated phenols (ug/g fresh tissue)		Total phenol (ug/g fresh tissue)	
	Tomato	Bean	Tomato	Bean	Tomato	Bean
Control	44	27	82	68	126	95
S .rolfsii	50.7	45	115	152	165.7	197
S. rolfsii + *T. harzianum*	31.7	31	100	72	131.7	103
S. rolfsii + *T. viride*	27.6	23	68.2	81	95.8	104
L.S.D	4.979	4.556	6.027	2.738	4.601	4.742

LSD at $p \leq 0.05$

From tables 12 and 13, twelve and nine free amino acids were detected in healthy tomato and bean, seven were common namely L- cystine , L- tyrosine, D-L-phenylalanine, glycine, D-L- aspartic, L- lycine HCl and L-arginine HCl. All amino acids were ranged from 100- 1000 mg /g fresh tissue except cystine in bean (> 3000 ug/gm) and lycine in bean (10 - 100 ug/gm). DL- methionine and DL- tryptophan (100-1000 ug/gm) only were recorded in bean. While DL- serine , DL- therionine, L- leucine, DL- norleucine, DL-histidine HCl were recorded only in tomato.

Infection of tomato with *S. rolfsii* resulted in appearance of four amino acids namely alanine, glutamic acid , hydroxyalanine and ornithine were ranged between 10-1000ug/g fresh tissue (table12) and disappearance of cystine and glycine.

In both bioinducer *T. harzianum* and *T. viride*, the same amino acids were generally detected in both system except only hydroxyproline was recorded in *T. viride* system. Cystein, therionine and alanine decreased to 10-100ug/g fresh tissue, histidine and glutamic disappear completely (table12).

In case of *S. rolfsii* - bean system, the infection was accompanied with appearance of cystein (1000-2000 ug /g fresh tissue),and norleucine (100-1000) and valine (10-100) . another amino acids namely methionine, and tryptophan disappeared. Only cystine was still more than 3000ug/g fresh tissue (table 13).

In both systems, , methionine, tryptophan, glutamic acid and histidine appeared while valine and norleucine disappeared. Additional changes were manifested only in *T. harzianum* system, cystein, leucine and ornithine were recorded while alanine, asparatic and arginine were detected in *T. viride*. Methionine and tryptophan recorded in both biocontrolled systems already occurred in healthy bean. Some amino acids were recorded, at the same rate, cystine (more than 3000 ug /g fresh tissue), glycine and lycine (100-1000 ug /g fresh tissue) in all systems (table 13).

Table 12. Individual amino acids (ug/g fresh tissue) in tomato tissue infected with *S..rolfsii* alone or with either *T. harzianum* or *T. viride* after 30 days from plantation under green house conditions at 25°C± 2

Free amino acids	Tomato			
	control	*S. rolfsii*	*S. rolfsii +T.harzianum*	*S.rolfsii +T.viride*
.L- cystein	-	++	+	+
.L- cystine	++	-	-	-
DL.methionine	-	-	-	-
DL-serine	+	++	++	++
DL- therionine	+	++	+	+
L- tyrosine	++	++	++	++
3,4 dihydroxy alanine	-	-	-	-
DL-Phenyl.alanine	++	++	++	+
DL- tryptophan	-	-	-	-
DL-valine:	-	-	-	-
L.- leucine	+	+	+	trace
DL-isoleucine	-	-	-	-
DL-norleucine	+	++	++	+
DL- alanine	-	++	+	+
Glycine	++	-	-	-
L-glutamic	-	trace	-	-
DL-aspartic.	++	++	++	++
Amino- n-butyric.	-	-	-	-
L- proline	-	-	-	-
L-hydroxyproline.	-	+	-	+
L-lycine HCl.	+	+	+	+
L- arginine- HCl	++	++	++	++
L-histidine HCl	+	+	-	-
DL- ornithine HCl	-	+	+	+

- - = not detected
- Trace = 0 – 10 ug
- + = 10 – 100 ug
- ++ = 100- 1000 ug
- +++ = 1000 – 2000 ug

Table 13. Individual amino acids (ug /g fresh tissue) in bean tissue infected with *S. rolfsii* alone or with either *T. harzianum* or *T. viride* after 30 days from plantation under green house conditions at 25°C± 2

Free amino acids	Bean			
	control	*S. rolfsii*	*S. rolfsii* + *T.harzianum*	*S.rolfsii +T.viride*
L- cystein	-	+++	++	-
L- cystine	+++++	+++++	+++++	+++++
DLmethionine	++	-	++	++
DL-serine	-	-	-	-
DL- therionine	-	-	-	-
L- tyrosine	++	++	+	++
3,4dihydroxalanine	-	-	-	-
DL-Phenylalanine	++	+++	+	+
DL-tryptophan	++	-	+	++
DL-valine	-	+	-	-
L- leucine-	-	-	+++	-
DL-isoleucine	-	-	-	-
DL-norleucine	-	++	-	-
DL- alanine	-	-	-	++
Glycine	++	++	++	++
L-glutamic	-	-	+	++
DL-aspartic	++	++	-	++
Amino- n-butyric	-	-	-	-
L- proline	-	-	-	-
L-hydroxyproline.	-	-	-	-
L-lycine HCl.	++	++	++	++
L- arginine- HCl	++	++	-	++
L-histidineHCl	-	-	++	++
DL- ornithine HCl	-	-	+	-

- - = not detected
- Trace = 0 – 10 ug
- + = 10 – 100 ug
- ++ = 100- 1000 ug
- +++ = 1000 – 2000 ug

Discussion

The possible use of *T. harzianum* and *T. viride* in biological control of three pathogenic fungi had been studied in laboratory. *R. solani* and *S. rolfsii* against *T. harzianum* and *T. viride* colonized from at least one third to about one half of the media surface (more than one third and less than two third). Some organisms appeared to dominate the other. *R . solani* was colonized at least two thirds of the surface and appeared with stand encroachment, but *T. harzianum* grew less than one third of the surface of media.

The highest inhibition of linear growth of pathogen against Trichoderma were recorded in *S. sclerotiorum* against *T. harzianum* (64.0%), then in *S. rolfsii* against *T. viride* (54.1%%). Inhibition of linear growth for *S. sclerotiorum* and *R. solani* against *T. viride* by 46.2 and 45.7% respectively. The recorded values of inhibition percent of linear growth for *T. harzianum* and *T. viride* were 66,3 and 54.7% respectively by *S. rolfsii*. While least value was recorded by *T. harzianum* (8.9%) by *R. solani*.

The greatest inhibition of sclerotia (83.1%) occurred in *R. solani* against *T. viride* and the lowest one(53.8%) occurred in *S. sclerotiorum* against *T. harzianum*. Antagonism of Trichoderma significantly inhibited the production of sclerotia in all pathogens. The inhibition here were similar to the finding of Amin *et al.,* (2010).

It is worthy to mention that the inhibition of conidia formation of Trichoderma occurred. The inhibition was germination affected slightly in cases of *T. harzianum* but significantly affected reached to 56% inhibition in conidia germination of *T. viride* by antagonism of *R. solani*. There were a mutual effect between antagonists and pathogens always negatively.

Survival of pathogens indicated by linear growth of *R. solani* after exposure to *T. harzianum* dropped by 45.5 % and S. sclerotiorum was also affected after exposure to *T. viride* by 16.6 %. While the growth of the other pathogens was obviously accelerated after exposure to the same antagonists. *R. solani and S. rolfsii* did not affect on survival of antagonists but *S. scleroltiorum* affected negatively on survival of *T. harzianum* and *T. viride* by 30.3 and 14.4 % respectively.

The inhibitory activity of antagonists against pathogens observed were mainly attributed to competition for space and nutrition (Amin *et al.,* 2010 and Abdollahzaden *et al.,* 2003). Antagonists may also inhibit growth of pathogens either through antibiosis or producing antifungal phenolic compounds (Saba Banday *et al.,* 2008).

Biological control gained by *T. harzianum* or *T. viride* against some soil borne fungi produced sclerotia as *R. solani, S. rolfsii* and *S. sclerotiorum* carried out in greenhouse, to control disease on some vegetable crops. *Trichoderma spp*. in general, had no significant effect on disease caused by *R. solani* in greenhouse experiments. *T. harzianum* significantly decreased the disease symptoms caused by *S. rolfsii* by 45% in bean but in tomato and cucumber 13.9 and 6.7% respectively. *T. viride* recorded the highest effect on *S. rolfsii* 30% in bean and the least one5.9% in cucumber. Trichoderma, generally, had no significant reduction in symptoms disease of plants in case of *S. sclerotiorum. T. harzianum* caused an increase of disease symptoms in bean by 10.8 % and *T. viride* also caused an increase in disease symptoms in tomato and bean by 17.2 and 13.9% respectively.

From previous results, *S. rolfsii* and *T. harzianum* were chosen for the clear influence ,as a pathogen and a as biological control respectively in beans and tomato plant making comparative study between biocontrolled systems ,in presence of *T. viride* and to study some of the biochemical contents of the plant and extent of changes in their presence inside the infected plant and treated plant.

In the present work , amino acids were higher in healthy bean plant than that in tomato but the protein contents was higher in tomato than that in bean. Bateman and Daly(1967) concluded that the high level of total amino acids output and low protein content can be correlated with the susceptibility of host .It is worthy to mentioned the susceptibility of bean was more high than that of tomato (74.2% and 40.1% respectively)

The free amino acid content increased after infection in two systems. In this respect, Chabdarove *et al.,* (1980) and Madovia *et al.,* (1990) found positive correlation between resistance and changes in amino acid content. The increase in amino acids spectrum was also detected in grains by Somani *et al*., (1993) suggesting that this may be caused by hydrolysis of weakened protein matrix.

Reduction of infection in pathogen - host- biocontrol systems was accompanied by a decreases in total amino acids. This draws the attention towards the rebalancing action of the biocontrol agent against the increased amount of amino acids during infection , an observation denoting the possible role of the bioinducer in host resistance.

Free and conjugated phenols were revealed higher in healthy tomato plant than healthy bean plant. Extra

production and accumulation of phenolic components occurred in all infected tissues. This evidence that the content of phenols in host prior to infection acts as a first chemical barrier against invasion by pathogen (Guesra and Anderson, 1985).

The importance of phenolic compounds in host - parasite systems may be due to its potentiality to inhibit essential hydrolytic enzymes (chamberlain and Baxton 1968). The difference in resistance between the susceptible hosts might be due to the inhibition of conversion of phenol precursors to the phytoalexins (Niem and Baayen, 1988). Some virulent pathogens possess enzymes which catalyze detoxification of phytoalexin (Ching *et al.,* 1995).

The use of Trichoderma as biocontrol agents was accompanied by a relevant decrease in phenol content in infected tissue , still higher than that of healthy ones in case of using *T. harzianum* . Such reduction in phenols may due to the capacity of the systems to convert the phenols to phytoalexins and / or to the rapid oxidation occurred by phenolase to quinone, then polymerizing to dark pigments as melanin and producing lesion and blackening tissue that functioned as infection barrier (Lovrekovich *et al.,* 1968).

On the other hand, reduction in disease by *T. harzianum* in infected tomato and bean. This may be due to the loss of regulatory properties of the allosteric enzymes involved in aromatic biosynthesis leading to accumulation of phenolic compounds in diseased plant tissue or to the inhibition of phenolic conversion to phytoalexin , or to increase in reductase activity that converts quinones to phenols (Kusage, 1969) .

Qualitative changes in amino acids within the host has been occurred as a result of interaction with the pathogen. Infection of tomato with *S. rolfsii* caused about 40% infection and resulted in appearance of four amino acids namely alanine, glutamic acid , hydroxyalanine and ornithine were ranged between 10-1000ug/g fresh tissue and disappearance of cystine and glycine.

Significant reduction of tomato root rot disease was occurred with both bioinducer *T. harzianum* and *T. viride* (26.3 and 30% respectively) , yet all the amino acids were generally detected the same in both system, except only hydroxyproline was recorded in *T. viride* system. Bioinducer *T. harzianum* accompanied by disappearing of cystine, hydroxyproline and histidine and decreasing of cystein, therionine and alanine to 10-100ug/g fresh tissue.

In case of *S. rolfsii* - bean system, the incidence of bean root rot was 74.2% . The infection was accompanied with appearance of cystein (1000-2000 ug/g fresh tissue),and norleucine (100-1000) and valine (10-100) . another amino acids namely methionine, and tryptophan disappeared. Only cystine was still more than 3000ug/g fresh tissue.

Incorporation of both Trichodema simultaneously with the mentioned pathogen caused a significant reduction of the disease (45% infection) in the *T. harzianum* - system and insignificant one (30 % infection) in *T. viride* – system . In both systems, methionine, tryptophan, glutamic acid and histidine appeared while valine and norleucine disappeared.

Methionine and tryptophan were recorded in biocontrolled system, already which existed in healthy bean . Some amino acids were actually recorded the same rate e.g. cystine (more than 3000 ug /g fresh tissue), glycine and lycine (100-1000 ug /g fresh tissue) in all systems of bean. The production of polypeptide by *Trichoderma spp.* has been recorded which acts as an inhibitor to plant pathogenic fungi.

A relationship may be exist between the proportion of biological resistance and the sum of biochemical changes incident inside the tissue of infected plant.

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