4. RESULTS

4.1 Isolation And Differentiation of Streptomyces

A preliminary study was carried out on 281 isolates from a wide range of different localities in Egypt (Table 2). The geographical sources were as indicated in Fig. 2. Since it is difficult to deal with such large number of isolates without grouping, the guidance of Prof. **Assem Mahmoud Hussein** in the differentiation of streptomyces isolates was followed. Grouping of these large number of isolates was based on the color of the obtained aerial mycelium. Four different groups were obtained based on color differentiation as shown in Table 3. It was decided to test the potentialities of the the two largest groups, namely, the red and gray groups.

Nineteen representative samples from the red group and eighty three representative samples from the gray group were chosen based on the color of their aerial mycelium, substrate mycelium. and their production of pigments, into pigmented and nonpigmented forms, see Tables 4 and 5 were the first test. Furthermore, these representative isolates were screened for their antifungal activity particularly towards the plant pathogenic fungi tested in this study.

The second test was carried out to reveal the morphology of the colony and antifungal potentialities of most of the streptomyces isolates which were listed in Tables 4 and 5. This test was a prelude to an antifungal potentiality bioassay trials towards the fungal pathogens used in this study

The third test was only carried on, the two most promising isolates from the point of view of having the highest fungitoxicity bioassay inhibition, namely, isolates 112 [red gray (non pigmented)], and isolate No. 729 [olive gray (pigmented)], see Tables 4 and 5. The other tests that were performed included, cultural morphology on different media (Table 6), their

Table 2 Origin of Egyptian isolates of streptomyces from eight localities where different crops were grown and the type of soil from which they were isolated.

Serial No. of soil sample	Soil sample location	Plant cover	Soil character	Isolate
1	Zagazig	Faba bean	Heavy clay	72*
2	Fakus	Faba bean	Heavy clay	52*
3	El-Salhea	Spinach	Heavy clay	31*
4	El-Wahat	Faba bean	Sandy	30*
5	Port said	Trifolium	Sandy	12*
6	Moshtuhor	Trifolium	Heavy clay	14*
7	Kafr Mouas	Trifolium	Heavy clay	25*
8	El-Salhea	Wheat	Sandy	45*

* refer to the number of isolates of streptomyces from each individual locality.

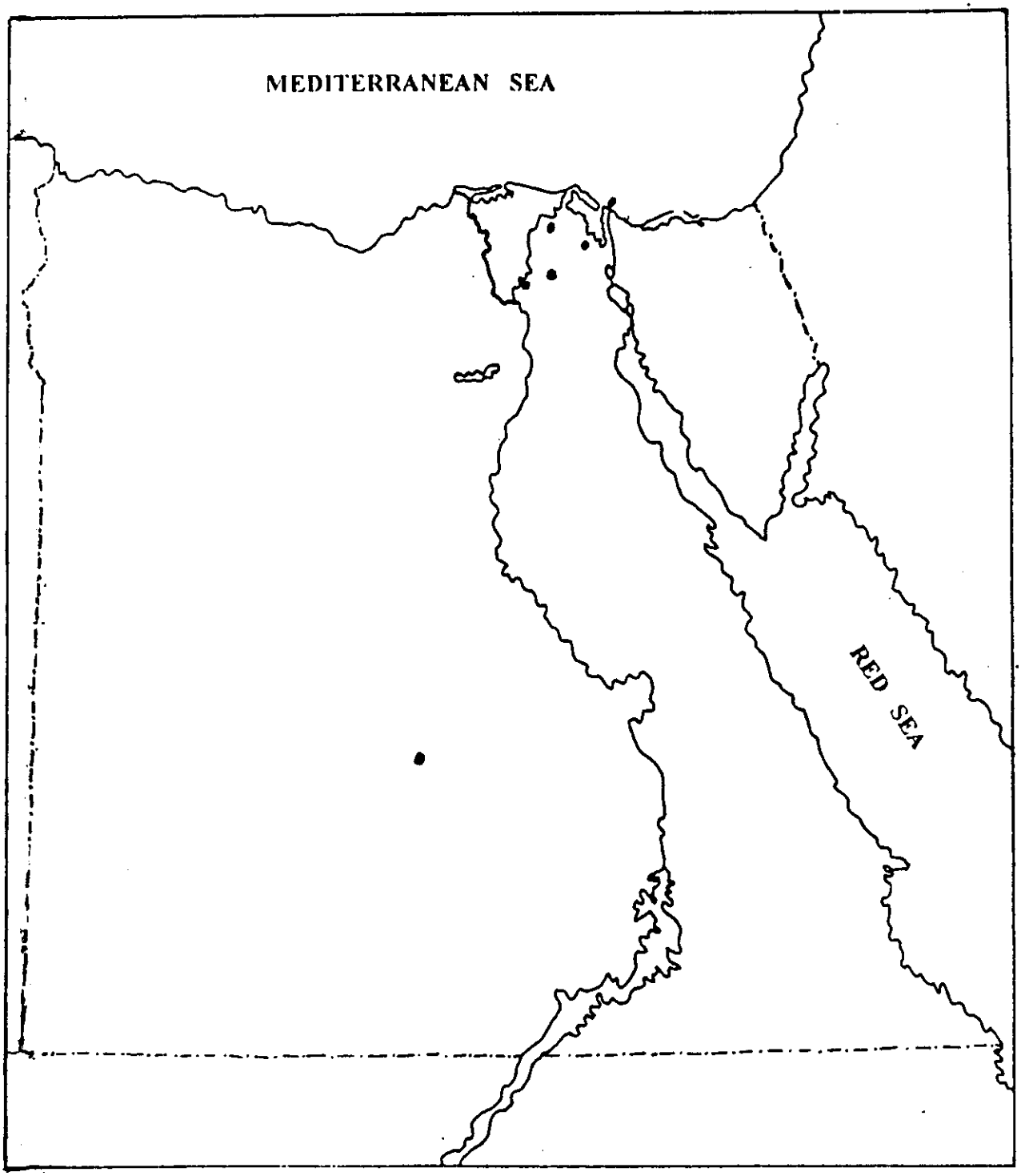


Fig. 2. Map of Egypt showing the area on the Nile valley where the isolates of streptomyces have been obtained.

Table 3. Screening of different types[a] of streptomyces isolates* obtained from the Egyptian soil at different localities.

Serial No. of soil	Serial No. of isolates		No. of isolates				No. of total isolates*
	from	to	yellow[a]	Red[a]	gray[a]	Blue green[a]	
1	1	100	-	(25)	(47)	-	72*
2	101	200	-	(8)	(43)	(1)	52*
3	201	300	(5)	(10)	(16)	-	31*
4	301	400	-	(7)	(21)	(2)	30*
5	401	500	(3)	(3)	(6)	-	12*
6	501	600	-	(5)	(8)	(1)	14*
7	601	700	(4)	(6)	(13)	(2)	25*
8	701	800	-	(25)	(18)	(2)	45*
Total No. of colored aerial mycelia			12**	89**	172**	8**	

a type of streptomyces isolates based on the color of aerial mycelium on the growth media.
* The No. of streptomyces isolates isolated from Egyptian soil at different localities (Table 2).
** The total No. of streptomyces isolates according to the color of their aerial mycelia.
( ) Value in brackets refer to No. of isolates of specific type out of the total No. of isolates isolated from each of the Egyptian localities that were chosen (Table 2)

Fig. 3. **(3.1 & 3.2)** Colony morphology of several streptomyces isolates of red and gray groups; Note: the inhibition activity of the two aggressive isolates (No. 112 and 279), as illustrated with arrows in **Fig 3.2**, against the plant fungal pathogens *M. phaseolina* **(M)** and *F. solani* f.sp. *phaseoli.* **(F).**

antimicrobial activity (Tables 7 and 8) and their other biochemical and physiological properties (Table 9).

4.2 Antagonistic Activity of Two Groups Isolates of *Streptomyces* spp.

4.2.1 *Antagonistic Properties of Red Group Isolates of Streptomyces*

Several isolates of the red group of streptomyces were screened for their antifungal activity towards *M. phaseolina* and *F. solani* f.sp. *phaseoli* pathogenic fungi. A significant differences between these isolates within the various groups were found.

The preliminary screening of the antifungal potentiality of the red group isolates revealed that isolates No. 50, 71, 115, 117 and 718 were active against the tested pathogens compared to the other ones (Table 4). All isolates of the red group which exhibited fungitoxicity were therefore discarded from the subsequent assessments with respect to their detailed cultural properties on different media as well as physiological properties, in addition to their antimicrobial activity *in vitro*.

Table 4. Antifungal potentiality of 19 isolates* of *Streptomyces* sp. (Red group type) that were cultivated on starch nitrate and fish malt extract.

19 isolates (Red group)*	Serial No. of representative isolate	Potential isolate ( )[a]	Fungitoxicity bioassay
I- Non pigmented			
Lavander	50, 115	(50)[a], (115)[a]	(++)
Rose with brown shade	313, 324	(313)[a],(324)[a]	(+)
Rose	69,71,117,824	(71)[a], (117[a]	(+++), (++)
Orange (insoluble)	838	-	(-)
Ten group	47	-	(-)
Eleven group	327	-	(-)
Twelve group	840	-	(-)
Thirteen group	710	-	(-)
II-pigmented			
Cinnamon	68,404	-	(-)
Violet	821	-	(-)
Red brown	700	-	(-)
Blue	314	-	(-)
Yellow	718	(718)[a]	(+++)

( )[a] isolate possesses fungitoxicity against the plant pathogenic fungi under this study

* refer to No. of streptomyces isolates of the red group type (Table 3).

(-) Non inhibitor (+) weak inhibitor

(++) moderate inhibitor (+++) intensive inhibitor

The degree of inhibition of the tested fungal growth as grown in the medium with the streptomyces isolates under study (+) width of inhibition zone < 1 mm, (++) < 2 mm, (+++) < 3 mm

4.2.2 *Antagonistic Properties of Gray Group Isolates of Streptomyces*

The gray group isolates of streptomyces were also screened for their antifungal activity against the previously mentioned plant pathogens, used in this study. Only two isolates No. 112 and 729 among the other tested isolates were shown to be the most active ones against the tested pathogens compared to the other ones (Table 5 and Fig 3). A closer attention was therefore paid to these two isolates (No.. 112 and 729) in subsequent *in vivo* trails study to test their potentiality under greenhouse conditions as well be indicated in the subsequent section.

Table 5. Antifungal potentiality of 83 isolates* of *Streptomyces* spp. (Gray group) cultivated on starch nitrate and fish extract.

83 isolates (Gray group)*	Serial No. of representative isolates	Potential isolates ( )[a]	Fungitoxicity bioassay
I- Non pigmented			
Red	155, 112, 219, 718, 85, 225, 305, 142, 6, 736, 153, 33, 220, 147, 16, 83, 101, 29, 312, 208, 88, 207, 301, 113, 75, 740, 505, 726	(112)[a], (85)[a] & (153)[a]	(++++) & (++)
Green	303, 329, 127, 29, 737, 514, 310, 515, 9, 1, 30, 835, 121, 15, 385, 77, 503,	(1)[a], (9)[a], (151)[a], (323)[a], (835)[a]	(++)
II- Pigmented			
Olive	729, 730	(729)[a]	(++++)
Yellow	14, 134, 25, 52, 86, 4, 104,	(104)[a],(134)[a]	(++)
Red	20, 23, 105, 213, 227,	-	(-)
Green	717,	-	(-)
Violet	312, 311, 328, 321, 316, 722, 133,	(133)[a]	(+)
Brown	506, 32, 501, 701, 702, 815, 510, 141, 300, 203,	(506)[a],(300)[a]	(++)
Blue	118, 209, 19, 719, 215, 316,	(118)[a]	(+)

( )[a] isolate possesses fungitoxicity against the plant pathogenic fungi under this study
* refer to No. of streptomyces isolates of the gray group type (Table 3).
(-) Non inhibitor (+) weak inhibitor
(++) moderate inhibitor (+++) intensive inhibitor
The degree of inhibition of the tested fungal growth as grown in the medium with the streptomyces isolates under study (+) width of inhibition zone < 1 mm, (++) < 2 mm, (+++) < 3 mm

4.2.2.1 *Detailed Culture Properties of The Two Selected Isolates of Streptomyces spp. (No. 112 & 729) on Different Media*

The two isolates (No. 112 & 729) were of completely different morphological forms with respect to their color pigmentation and their growth pattern on the various media that grew on them (Table 6). This has proved to be a consistent phenomenon during the subsequent studies with the two isolates. Isolate No. 112 was of predominately reddish gray color of varying degrees. Its growth was flat-powdery in appearance on starch nitrate medium (SNA), and fish meal medium (FMA), but it was of elevated pattern on glycerol nitrate medium (GNA). Also, this isolate possesses a different morphological form with respect to its color, particularly when it was grown on fish extract medium (FMA) where it appeared as yellow color form in FMA medium.

The other selected isolate (No. 729) was of predominantly olive gray color (insoluble). Its growth was of velvety appearance on the different tested media (Table 6) but it was of cottony growth and produced an olive greenish pigmentation on FMA medium. This isolate did not produce any pigmentation on the other tested media (Table 6).

Table 6. Culture properties of 15 day old cultures of selected Streptomyces isolates[a] (No. 112) and (No. 729) on different media.

Medium	Isolate[a]	Color pigmentation of			Type of growth[b]
		Aerial mycelium	Substrate mycelium	Medium	
(SNA)	112	Light reddish gray	Light yellow	Non pigmented	Flat
	729	White to light gray	Non pigmented	Pale yellow green in the bottom	Velvety
(GNA)	112	Reddish medium gray	Pale yellow	Non pigmented	Elevated
	729	Medium gray	Pale greenish yellow	Non pigmented	Velvety
(SEA)	112	Light reddish gray	Non pigmented	Non pigmented	Rare
	729	Medium gray	Non pigmented	Non pigmented	Velvety
(FMA)	112	Purplish white	Light yellow	Non pigmented	Flat
	729	Light olive gray	Dark greenish olive	Light greenish olive	Cottony

a isolates (No. 112 & 729) were selected based on their inhibition activity against the tested plant pathogenic fungi under study as indicated in Table (5).

b as grown in each of the tested mediums of SNA, starch nitrate agar; GNA, glycerol nitrate agar; SEA, soybean extract agar; and FMA, fish meal agar.

4.2.2.2 *Antimicrobial potentialities of the selected isolates of Stryptomyces spp. (No. 112 & 729)*

As was previously stated in subsection (4. 2. 2) further studies were only performed on the two isolates of streptomyces (No. 112 & 729) which were a prelude to biocontrol trails *in vitro* (Table 5). They were subjected to other tests in more detail to further examine their antimicrobial potentialities in various media towards *M. phaseolina* and *F. solani* f.sp. *phaseoli* pathogens, in particular, and to some other non phytopathogenic micro-organisms. The list of these microorganisms and their results are shown in Table 7 and 8. These results showed that SNA was superior to all other media used in terms of diameters (mm) of inhibition zones produced, after the incubation period for each individual micro-organism tested was ended on all the media used. Considerable inhibition of the growth of the target pathogens as well as the other tested micro-organisms, except for *E. coli, P. fluorescens* and *S. cervisiae,* were seen. The tested streptomyces isolates did not affect the growth of these later micro-organisms. It was of great interest that both of the tested isolates (No. 112 & 729) were more active against *M. phaseolina* and *F. solani* f.sp. *phaseoli* pathogens growth; as indicated by the largest inhibition zones as compared to the other tested micro-organisms (Table 7 & 8). Isolate (No. 729) showed one exception, in which it produced the largest inhibition zone only with the *M. phaseolina* (4 mm) while with the *F. solani* f.sp. *phaseoli* pathogen, the inhibition zone was of similar diameter of 2.5 mm approximately.

Table 7. Antimicrobial potentialities of streptomyces (isolate No. 112) as cultivated on different media[a]

Tested microorganism[b]	Zone of inhibition on different media[a] (mm)[c]		
	SNA[a]	FMA[a]	SEA[a]
M. phaseolina†	3.5	3	2.8
F.solani. f.sp. *phaseoli*†	2.3	2.1	1.5
B. subtilis	1.5	1.5	1.1
B. cereus	1.7	1.4	1.4
E. coli	0	0	0
P. fluorescens	0	0	0
C. albicans	2	1.5	1.8
S. cerevisiae	0	0	0

† antifungal potentially.

a- Full names of the medium as outlined in the text, see pp. 54-55 and Table 6.

b- Full names of the tested microorganism as out lined in the text.

c- Width of zones of inhibition in mm after 24-48 hr of incubation for bacteria and yeast and 7 day for plant pathogenic fungi.

Table 8. Antimicrobial potentialities of streptomyces (isolate No. 729) cultivated on different media[a]

Tested microorganism[b]	Zone of inhibition on different media[a] (mm)[c]		
	SNA[a]	FMA[a]	SEA[a]
M. phaseolina†	4	2.2	2.2
F.solani. f.sp. *phaseoli*†	2.5	2.3	2.1
B. subtilis	2.5	2.2	2
B. cereus	2	1.8	1.5
E. coli	0	0	0
P. fluorescens	0	0	0
C. albicans	1.8	1.5	1.2
S. cerevisiae	0	0	0

† antifungal potentially.

a- Full names of the medium as outlined in the text.

b- Full names of the tested microorganism as out lined in the text.

c- Width of zones of inhibition in mm after 24-48 hr of incubation for bacteria and yeast and 7 day for plant pathogenic fungi.

4.2.2.3 *Identification of The Selected Isolates of Streptomyces spp. (No. 112 & 729)*

The main characteristics used to identify the isolated isolates of streptomyces (No. 112 & 729) are described in this section (Table 9). These involved cultural properties ,i.e. , spore morphology, under (S.E.M) in addition to physiological properties in order to place these isolates into its family and in its appropriate genera. Based on these previous characteristics and following the keys of **Waksman and Lechevalier (1953), Pridham 1970 and Bergey's Mannuals (1984, 1989),** it appeared that the isolated organism No. 112 and isolate No. 729 were similar to the species *roseodiastaticus* and *olivaceiscleroticus,* respectively. Since the previous properties can not be used alone to separate the isolates; the cell wall chemotaxonomy and the use of polymorphism in rDNA (restriction fragment pattern) are regarded to be a crucial supplemented taxonomic technique.

Table 9. Identification of the selected isolates[a] of streptomyces using the classical methodology.

Test	Reaction of isolate[a]	
	No. 112[a]	No. 729[a]
Melanin production	-	-
H_2S formation	-	-
Growth on medium[c]	flat	velvety
Spore chain [d]	short loose spiral	short primitive spiral
Spore surface[d]	smooth	smooth
Color of aerial mycelium[c]	Red	Gray
Color of substrate mycelium[c]	yellow	olive
Antifungal activity[E]	+	+
Antibiotic production[f]	+	+
Color of pigmentation[c]	non-pigment	olive
Suspect name[b]	*S. roseodiastaticus Bergy's p.779No 180*	*S. olivaceiscleroticus Bergy's p.778 No 168*

a- The selected isolates of streptomyces were No. 112&729. Table (6,7) for more detailed information.
b- Following the keys of **Waksman & Lechevalier (1953)** and **Pridham (1970)**.
C- Growth on different medium. (Table 6)
d- Morphology of spore under S.E.M. (scanning electron microscopy)
E,f- In vitro against the tested plant pathogenic fungi. (Table 7&8)

4.3 Phytotoxicity Bioassay

4.3.1 Phytotoxicity on Soybean

Culture filtrates (CFs) of the biocontrol agents to be tested in this work, were assayed for phytotoxicity in soybean seeds, *in vitro* (by the germination and radical growth assay). Seeds were dipped in each of the CFs at various serial concentrations which were conducted and employed as outlined in materials and methods. The results are shown in (Table 10).

Generally, a considerable reductions of the length of the radical were apparent with the CFs used at higher concentrations. These inhibitory effects can be considered moderate at lower concentrations (ranged from 60-10%) see (Table 10). It was also apparent, that adventitious roots were produced but with the CF of the biocontrol agents *S. roseodiastaticus* (isolate No. 112) at very low concentration (10%).

Closer examinations of the results in (Table 10), indicated that the CFs of the biocontrol bacteria at the higher concentrations was the most toxic among the other biocontrol agents tested. It caused reduction of the radical length by 91.5% of the water control, while the CFs of the *S. roseodiastaticus* (isolate No. 112) caused reduction by 71.5-56% compared with water control.

CF of the other isolate of streptomyses used in this assay, i.e., (*S. olivaceiscleroticus* (isolate No. 729)) was slightly more toxic at the same concentration as compared to the other tested CF of the streptomyces isolate (*S. roseodiastaticus* (isolate No. 112)).

Similarly, CF of the Trichoderma biocontrol maintained similar trend in comparison to the *S. roseodiastaticus* (isolate No. 112) up to 60% concentration.

Table 10. Effect of biological control agent culture filtrate at different concentrations on germination of soybean seeds cv. (Crowford) in the laboratory.

Treatment	*B. subtilis*[a]		*S. roseodiastaticus*[b]		*S. olivaciescleroticus*[b]		*T. harzianum*[c]	
	No. of seeds germinated	Length of the radical	No. of seeds germinated	Length of the radical	No. of seeds germinated	Length of the radical	No. of seeds germinated	Length of the radical
10%	10	2	10	3.8	10	5.5	10	4
20%	9	1.5	10	3	9	3	9	2
30%	9	1.5	9	3.5	8	4	9	3
40%	8	1.3	8	2	9	2.5	8	2.5
50%	8	1	9	1.5	7	3	8	2.5
60%	7	0.5	9	2.5	8	2.5	9	2.5
70%	7	0.7	9	2	7	1.5	9	2
80%	5	0.7	8	2	7	1	10	2.5
90%	10	0.3	10	5.5*	7	2	10	2
100%	4	0.4	10	1.5	8	2	10	1.5
Control water	10	4.5	10	4.5	10	4.5	10	4.5

a,b,c The full name of the organism see the text
* adventitious root
N.E Not examined

At lesser concentrations (30%), the *S. olivaceiscleroticus* (isolate No. 729) CF was more toxic than *S. roseodiastaticus* (isolate No. 112) CF. The radicals were reduced by 56% with the former and by 20% with the latter. This evidence indicates that the *S. olivacescleroticus* CF has a potential ability to inhibit the radical growth at low concentrations (10-20%).

4.3.2 *Phytotoxicity on Sunflower*

The phytotoxicity of the biocontrol culture filtrates on the sunflower seeds plants was tested. The phytotoxicity bioassay was similarly performed as the soybean phytotoxicity assay and as outlined in the materials and methods (section 3.9). The results are shown in (Table 11). In this second crop under study, similar results were obtained, as with the previous tested crop (soybean, see the preceding section), in the form of apparent visible damage to the sunflower radicals (Table11).

Drastic changes in the length of radical, in all the biocontrols used at all different concentration levels, were observed, however, these changes were more noticeable at higher concentrations, (Table 11).

Also, as it was observed in the soybean phytotoxicity trial, a biologically active constituent(s) other than the toxins were detected in the CFs as indicated by the presence of adventitious roots. This suggests that not all the biological factors of the biocontrol agents, produced in culture, used in this work are necessarily phytotoxic. The possible effects of the toxins in addition to the biologically active constituent(s), other than the toxins that their activities have been demonstrated in the (CFs) of these biocontrols, will be discussed later in chapter 5.

Table 11. Effect of biological control agent culture filtrate at different concentrations on germination of sunflower cv. (Miak) in the laboratory.

Treatment	*B. subtilis*[a]		*S. roseodiastaticus*[b]		*S. olivaciescleroticus*[b]		*T. harzianum*[c]	
	No. of seeds germinated	Length of the radical	No. of seeds germinated	Length of the radical	No. of seeds germinated	Length of the radical	No. of seeds germinated	Length of the radical
10%	10	0.8	9	2.5*	10	2	10	1.9
20%	9	1.0	9	1.8	10	2.5	10	1.8
30%	9	0.7	9	3	10	1.5	10	2
40%	9	0.6	9	1.5	10	2	10	1.7
50%	8	0.5	10	1.0	10	2	10	2.2
60%	9	0.4	9	2	9	1.5	10	1.7
70%	9	0.6	10	2	8	1.3	10	1.5
80%	10	0.5	10	2	9	1	10	1.2
90%	9	0.4	10	1	9	1.5	10	1
100%	9	0.3	10	1.5	9	1	10	0.9
Control water	10	3.5	10	3.5	10	3.5	10	3.5

a,b,c The full name of the organism see the text
* adventitious root
N.E Not examined

4.4. Biocontrol Studies

4.4.1 *In Vitro*

4. 4.1.1 *Antagonistic Studies*

Antagonism between several microorganisms; *Bacillus subtilis, Streptomyces roseodiastaticus* (isolate No. 112), *Streptomyces olivaceiscleroticus* (isolate No. 729) and *Trichoderma harzianum* which were non-pathogenic to the target plants of this study, and *M. phaseolina* and *F. solani f. sp. phaseoli;* the fungal pathogens of the target plants, i.e., the soybean and sunflower plants, were firstly investigated *in vitro.*

Reduction in linear growth of each of the tested pathogen on the side near each of the antagonist to be tested was recorded. Figs. 4.1 and 5.1 showed that *B. subtilis* has greatly reduced the linear growth of *M. phaseolina* and *F. solani* f.sp. *phaseoli.* Also, *Streptomyces roseodiastaticus* and *streptomyces olivaceiscleroticus* have antagonistic effects on *F. solani* f.sp. *phaseoli* and *M. phaseolina.* On the other hand *T. harzianum* overgrew over more than two third of the petri dishes and it spreaded rapidly and reduced the linear growth of *M. phaseolina.* Moreover, its overgrowth over the *M. phaseolina* can be seen from Fig. 4.2. While in the *F. solani* f.sp. *phaseoli* case, it was limiting its growth to small area. . No. inhibition zones were observed, in addition, yellow pigment was seen on the tested medium (Fig. 5.2). Furthermore, direct contact was also observed between the *T. harzianum* and the two tested fungi.

Fig. 4. Antagonism between *M. phaseolina (M)* and : **4.1**, *B. subtilis* **(B); 4.2**, *T harzianum* **(T)**

Fig. 5 Antagonism between *F. solani* f.sp. *phaseoli* **(F) and: 5.1,** *B. subtilis* ; 5.2, *T. harzianum* **(T).**

4.4.2 *In Vivo*

4.4.2.1 *Pathogenicity Test And Disease Evaluation*

Pathogenicity of each of the isolates of *M. phaseolina* and the isolates of *F. solani* f.sp. *phaseoli* which were used throughout this study, against soybean cv. (Crowford) and sunflower cv. (Miak) respectively, were tested as outlined previously in the materials and methods. Specific pathogenicity was shown by these fungi isolates under study. Firstly, under a growth cabinet incubation condition using 10 day old excised hypocotyls of cv. Crowford, (susceptible to the two previously tested isolates by **(Emara 1987)**), using the droplet technique. Secondly, under greenhouse condition using also the plants of the cv. crowford that were grown from seeds sown in the soil which were also used throughout this work using the soil infestation technique as outlined in the materials and methods. The results of subsequent experiments of these preliminary pathogenicity test are given in the subsequent subsection.

4.4.2.2 *Pathogenicity Test By Drop Technique In Growth Cabinet.*

Soybean (cv. Crowford) seedling excised hypocotyls were tested with the two desired isolates of the pathogenic fungi, *M. phaseolina* and *F. solani* f.sp. *phaseoli* to check their susceptibility and resistance. Excised hypocotyls from ten day seedlings, grown at environmental control condition, were inoculated by the droplet technique as outlined in materials & methods and their macroscopic symptoms were periodically examined.

The observed disease symptoms on soybean hypocotyls, ten days after inoculation and incubation at $30^{o}C$, were recorded as indicated in (Table 12) and was classified as :- Susceptible, if brown and gray blackening of hypocotyls associated with mycelium and sclerotia formation was observed with the former pathogen and if extensive browning and rotting together with water soaking associated with mycelia formation on the surface of hypocotyl were observed with the later pathogen.

Table 12. Soybean cv. (Crowford) reaction to *M. phaseolina* and *F. solani* f.sp. *phaseoli* at 30^{0}C in growth cabinet.

Pathogen**	Host* reaction
*M. phaseolina***	S
F. solani f.sp. *phaseoli***	(S)

* 7-10 day old excised hypocotyls of soybean cv. Crowford

** Age of inoculum 7-10 day.

S Susceptible, rotting and gray blackening of the inoculated hypocotyls associated with mycelia and sclerotia.

(S) Susceptible, browning and rotting of the inoculated hypocotyls associated with mycelial formation

Fig. 6. Various symptoms on excised soybean hypocotyls of 10 day old seedlings, artificially inoculated with: 6.1, *M. phaseolina*; 6.2, *F. solani* f.sp. *phaseoli* and were incubated in the growth cabinet at 30o C.

Accordingly the tested *M. phaseolina* used in the present work caused the charcoal rot disease characteristic in soybean. While, the *F. solani* f.sp. *phaseoli* produced the root and stem rot disease characteristic in soybean.

4.4.2.3 *Evaluation of Disease Symptoms Development in Soybean (Excised) Hypocotyls in Growth Cabinet.*

Soybean excised hypocotyls cv. Crowford were inoculated with *M. phaseolina* and *F. solani* f.sp. *phaseoli* using the droplet technique previously described in the Materials & methods and incubated at 30°C. Daily observations of the symptoms development at the inoculated sites were carried out.

The results obtained (Table 13) showed that soybean (excised) hypocotyls, when inoculated by *M. phaseolina* and incubated at 30°C became susceptible. These observations also revealed that light brown flecks were formed two days after inoculation. However, extensive browning of hypocotyls was observed after four days. After six days, rotting of the hypocotyls and sclerotia formation on the surface were also observed, see Fig. 6.1.

Soybean (excised) hypocotyls reaction to inoculation by the other pathogenic fungus, i.e., *F. solani* f.sp. *phaseoli* and incubation at 30°C, also, confirmed their susceptibility. The time course of symptom development showed that two days after inoculation, soybean hypocotyls cv.(Crowford) exhibited slight light brown flecks. After four days, the long light brown streaks appeared; while six days after inoculation, the long dark brown streaks were observed; seven to ten days after inoculation, rotting of the hypocotyls associated with mycelial growth were observed, see Fig. 6.2.

Table 13. Time course of development of disease symptoms on soybean excised hypocotyls[a] after inoculation[b] by *M. phaseolina* and *F.solani* f.sp. *phaseoli* at 30^{0}C in the dark.

Time after inoculation[b]	Disease symptoms of soybean[a] with fungal pathogen	
	M. phaseolina[b]	*F. solani* f.sp. *phaseoli*[b]
0-1	no apparent symptoms	no apparent symptoms
2	numerous light brown flecks	minute light brown flecks
3	long light brown streaks	long light brown streaks
4	long dark brown streaks	long light brown streaks
5	browning of the hypocotyls and rotting begin	long light brown streaks
6	formation of sclerotia on the hypocotyls surface	intense dark browning of the hypocotyls
7-10	increased rotting and gray blackening of the hypocotyls	browning, associated with rotting and mycelium formation on the surface

a- 7-10 day old excised hypocotyls of soybean cv. Crowford
b- Inoculation was applied at concentration of 1x105 sclerotia/ml for *M. phaseolina* and 1x10^6 spores/ml for *F. solani* f.sp. *phaseoli*.

4.4.2.4 *Pathogenicity Assessment Under Greenhouse.*

The data obtained, which are presented in (Table 14), indicate that the two tested fungi were generally pathogenic to soybean and sunflower seedling. Thus, the isolate of *F. solani* f.sp. *phaseoli* used in this study caused root rot and wilt disease on soybean and sunflower seedling. The Fusarium pathogen appeared to be more virulent than the Macrophomina pathogen to soybean than to sunflower, especially at the pre and post emergence stages of plant development (Table 14). It produced severe symptoms of chlorosis and root rot leading to death of infected plants. It restricted the growth of the plant and reduced leaves size. Moreover it also reduced pods number or head diameter per plant within the soybean and sunflower plants.

It should be noted that the etiology of the disease caused by the *F. solani* f.sp. *phaseoli* isolate under study on soybean and sunflower plants in greenhouse growing plants was established as follows. The first case concerned young plants of 15 day old (seedling stage) in which plants survived but were most seriously affected by the infection in the adult plants. This was probably caused by the pathogen from the young plants. The second one was on vegetative stage of 30 day old plants after inoculation, a high proportion of apparently healthy plants was observed as compared with the infected plants. Also, there were no external symptoms yet at this stage. However, internal brown discoloration of the vascular tissue was seen. Slight browning of the root system and slight yellowing of the leaves were visible but no progressive symptoms was noticed. Later on, at the fruiting stage of 60 day old plants, large portion of the leaves were found shriveled. Also, characteristic dark brown spots were noticed. Shoots often wilt, curled and retained yellowing and furthermore dead leaves were also seen (Figs. 8 and 10). Several shoots appeared wilt during this development stage. Generally, the most conspicuous symptom was browning of the root system and typical symptoms of root rot and wilt appeared.

Table 14. Effect of *M. phaseolina* and *F. solani* f.sp. *phaseoli* inoculation on seedlings emergence and yield of soybean cv. (Crowford) and sunflower cv. (Miak) under greenhouse condition.

Pathogen[b]	Host[a]					
	soybean			sunflower		
	pre-emergence damping off	post-emergence damping off	surviving (apparent healthy)[c]	pre-emergence damping off	post-emergence damping off	surviving (apparent healthy)[c]
M. phaseolina[b]	1/15	2/15	12/15	1/15	4/15	10/15
F.solani f.sp. *phaseolina*[b]	3/15	-/15	12/15	-/15	2/15	13/15
Control	-/15	-/15	15/15	-/15	-/15	15/15

a- 15 seeds of either soybean or sunflower were sown in sterile sandy clay at rate 1:2 (v/v).
b- Age of the fungal pathogen used was 15 day at the rate of 3% (w/w) sandy clay soil.
c- Mean No. of seedlings emerged out of 15 sowing seeds and looked apparently healthy at the time of recording date which was at the fruiting stage.

Fig. 8. Various symptoms on soybean artificially inoculated with *F. solani* f.sp. *phaseoli*: **(A),** healthy; **(B),** diseased; compare the difference in leaves size (head arrow) and the blight of leaves (arrow).

Fig. 9. Various symptoms of charcoal rot disease on sunflower artificially inoculated with *M. phaseolina:* **(A),** healthy; **(B),** diseased; showing a tattered and blight plant; capony leaves are brown in appearance.

Fig. 10. Various symptoms of Fusarium root rot disease on sunflower artificially inoculated with *F. solani* f.sp. *phaseoli*: **(A),** healthy; **(B),** diseased; showing browning of the stem; a tattered and blight capony appearrance of leaves; accelerated flowering rate as compared to the control.

Moreover, the isolate of the *M. phaseolina* pathogen used in this study caused root and stem rot disease on soybean and sunflower. The symptoms of the seedling and vegetative stages were more or less similar to that with *F. solani* f.sp. *phaseoli*. The differences in the later stage (fruiting) were: leaves became more yellowing. The comparable shoots were often dead, (Figs. 7.1, 7.2 and 9) and visible die-back was produced by a dark browning of the pith and the immediate surrounding sapwood in proximate stem portions. The root system was dark brown. These were the main features of the symptoms of *Machrophomina pathogen* which were associated with gray blackening of the infected tissue.

No. symptoms of the disease were recorded in the control plants which continued to produce pods. The control plants produced three times the number of pods per plant or head per plant 60 and 85 days for soybean and sunflower plants, respectively, as compared with the inoculated plants. Re-isolation from the inoculated plants yielded the same fungi that gave the same symptoms resulted from the inoculated isolates of both the tested fungal pathogens; thus fulfilling Koch's postulates.

It could be concluded that *F. solani* f.sp. *phaseoli* caused extensive root rot of soybean plants than *M. phaseolina* under greenhouse condition irrespective of plant stage development, unlike the result with sunflower plant, where rotting was severe leading to pronounced symptoms with *M. phaseolina* than with *F. solani* f.sp. *phaseoli*.

4.4.3 *Efficacy of Several Biocontrol Agents on Disease Severity And Plant Growth*

Data in (Tables 15 and 16) show the disease expression of soybean and sunflower when the seeds were coated separately with each of the spore suspension of *B. subtilis, S. roseodiastaticus, S. olivaceiscleroticus* and *T. harzianum*. These data illustrate that seeds, that were treated with each antagonist singly and grown in soil infested at a rate of 3% (w/w) with *M. phaseolina* or *F. solani* f.sp. *phaseoli*, proved to be quite effective in reducing the pre-

emergence damping off incidence. Also, plant growth was significantly increased . Such increase was in fresh weight of shoot bearing leaves and roots per plant.

The incidence of both diseases of soybean and sunflower (numbers) and fresh weight of plants and also, the numbers and weights of apparently healthy plants were recorded over 18 days harvest period following inoculation with either *M. phaseolina* or *F. solani* f.sp. *phaseoli.*

The yield of survival plants (apparently healthy) from soybean pots inoculated with *M. phaseolina* or *F. solani* differed slightly from the uninoculated controls, although soybean infection at the growing seedling stage was detected. This is unlike the sunflower plants-infected with *M. phaseolina* or *F. solani* f.sp. *phaseoli* where few plants died following their emergence.

The yield from pots of soybean plants infected with either *M. phaseolina* or *F. solani* f.sp. *phaseoli* was slightly less than both the controls (untreated-uninfected) and those of the other infected hosts (sunflower). Further, compared with the controls, the number of diseased plant at the post emergence stage of sunflower plants differed greatly from the protected ones.

The uninoculated control treatment remained diseases free throughout the experiment. Also, none of the biocontrol agents produced any symptoms. Each individual biocontrol agents used was effective in varying degrees and they all showed an increase in fresh weight.

S. roseodiastaticus (isolate No. 112) and *S. olivaceiscleroticus* (isolate No. 729) treatments increased the fresh weight of soybean and sunflower plants upon their simultaneous application with the pathogen. Differences in fresh weight between the various biocontrol agents could be seen from Tables 15 and 16.

Table 15. Effect of biocontrol[a] on pre and post emergence damping off caused by *M. phaseolina* in soybean cv. (Crowford) and sunflower cv. (Miak).

	Treatment	Soybean			Fresh wt. (gm)/ 5 plants[d]	Sunflower			Fresh wt. (gm)/ 5 plants[d]
		pre-emergence damping off	post-emergence damping off[b]	surviving (apparent healthy)[c]		pre-emergence damping off	post-emergence damping off[b]	surviving (apparent healthy)[c]	
A	**Pathogen only**								
	M .phaseolina	1	-	9	4.8	2	2	6	13
B	**Pathogen + Biological agent**								
B1	*M+B. subtilis*	1	-	9	7.0	1	1	8	17
B2	*M+ S. roseodiastaticus*	-	-	10	7.5	-	-	10	23
B2\	*M + S. olivaceiscleroticus*	-	-	10	7.2	-	-	10	18.5
B3	*M+ T. harzianum*	-	-	10	5.5	1	-	9	15
C	**Biological agent**								
C1	*B .subtilis*	-	-	10	7.8	-	-	10	18
C2	*S. roseodiastaticus*	-	-	10	8.7	-	-	10	23.5
C2\	*S. olivaceiscleroticus*	-	-	10	8.3	-	-	10	19.8
C3	*T. harzianum*	-	-	10	7.0	-	-	10	17.0
D	*Control (water) only*	-	-	10	5.0	-	-	10	15.0

a- Seed coated by biological agent as indicated in the text
b- Result recorded at the seedling stage, 18 day old plant
c- 10 seed of either soybean or sunflower were sown in sterile sandy clay 1:2 (v/v) at rate of 3% (w/w) inoculum.
d- Represent the fresh weight(gm) / 5 random plants which were chosen out of 10 plants.

Table 16. Effect of biocontrol[a] on pre and post emergence damping off caused by *F. solani* f.sp .*phaseoli* in soybean cv. (Crowford) and sunflower cv. (Miak).

	Treatment	Soybean			Fresh wt. (gm)/ 5 plants[d]	Sunflower			Fresh wt. (gm)/ 5 plants[d]
		pre-emergence damping off	post-emergence damping off[b]	surviving (apparent healthy)[c]		pre-emergence damping off	post-emergence damping off[b]	surviving (apparent healthy)[c]	
A	**Pathogen only**								
	F. solani f.sp. *phaseoli*	2	-	8	4.5	1	1	8	14
B	**Pathogen + Biological agent**								
B1	*F + B. subtilis*	-	-	10	7	2	-	8	15
B2	*F+ S .roseodiastaticus*	1	-	9	6.6	-	-	10	21
B2	*F + S. olivaceiscleroticus*	-	-	10	6.2	1	-	9	18
B3	*F+ T. harzianum*	1	-	9	5.0	-	-	10	15
C	**Biological agent**								
C1	*B. subtilis*	-	-	10	7.8	-	-	10	18
C2	*S. roseodiastaticus*	-	-	10	8.7	-	-	10	23.5
C2	*S. olivaceiscleroticus*	-	-	10	8.3	-	-	10	19.8
C3	*T. harzianum*	-	-	10	7.0	-	-	10	17.0
D	*Control (water) only*	-	-	10	5.0	-	-	10	15.0

a- Seed coated by biological agent as indicated in the text
b- Result recorded at the seedling stage, 18 day old plant
c- 10 seed of either soybean or sunflower were sown in sterile sandy clay 1:2 (v/v) at rate of 3% (w/w) inoculum.
d- Represent the fresh weight(gm) / 5 random plants which were chosen out of 10 plants.

4.4.3.1 *Effect of Biocontrol (Seed Treatment) on Soybean* Macrophomina *Charcoal Rot Severity And Plant Growth Under Greenhouse Conditions*

The susceptibility of cv. Crowford of soybean seeds to *M. phaseolina* was tested. Seeds of soybean were coated with different bicontrol agents and sown in the soil infested with the Machrophomina charcoal rot pathogen at rate of 3% of sandy clay soil as outlined in (Materials and methods). The susceptibility was noted after the pre emergence and post emergence stages till the fruiting stage. Results are shown in Table 17 and Fig. 11.

No. sign of infection was seen on the plants grown from the seeds inoculated only with any of the tested biocontrol agents. However, plants grown from the seeds treated using each of the biocontrol agents and which were planted in the infested in the soil pots incorporated with the Machrophomina pathogen were affected in varying degrees. They demonstrated an increase in various plant growth parameters and yield production, see Figs. 11, 12 and 13. The uninoculated control plants remained disease free throughout the experiment.

The overall appearance of plants showed similarity among the four biocontrol agents used. However, in the *B. subtilis* and the *S. roseodiastaticus* (isolate No. 112) treatments the plants showed the greatest level of growth parameters and yield production than with the cases of *S. olivaceiscleroticus* and the *T. harzianum* treatments when compared with Machrophomina infected plants (control) that showed much disease progress and greater yield reduction as a result of symptoms caused by the Machrophomina charcoal rot pathogen, see Fig. 11. This charcoal rot causing pathogen produced few rotted rot tips, brown streak were observed on stem, node and branches and heavily infected plants exhibited a tattered and blight capony appearance of foliage parts. Brown spots or circulars in shape were seen on yellow leaves and on pods. Seeds that were also infected in the immature green stages failed to develop further.

Table 17. Effect of biocontrol (seed treatment) soybean cv.(Crowford) on charcoal rot severity[a] and plant growth[b] under greenhouse

Treatment		Soybean[c]											
		Stems			leaves			Flowering rate	pods[b]		state of seeds[s]		
		Thickness (hardness)	length	Branching	size	Thickness (texture)	color		size	color	No.	type	fresh wt (gm)[b]
A	Pathogen only *M. phaseolina*	++	++±	5	less	++±	yellow[b]	63 day	less	brown	(2)	shrinked	0.53
B	Pathogen + Biological agent												
B1	*M+B. subtilis*	++	+++	7	large	++*	spots green	58 day	larger	green	(3)$^{+}$	full	0.92
B2	*M+ S. roseodiastaticus*	+++	++++	7	large	++*	spots green	57 day	larger	green	(3)$^{++}$	full	1.15
B2\	*M+S. olivaceiscleroticus*	+++	++	5	large	++	spots green	58 day	large	y.s. green	(3)$^{++}$	full	1.06
B3	*M+ T. harzianum*	+++	++	5	normal	++±	green	60 day	normal	B.green	(3)	full	0.75
C	Biological agent												
C1	*B subtilis*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C2	*S. roseodiastaticus*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C2\	*S. olivaceiscleroticus*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C3	*T. harzianum*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
D	*Control (water) only*	++	++	5	normal	++	green[a]	60 day	normal	green	(3)	full	0.72

a- assesment of disease severity grade based on: 0,1,2 &3 ;< 0.72= normal (healthy) ; >0.053 disease

b- based on the weight of 5 random seeds

c- result, recorded after flowering and fruiting stage of plant (soybean i.e. after 60 days from the time sowing

±less ++ moderate +++high +*more B brown Y.S yellow spots

Fig. 11. Various symptoms and plant growth parameters on soybean artificially inoculated with: **(B),** *M. phaseolina*; **(C),** *B. subtilis*; **(D),** *S. roseodiastaticus* (isolate No. 112); **(E),** *S. olivaceiscleroticus* (isolate No. 729); and **(F),** *T. harzianum*. Note:- **[C, D, E and F]** were simultaneouly subjected to the *Macrophomina* pathogen compared to **(A)** which represents the untreated uninfected control (water); **[C & D]** show the greatest level of plant growth parameter (plant hight and yield production).

Heavy infection was also present in the maturing pods. Small size and light weight infected seeds were not able to germinate. Moreover, great reduction in growth parameters, e.g., stems (thickness, length and branching), leaves (size, texture and color) and pods (size and color) and state of seeds (number., typeand fresh weight) were observed as compared to the untreated uninfected seed grown plants (controls).

4.4.3.2 *Effect of Biocontrol (Seed Treatment) on Soybean* Fusarium *Root Rot Severity And Plant Growth Under Greenhouse Conditions*

The susceptibility of cv. Crowford of soybean to *F. solani* f.sp. *phaseoli* was tested. Seeds of soybean were coated with different biocontrol agents and sown in infested soil with the Fusarium rot pathogen as outlined in (Materials and Methods). The susceptibility was noted at the pre-emergence stage of seedling and post emergence stage till fruiting stage. Results are shown in (Table 18).

Difference between the various biocontrol agents could be seen in disease progress symptoms and plant growth parameters up to seedling stage, see Fig. 14.

S. roseodiastaticus (isolate No. 112) and *T. harzianum* treatments showed similar and the highest level of yield production and plant growth parameters compared with S. olivaceiscleroticus (isolate No. 729) and *B. subtilis* treatments which showed lesser levels. While the Fusarium rot pathogen continued to cause progressive disease and much greater yield reduction as a result of symptoms caused by it. The disease was expressed by the production of root rot on the main root in addition to lateral root. Dark brown spots and necrosis were noticed on stem. Small size and yellowing leaves appearance of the whole plant were also seen. Several shoots often curled and retained dead leaves. Heavily infected plants exhibited blight appearance capony of foliage parts; pods and seeds appeared shrinked; also, infected seeds did not germinate. Moreover, great reduction on growth parameters, stems (thickness and length),

Table 18. Effect of biocontrol (seed treatment) soybean cv.(Crowford) on Fusarium root rot severity[a] and plant growth[b] under greenhouse

	Treatment	Soybean[c]											
		Stems			leaves			Flowering	pods[b]		state of seeds[b]		
		Thickness (hardness)	length	Branching	size	Thickness (texture)	color	rate	size	color	No.	type	fresh wt (gm)[b]
A	Pathogen only *F.solani f.sp.phaseoli*	++_	++	5	less	++_	yellow[b]	62 day	less	yellow	(2-3)	shrinked	0.69
B	Pathogen + Biological agent												
B1	*F+B.subtilis*	+++	+++	6	large	+++	pale green	58 day	larger	green	$(3)^{++}$	full	1.20
B2	*F+ S.roseodiastaticus*	++	++++	7	large	+++	pale green	58 day	larger	green	$(3)^{+++}$	full	1.30
B2\	*F+ S.olivaceiscleroticus*	+++*	++	5	normal	++	pale green	58 day	normal	green	$(3)^{+}$	full	1.15
B3	*F+ T.harzianum*	+++	+++*	7	large	+++	pale green	60 day	normal	green	$(3)^{+}$	full	1.25
C	Biological agent												
C1	*B.subtilis*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C2	*S.roseodiastaticus*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C2\	*S.olivaceiscleroticus*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C3	*T.harzianum*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
D	*Control (water) only*	++	++	5	normal	++	green[a]	60 day	nrmal	green	(3)	full	0.72

a- assesment of disease severity grade based on: 0,1,2 &3 ;< 0.72= nornal (healthy) ; > 0.69 disease

b- based on the weight of 5 random seeds

c- result, recorded after flowering and fruiting stage of plant (soybean i.e after 60 days from the time sowing

+_less ++ moderate +++high +*more

Fig. 12. Various symptoms and plant growth parameters on soybean artificially inoculated with: **(B),** *M. phaseolina*; **(A),** *S. roseodiastaticus* (isolate No. 112) and simultaneously subjected to *M. phaseolina*. Note: plant hights in **(A)** and **(B).**

Fig. 13. Various symptoms and plant growth parameters on soybean artificially inoculated with *M. phaseolina* and simultaneously subjected to: **(A),** *S. olivaceiscleroticus* (isolate No. 729); **(B),** *T. harzianum*, e.g., compare the size of pods in **(A)** and **(B),** (see arrows).

Fig. 14. Various symptoms and plant growth parameters on soybean artificially inoculated with: **(B),** *F. solani* f.sp. *phaseoli*; **(C),** *B. subtilis*; **(D),** *S. roseodiastaticus* (isolate No. 112); **(E),** *S. olivaceiscleroticus* (isolate No. 729); and **(F),** *T. harzianum*. **[C, D, E and F]** were simultaneously subjected to the Fusarium pathogen compared to **(A)** control (water). Note:. **[D & F]** show the greatest level of plant growth parameter (plant hight and yield production).

leaves (branching, size, texture and color), pods (size and color) and state of seed (number, type and fresh weight/gm) compared to the controls (water), untreated and uninfected seed grown plants.

However, yield comparison with soybean plants, infected with either *M. phaseolina* or *F. solani* f.sp. *phaseoli* revealed that there was much greater effect with *S. roseodiastaticus* (isolate No. 112) than with the other biocontrol agents used. Thus in practical terms the *S. roseodiastaticus* (isolate No. 112) has induced protection against both pathogens and could be said to be more efficient.

B. subtilis also induced considerable protection against the *M. phaseolina* pathogen only and with lesser efficiency against the Fusarium rot pathogen. But, *T. harzianum* induced remarkable protection against *F. solani* f.sp. *phaseoli.*

4.4.3.3 *Effect of Biocontrol (Seed Treatment) on Sunflower Charcoal Rot Severity And Plant Growth Under Greenhouse Conditions*

The susceptibility of cv. Miak of sunflower to *M. phaseolina* was tested. Seeds of sunflower were coated with different bicontrol agents and sown in infested soil with the pathogen as outlined in (Materials & Methods). The biocontrol agents used are those which were used in the previous experiments with soybean. The susceptibility was noted at the pre-emergence stage of seedling and post-emergence stage till fruiting stage. Results are shown in Table 19.

Difference between the various biocontrol agents could be seen in disease progress symptoms and plant growth parameters up to seedling stage (Fig. 15)

S. roseodiastaticus (isolate No. 112) treatment showed the highest level of plant growth parameters and yield production compared with the other treatments. While *M. phaseolina* caused progressive disease and much greater yield reduction as a result of symptoms caused by Macrophomina *pathogen* which produced a few rotted root tips and brown streak were observed on stem. Heavily infected plants exhibited a tattered and blight, capony appearance of foliage leaves. Seeds appeared shrinked and infected and did not germinate. Superficial mycelium growing on living plants was seen (loose, freely web). Production of sclerotia under which infection occurs was also observed. This was considered to be the most striking feature of the infected plants: head bearing shrinked seeds did not germinate. Moreover, great reduction in growth parameters, stems (thickness and length) leaves (size, texture and color) and head diameter including state of seeds (type and fresh weight) compared to the controls (water), (untreated - uninoculated) seed grown plants.

Table 19. Effect of biocontrol (seed treatment) sunflower cv.(Miak) on charcoal rot severity[a] and plant growth[b] under greenhouse

	Treatment	Sunflower[c]									
		Stems		leaves			Flowring	Head[b]		state of seeds[s]	
		Thickness (hardness)	length	size	Thickness (texture)	color	[illegible]	diameter (cm)	color	type	fresh wt(gm)[b]
A	Pathogen only										
	M.phaseolina	++	++±	less	less	yellow green[b]	90 day	1.5-2	green	shrinked	0.23
B	Pathogen + Biological agent										
B1	*M+B.subtilis*	++	+++	large	++	yellow green	85 day	2	green	full	0.50
B2	*M+ S.roseodiastaticus*	++++	++++	large	++++	yellow green	85 day	2	green	full	0.55
B2\	*M+ S.olivaceiscleroticus*	+++	+++	large	+++	yellow green	85 day	3.5	green	full	0.56
B3	*M+ T.harzianum*	++	+++*	normal	++	yellow green	85 day	3	green	full	0.55
C	Biological agent										
C1	*B.subtilis*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C2	*S.roseodiastaticus*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C2\	*S.olivaceiscleroticus*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C3	*T.harzianum*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
D	*Control (water) only*	++	++	normal	++	green[a]	85 day	2	green	full	0.50

a- assesment of disease severity grade based on: 0,1,2 &3 as oulined in Materials and Methods; normal (healthy) if seed fresh weight in gm ≥ 0.5 disease, otherwise disease

b- based on the weight of 5 random seeds

c- results, recorded after flowering and fruiting stage of plant (sunflower i.e after 90 days from the time of sowing based on organs thicknesses

±less ++ moderate +++high +*more

NE not examined at the time of this stage

Fig. 15. Various symptoms and plant growth parameters on sunflower artificially inoculated with *M. phaseolina*, **(B),** and simultaneously subjected to: **(C),** *B. subtilis*; **(D)** *S. roseodiastaticus* (isolate No. 112); **(E),** *S. olivaceiscleroticus* (isolate No. 729), and **(F),** *T. harzianum*. Note: **(D)** shows the greatest level of plant growth parameter, i.e., plant height.

4.4.3.4 *Effect of Biocontrol (Seed Treatment) on Sunflower Fusarium Root Rot Severity And Plant Growth Under Greenhouse Conditions*

The susceptibility of cv. Miak of sunflower plant to *F. solani* f.sp. *phaseoli* was tested. Seeds of sunflower were coated with different biocontrol agents and sown in infested soil with the pathogen as outlined in (Materials & Method). The biocontrol agents used are those which were used in the previous experiment with soybean. The susceptibility was noted at the pre-emergence stage of seedling and post-emergence state till fruiting stage. Results are shown in (Table 20).

Difference between the various biocontrol agents could be seen in disease progress symptoms and plant growth parameters up to seedling stage, see Figs. 16 and 17.

S. roseodiastaticus (isolate No. 112) and *S. olivaceiscleroticus* (isolate No. 729) treatments showed similar and the highest level of yield production and plant growth parameters compared with other treatments. While *F. solani* f.sp. *phaseoli;* the root rot causing pathogen, continued to cause progressive disease and much greater yield reduction as a result of symptom caused by Fusarium pathogen. The symptoms were expressed in the form of rottening of a few of the main and lateral roots. Dark brown streaks were noticed on the stem with small size and yellowing leaves appearance of the whole plant. Heavily infected plant were tattered and blighted. Superficial white mycelium growing on living plants was observed and production of apperesoria under which infection occurs was also noticed. Heads bearing shrinked seeds did not germinate. Moreover, great reduction in growth parameters stems (thickness and length, see Fig. 17), leaves (size, texture and color) and head diameter including state of seeds (type and fresh weight) was also observed.

Table 20. Effect of biocontrol (seed treatment) sunflower cv.(Miak) on Fusarium root rot severity[a] and plant growth[b] under greenhouse

	Treatment	Sunflower[c]									
		Stems		leaves			Flowring rate	Head[b]		state of seeds[b]	
		Thickness (hardness)	length	size	Thickness (texture)	color		diameter (cm)	color	type	fresh wt/gm[b]
A	Pathogen only										
	F.solani f.sp.phaseoli	++±	++	normal	++	yellow brown[b]	85 day	1-1.5	green	shrinked	0.38
B	Pathogen + Biological agent										
B1	*F+B.subtilis*	++	+++*	large	++	yellow	83 day	2	green	full	0.55
B2	*F+ S.roseodiastaticus*	+++±	++++	large	+++	yellow	83 day	2.5	green	full	0.55
B2	*s*	+++	+++±	large	+++±	green	83 day	2.8	green	full	0.58
B3	*F+ T.harzianum*	++	++++*	normal	++	green	83 day	2	green	full	0.50
C	Biological agent										
C1	*B.subtilis*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C2	*S.roseodiastaticus*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C2\	*S.olivaceiscleroticus*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
C3	*T.harzianum*	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
D	*Control (water) only*	++	+++	normal	++	green[a]	85 day	2cm	green	full	0.50

a- assesment of disease severity grade based on: 0,1,2 &3 as oulined in Materials and Methods; normal (healthy) if seed fresh weight in gm ≥ 0.5 disease, otherwise disease

b- based on the weight of 5 random seeds

c- results, recorded after flowering and fruiting stage of plant (sunflower i.e after 90 days from the time of sowing based on organs thicknesses

±less ++ moderate +++high +*more

NE not examined at the time of this stage

Fig. 16. Various symptoms and plant growth parameters on sunflower artificially inoculated with *F. solani* f.sp. *phaseoli* **(B),** and simultaneouly subjected to: **(C),** *B. subtilis*; **(D),** *S. roseodiastaticus* (isolate No. 112) **(E),** *S. olivaceiscleroticus* (isolate No. 729) and (F), *T. harzianum*. **(D)** and **(E)** show the greatest level of plant growth parameter, i.e., plant hight and yield production.

Fig. 17. Thickness of sunflower stems as a result of artifical inoculation with *F. solani* f.sp. *phaseoli* and simultaneously subjected to: **(17.1), (A),** *S. olivaceiscleroticus* (isolate No. 729); **(B),** *B. subtilis*; **(17.2), (A),** *S. roseodiastaticus* (isolate No. 112) ***and (B),*** *S. olivaceiscleroticus* (isolate No. 729).

However, yield comparison with sunflower plants, infected with either *M. phaseolina* or *F. solani* f.sp. *phaseoli*, indicated that there was much greater effect to *S. roseodiastaticus* (isolate No. 112) treatment than the other biocontrol agents. Thus in practical terms *S. roseodiastaticus* has induced protection against both pathogens. While *S. olivaceiscleroticus* has induced a considerable protection against the Fusarium pathogen causing the root rot of sunflower but with lesser efficiency towards the control of Macrophomina pathogen causing the charcoal rot disease of sunflower.

Generally, it can be concluded that *S. roseodiastaticus* (isolate No. 112) treatment has induced the greatest protection among the other tested biocontrol agents, against both pathogens causing charcoal rot and root rot diseases of soybean and sunflower.

4.5 Phytoalexins

4.5.1 *Phytoalexins Detection by TLC*

4.5.1.1 Screening of Phytoalexins Production in Soybean (Hypocotyls)-Pathogens/Biocontrol Agents Interaction Systems, in Greenhouse.

Data presented in (Table 21) showed the presence of several compounds. Six constitutive compounds were detected in healthy (untreated-uninoculated) plant hypocotyls at R_f 0.88, 0.77, 0.72, 0.66, 0.11 and 0.05. The first compound at Rf 0.88 was also present with the M. system, see Fig. 19 for its U.V. spectrum. The U.V. sepectra of the last five compounds are shown in Fig. 18. The constitutive compound at R_f 0.11 was also detected in soybean (*B. subtilis* and *S. roseodiastaticus* (isolate No. 112)) treatments and it has different characterization under the long U.V. It exhibited dark red and red color in the previously mentioned treatments, respectively. The same constitutive compound at R_f 0.11 was also detected in *F./S. roseodiastaticus* (isolate No. 112) system and *F./S. olivaceiscleroticus* (isolate No. 729) system but it has different characterization under the long U.V., it exhibited bright fluorescent, see Table 22 and Fig. 28 for its U.V. spectrum in *F./S. olivaceiscleroticus system.* Two constitutive compounds of R_f 0.88, and 0.77 were detected in *T. harzianum* tissue

system. Also these two compounds (constitutive) were also detected in all interaction systems which involve the other biocontrol agents separately or with each of the two pathogens to be tested. These compounds were not detected with the *Fusarium (F) pathogen* system tissue alone. The compound at R_f 0.88 exhibited a dark red color under the long UV with water, *T. harzianum, Macrophomina (M) pathogen, M/B* and *M/S. roseodiastaticus* (isolate No. 112). While it was of different colors (faint rose) with the tissue of the *M/S. olivaceiscleroticus* (isolate No. 729), *M/T. harzianum, F/B, F/S. olivaceiscleroticus* (isolate No. 729) and *F/T. harzianum* interactions systems. Another two constitutive compounds of R_f 0.72 and 0.66 were only detected in soybean *S. roseodiastaticus* (isolate No. 112) tissue as rose red, whereas with the F. pathogen alone, F/B, F/*S. roseodiastaticus* (isolate No. 112) and *F/T. harzianum* tissue interaction systems, these two compounds were detected as rose color. Other constitutive compound at R_f 0.66 in the tissue of F/B and F/*T. harzianum* interaction systems was detected as faint rose, while with F/*S. roseodiastaticus* (isolate No. 112) tissue system it was detected as rose under the long U.V. The latter constitutive compound at R_f 0.05 in *T. harzianum* tissue system was detected and it has different characterization under the long U.V. as it exhibited red color under the long UV.

Four inducible compounds in the tissue of F/*S. roseodiastaticus* (isolate No. 112) interaction system were detected at R_f 0.91, 0.50, 0.19 and 0.08. The predominant one and specifically with this system was that detected at R_f 0.50 as it exhibited substantial absorbance at λ_{max} 237 nm, Fig. 27, whereas the other three compounds were detected at lesser concentrations. Only one compound of the same R_f 0.91 was also detected with the F. pathogen interaction system, it exhibited a faint fluorescent under the long UV and has different absorbence at λ_{max} (248, 218 nm), Fig. 25, wheras it exhibited absorbane at λ_{max} (215, 198 nm), Fig. 27 . Another specific inducible compound at R_f 0.19, was detected as bright flourescent under the long U.V., and exhibited substantial absorbency at λ_{max} 235 and 206 nm, Fig. 27. The latter one at R_f 0.08 was also detected in F. pathogen interaction system with each of the biocontrol agents (B and *T. harzianum*) as violet and violet red with λ_{max} 200 and 250/204 nm, see Figs. 26 and 29, respectively. Also, the same compound was only

detected, but as red color, in the tissue of *S. roseodiastaticus* (isolate No. 112) interaction system.

Two inducible compounds that were only specific to F. pathogen tissue were detected at R_f 0.27 and 0.19 as faint fluorescent with different λ_{max} (245, 200 nm) and (215, 197 nm), Fig. 25.

Eight inducible compounds in the M/*S. olivaceiscleroticus* (isolate No. 729) interaction system were detected at R_f 0.86, 0.83, 0.69, 0.58, 0.55, 0.33, 0.27 and 0.027. The compound at R_f 0.86 was also detected with the Fusarium system (s) pathogen, e.g., F/*S. olivaceiscleroticus* (isolate No. 729) interaction system This compound was of rose color under the long U.V., with λ_{max} 268 and 194 nm, Fig. 22. The second compound at R_f 0.83 was also detected with the other systems, which involved the bacterial biocontrol agents only, as red color and with M/*S. roseodiastaticus* (isolate No. 112) system as rose color with λ_{max} 315 and 194 nm, Fig. 21. This compound was also detected with M/*S. olivaceiscleroticus* (isolate No. 729) and *T. harzianum* but as rose color under the long U.V. The third compound at R_f 0.69 was detected in *S. roseodiastaticus* (isolate No. 112) ,tissue only and appeared as rose red with λ_{max} 268 and 200 nm, Fig. 23, whereas with Macrophomina pathogen only it was of red color, but it was a dark red with M./*B. subtilis* and M./*S. roseodiastaticus* (isolate No. 112) interaction systems. Also this compound was detected as rose color with M./ *S. olivaceiscleroticus* (isolate No. 729) and M./*T. harzianum* with λ_{max} 295 and 194 nm, Fig. 24. The fourth compound at R_f 0.58 was detected in the *M/S. olivaceiscleroticus* (isolate No. 729) interaction systems tissue only. The fifth inducible compound at R_f 0.55 was present with M./*B. subtilis*, M./*S. roseodiastaticus* (isolate No. 112) and M./*S. olivaceiscleroticus* (isolate No. 729) as rose color under the long U.V. The six inducible compound at R_f 0.33 was detected in M./*B. subtilis* and M./*S. olivaceiscleroticus* (isolate No. 729) interaction systems as bright fluorescent and rose color, respectively. The seventh inducible compound at R_f 0.27 was detected in the M./*B. subtilis*, Fig. 20, and M./*S. roseodiastaticus* (isolate No. 112) interaction

and 194 nm, respectively. The last compound at R_f 0.027 was present with the M./*S. roseodiastaticus* (isolate No. 112) interaction, Fig. 21 and Table 21, and M./*S. olivaceiscleroticus* (isolate No. 729) systems, Table 21, as dark red of λ_{max} 260, 225 and 200 nm and it was of fluorescent color under the long U.V.

Table 21. Screening of phytoalexins production in soybean- *M. phaseolina* / biocontrol agents interaction systems in greenhouse by TLC

No.of band	Soybean-hypocotyls* /in gm fresh weight													
	Control (water) (1.5gm)			*B.subtilis* (1.8gm)			*S.roseodiastaticus* (2.6gm)			*S.olivaceiscleroticus* (4gm)			*T.harzianum* (2.5gm)		
	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max
(1)a															
(2)a	0.88	dR^C	N.E										0.88	dR^C	N.E
(3)a															
(4)a				(0.83)	R^I	N.E									
(5)a							0.80	rR^I	N.E						
(6)a	0.77	R^C	198 218 sh										(0.77)	rR^C	N.E
(7)a	0.72	R^C	250 202				0.72	rR^I	N.E						
[8]															
(9)a	0.66	dR^C	250 200												
[10]										0.69	rR^I	268 200			
[11]															
[12]										0.63	rR^I	260			
(13)a															
(14)a															
(15)a															
[16]															
[17]															
(18)a	0.11	PV^C	248 200	0.11	dR^C	N.E	0.11	R^I	N.E						
(19)a							0.08	R^I	N.E						
(20)a	0.05	BV^C	197										0.05	R^C	NE

a represents the same suspected compounds in different treatments and also with the other tested host (sunflower) for the examined ones only.
[ ] refers to the specific compound for (soybean)
( ) refer to coresponding compound detected from excised hypocotyls pr evious work (Emara 1987)
* age of plant 18 day, grown in greenhous ** solvent system (ethyle acetate- hexan- methanol 60: 40: 1 (v/v/v)
*** observation of band under long UV 366 nm I inducible C constitutive
R red dR dark red bF bright fluorescent fF faint fluoresce r rose NE not examined

Cont.

Table 21. Screening of phytoalexins production in soybean- *M. phaseolina* / biocontrol agents interaction systems in greenhouse by TLC

No.of band	Soybean-hypocotyle* /in gm fresh weight														
	M.phaseolina (1.5gm)			*M+B.subtilis* (1 gm)			*M + S.roseodiasticus* (3.5gm)			*M+S.oliviaceiscleroticus* (2.7gm)			*M+ T.harzianum* (2.5gm)		
	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (EtoH) max	R_f**	U.V***	λ (Et oH) max
(1)a															
(2)a	0.88	dR^C	265 200	0.88	dR^C	N.E	0.88	dR^C	N.E	0.88	fr^C	N.E	0.88	dR^C	N.E
(3)a										(0.86)	r^1	268			
(4)a							0.83	r^1	315 194	(0.83)	r^1	N.E	(0.83)	r^1	N.E
(5)a							0.80	rR^1	N.E						
(6)a	(0.77)	R^C	N.E	(0.77)	r^C	N.E	(0.77)	r^C	N.E	(0.77)	r^C	N.E	(0.77)	rR^C	N.E
(7)a															
[8]	0.69	R^1	N.E	0.69	dR^1	194	0.69	dR^1	N.E	0.69	r^1	N.E	0.69	r^1	265 194
(9)a															
[10]													0.63	r^1	N.E
[11]				(0.61)	r^1	200	0.61	r^1	N.E						
[12]										0.58	fF^1	194			
(13)a				0.55	r^1	314 194	0.55	r^1	N.E	0.55	r^1	194			
(14)a				0.33	bF^1	314 194				(0.33)	r^1	260 220			
(15)a				0.27	bF^1	314 225 194				0.27	F^1	NE			
[16]							0.25	F^1	314 191						
[17]				0.17	r^1	N.E									
(18)a															
(19)a															
(20)a							0.027	dR^1	260 225 200	0.027	F^1	N.E			

a represents the same suspected compounds in different.treatments and also with the other tested host (sunflower)for the examined ones only.

[ ] refers to the specific compound for(soybean)

( ) refer to coresponding compound detected from excised hypocotyls pr evious work (Emara 1987)

* age of plant 18 day, grown in greenhous ** solvent system (ethyle acetate- hexan- methanol 60: 40: 1 (v/v/v)

*** observation of band under long UV 366 nm I inducible C constitutive

R red dR dark red bF bright fluorescent fF faint fluoresce r rose NE not examined

Table 22. Screening of phytoalexins production in soybean- *F.solani* f.sp. *phaseoli* / biocontrol interaction systems in greenhouse by TLC

No.of band	Soybean-hypocotyls* /in gm fresh weight													
	F.solani f.sp.phaseoli (1.5gm)			*F+B.subtilis* (0.7 gm)			*F + S.roseodiasticus* (1.6gm)			*F+S.olivaceiscleroticus* (2.3gm)			*F+ T.harzianum* (2.5gm)		
	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max
(1)a	0.91	fF^1	215 198				0.91	fF^1	248 218						
(2)a				0.88	fr^C	N.E	0.88	fr^C	248sh 198	0.88	fr^C	N.E	0.88	fr^C	N.E
(3)a										(0.86)	r^1	N.E			
(4)a															
(5)a															
(6)a				(0.77)	r^C	N.E	(0.77)	r^C	248 198				(0.77)	r^C	N.E
(7)a				0.72	r^1	N.E	0.72	r^1	248 200	0.72	r^1	N.E	0.72	r^1	N.E
[8]															
(9)a				0.66	fr^C	N.E	0.66	r^C	N.E				0.66	fr^C	N.E
[10]															
[11]															
[12]															
(13)a							0.50	r^1	237 195sh						
(14)a															
(15)a	0.27	fF^1	245 200												
[16]															
[17]	0.19	fF^1	215 197				0.19	bF^1	235 206						
(18)a							0.11	bF^1	250	0.11	bF^1	198			
(19)a				0.08	V^1	200	0.08	F^1	220 200				0.08	VR^1	250 204
(20)a															

a represents the same suspected compounds in different.treatments and also with the other tested host (sunflower)for the examined ones only.
[ ] refers to the specific compound for(soybean)
( ) refer to coresponding compound detected from excised hypocotyls pr evious work (Emara 1987)
* age of plant 18 day, grown in greenhous ** solvent system (ethyle acetate- hexan- methanol 60: 40: 1 (v/v/v)
*** observation of band under long UV 366 nm I inducible C constitutive
R red dR dark red bF bright fluorescent fF faint fluoresce r rose NE not examined

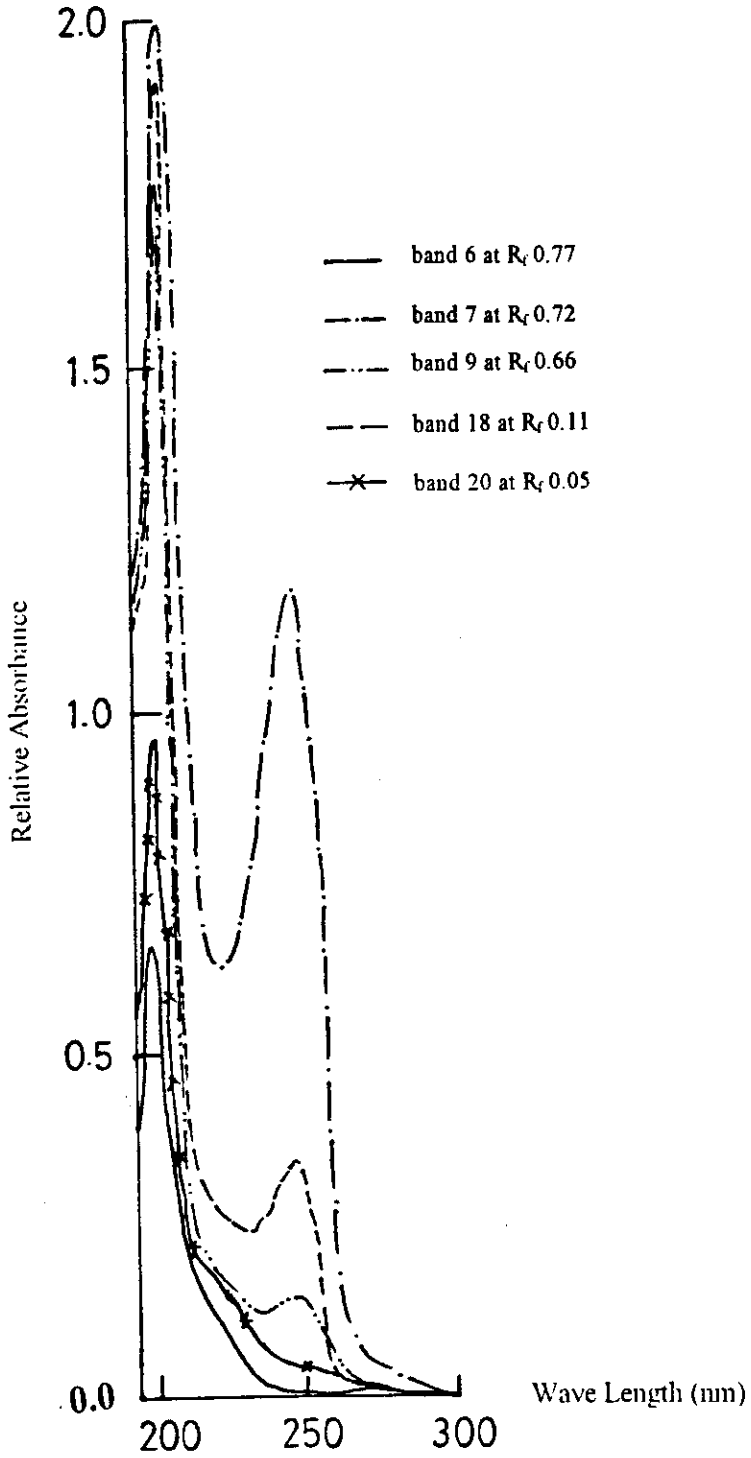


Fig. 18. U.V spectra of phytoalexin compounds of bands 6, 7, 9, 18 and 20 at R_f 0.77, 0.72, 0.66, 0.11 and 0.05, respectively, from TLC plate of the extract of soybean (cv. Crowford) hypocotyl of 18 days old, grown under greenhouse condition, control (untreated- uninfected).

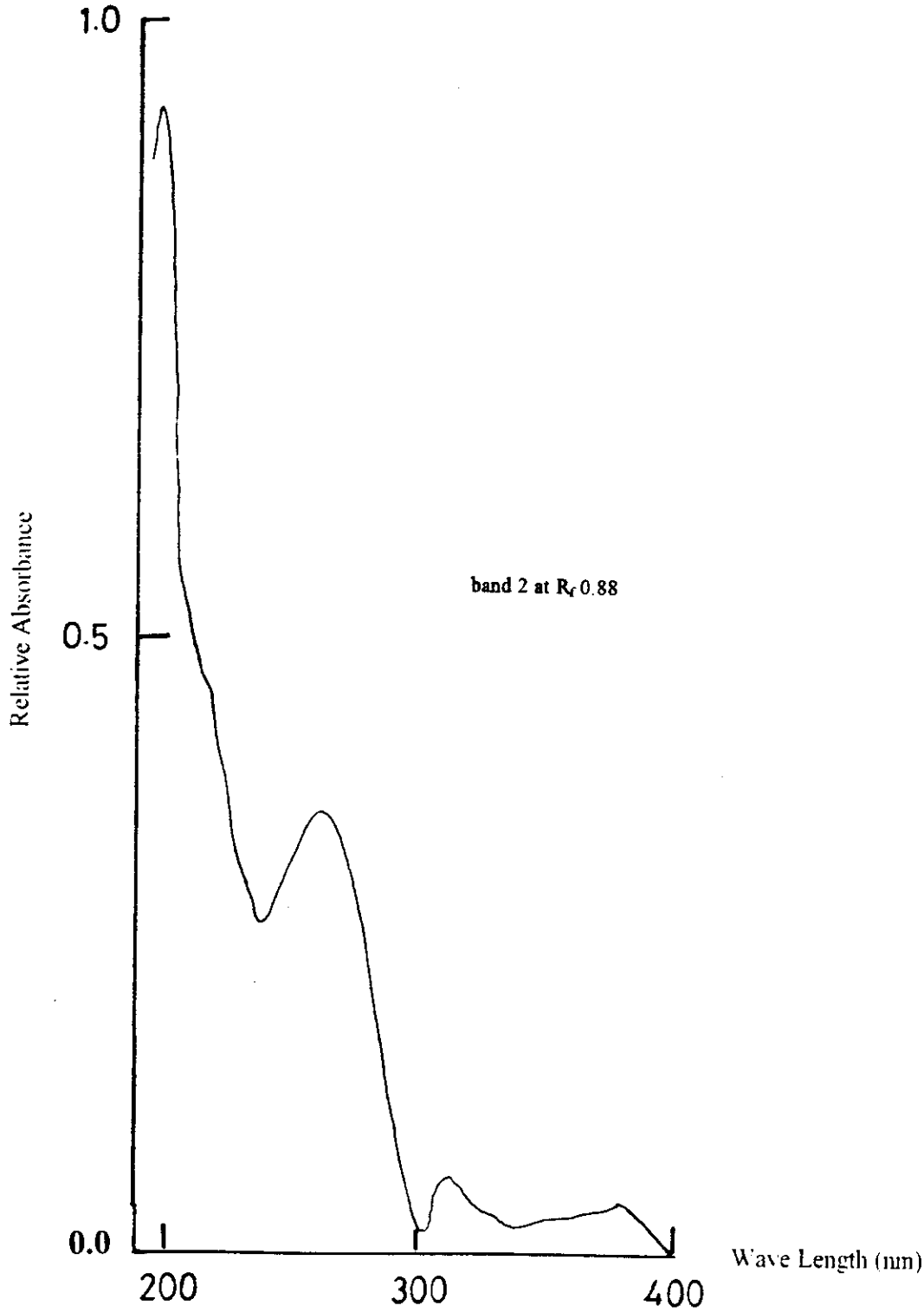


Fig. 19. U.V spectrum of phytoalexin compound of band 2 at R_f 0.88 from TLC plate of the extract of soybean (cv. Crowford) hypocotyl of 18 days old grown under greenhouse condition after inoculation with *M. phaseolina*.

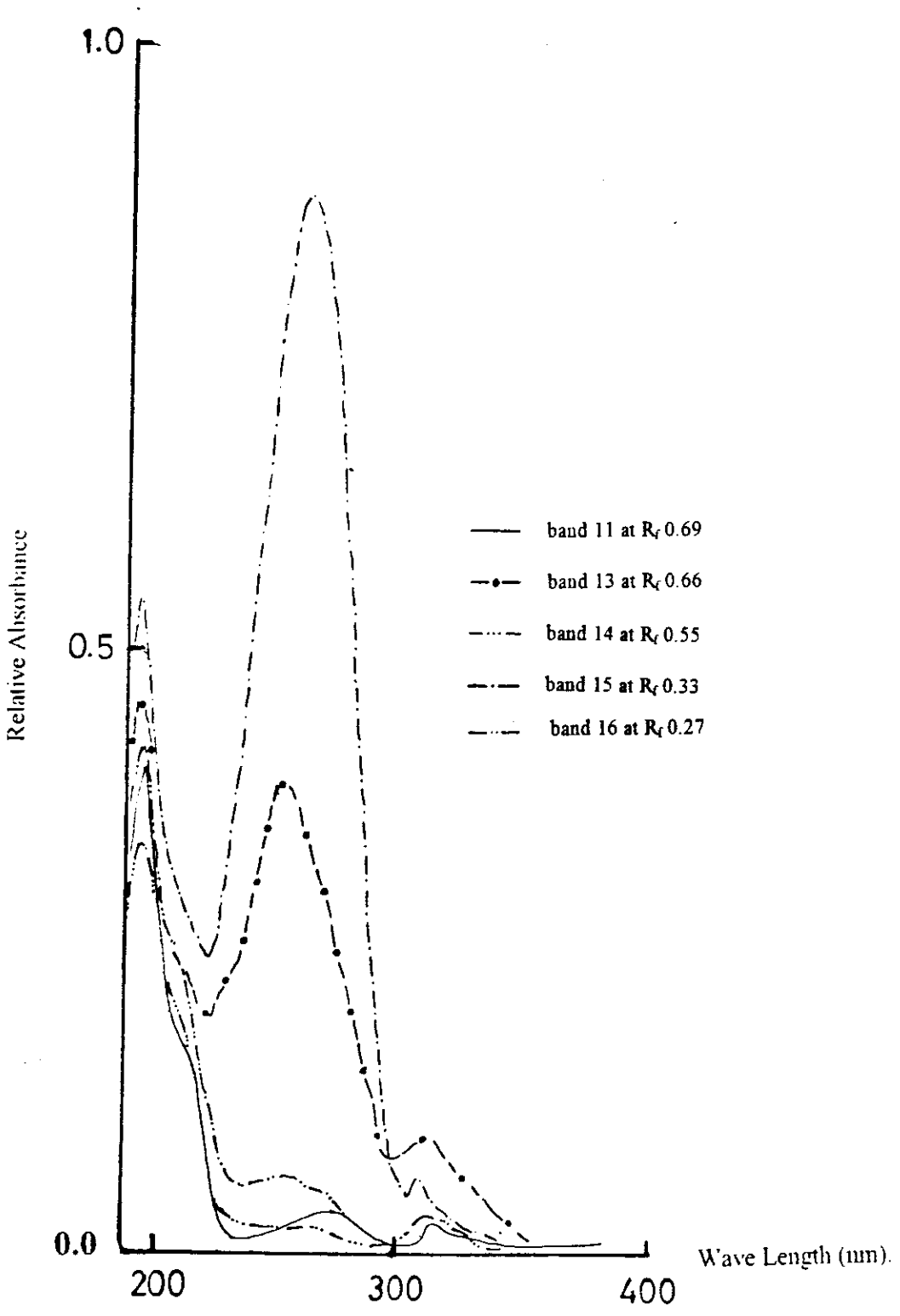


Fig. 20. U.V spectra of phyutoalexin compounds of bands 11, 13, 14, 15 and 16 at R_f 0.69, 0.61, 0.55, 0.33 and 0.27, respectively, from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse codition after inoculation with *M. phaseolina* simultaneeously with *B. subtilis*.

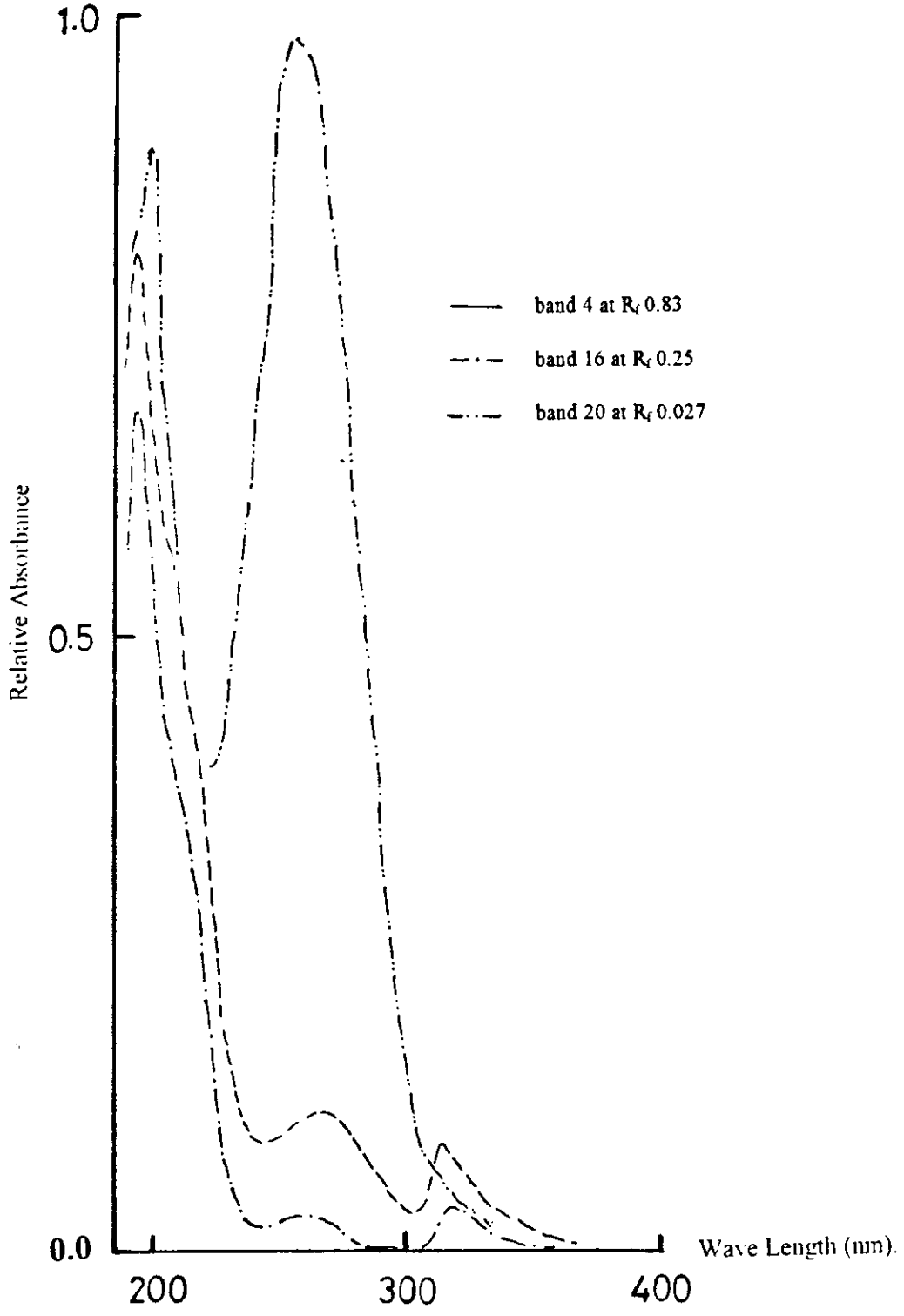


Fig. 21. U.V spectra of phytoalexin compounds of bands 4, 16 and 20 at R_f 0.83, 0.25 and 0.027, respectively from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with *M. phaseolina* simultaneously with *S. roseodiastaticus*.

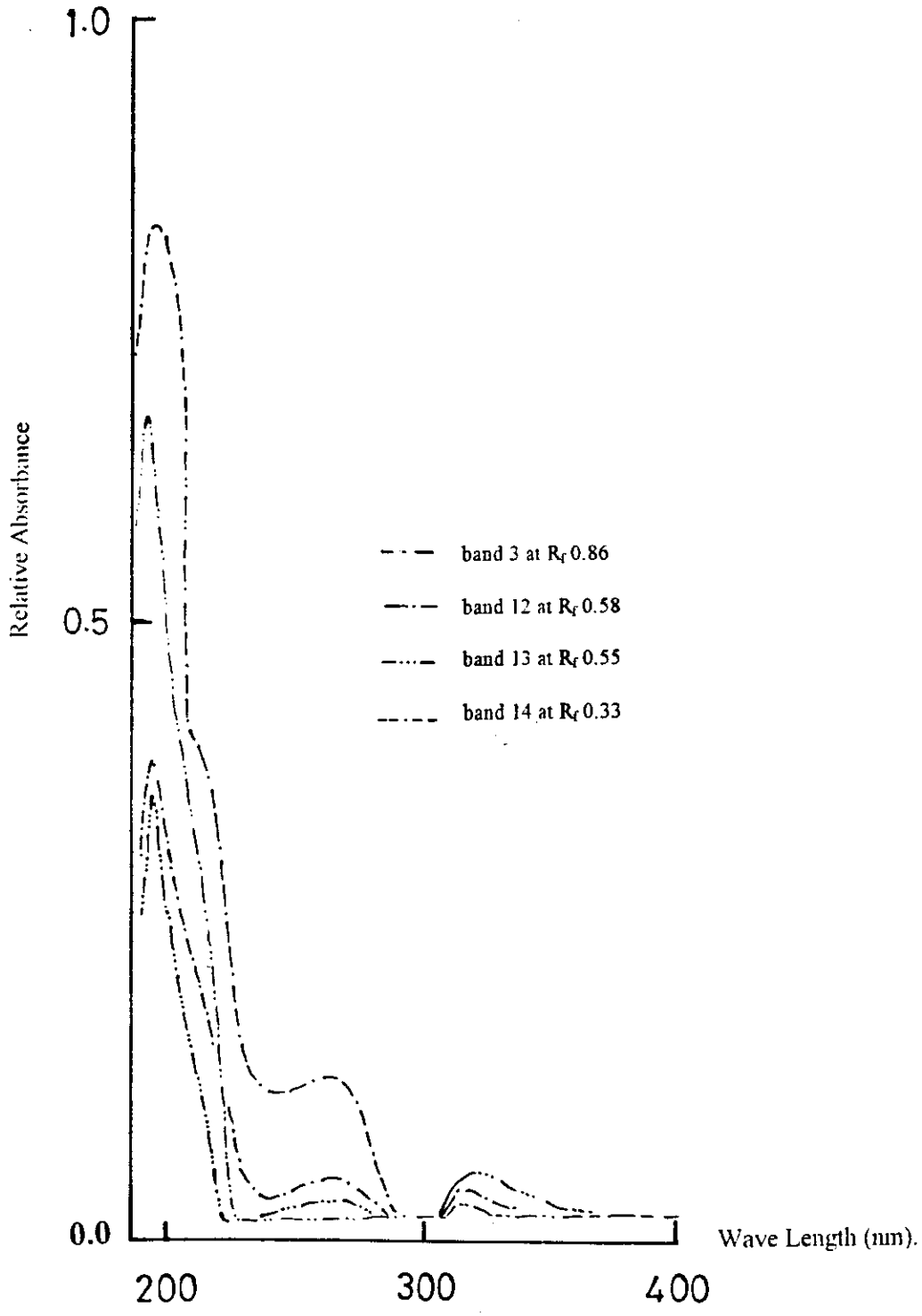


Fig. 22. U.V spectra of phytoalexin compounds of bands 3, 12, 13 and 14 at R_f 0.86, 0.58, 0.55 and 0.33, respectively, from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with *M. phaseolina* simultaneously with *S. olivaceiscleroticus.*

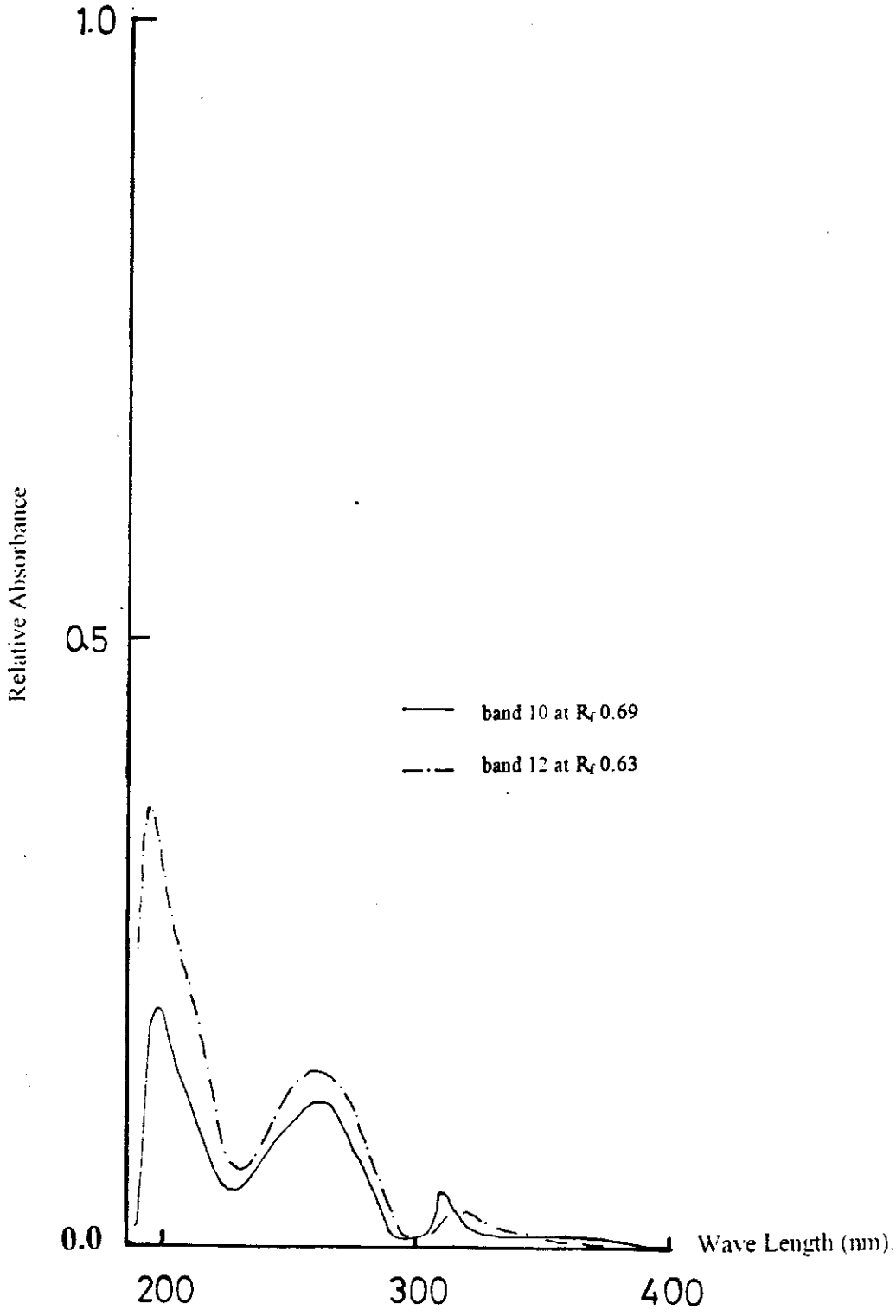


Fig. 23. U.V spectra of phytoalexin compounds of bands 8 and 12 at R_f 0.69 and 0.63, respectively, from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with *S. olivaceiscleroticus.*

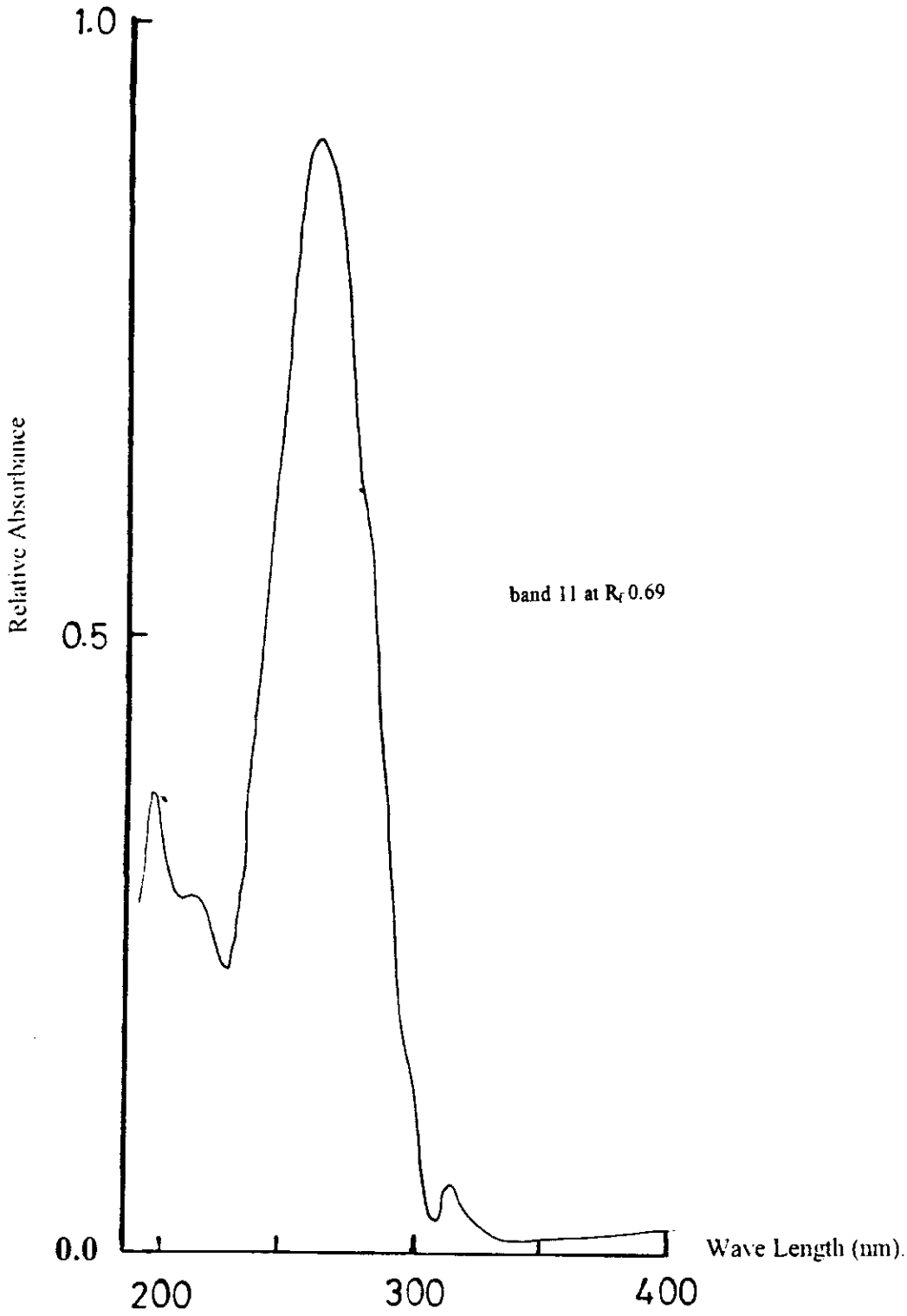


Fig. 24. U.V spectrum of phytoalexin compound of band 8 at R_f 0.69 from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with *M. phaseolina* simultaneously with *T. harzianum*.

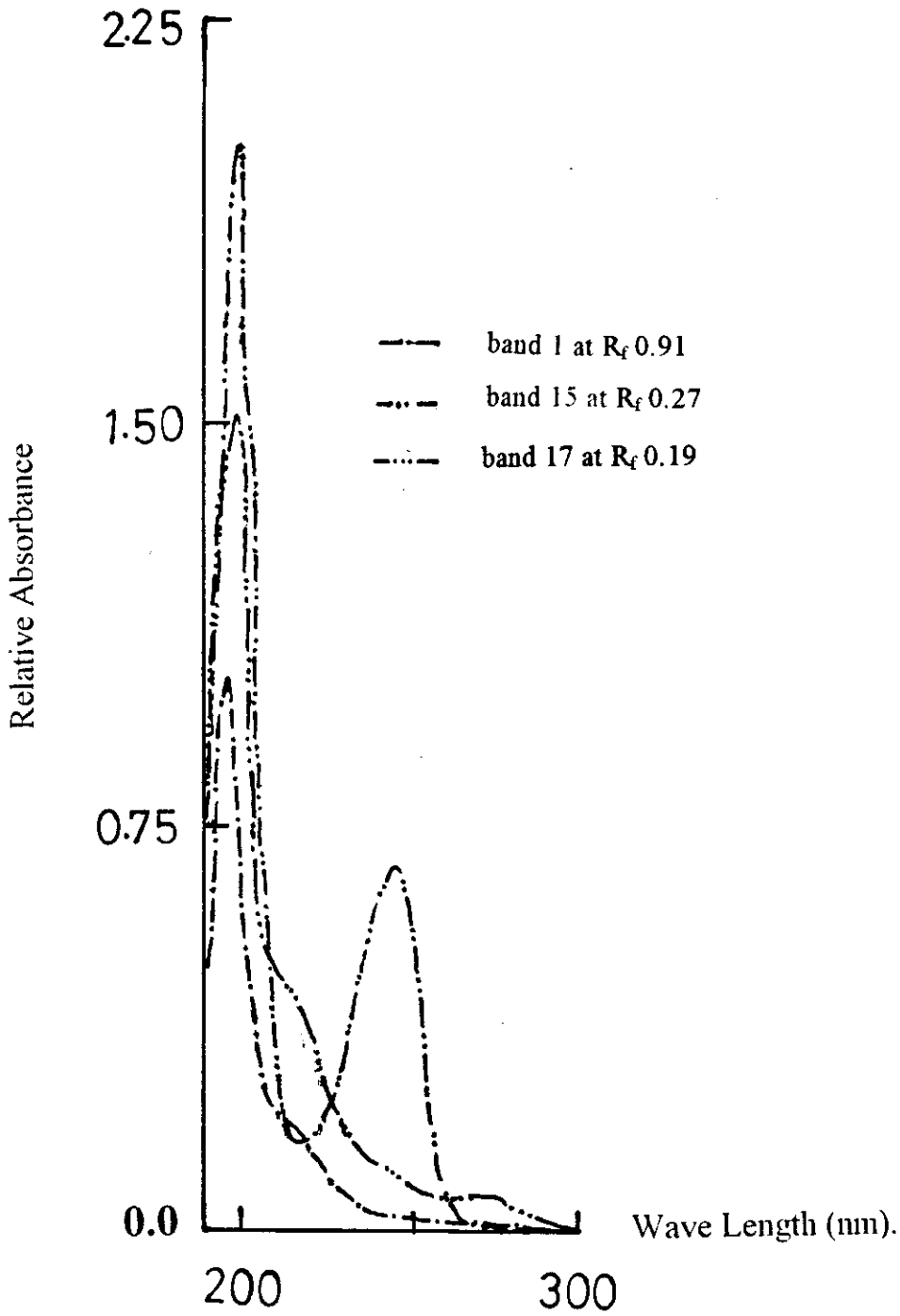


Fig. 25. U.V spectra of phytoalexin compounds of bands 1, 15 and 17 at Rf 0.91, 0.27 and 0.19, respectively, from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with *F. solani* f. sp. *phaseoli*.

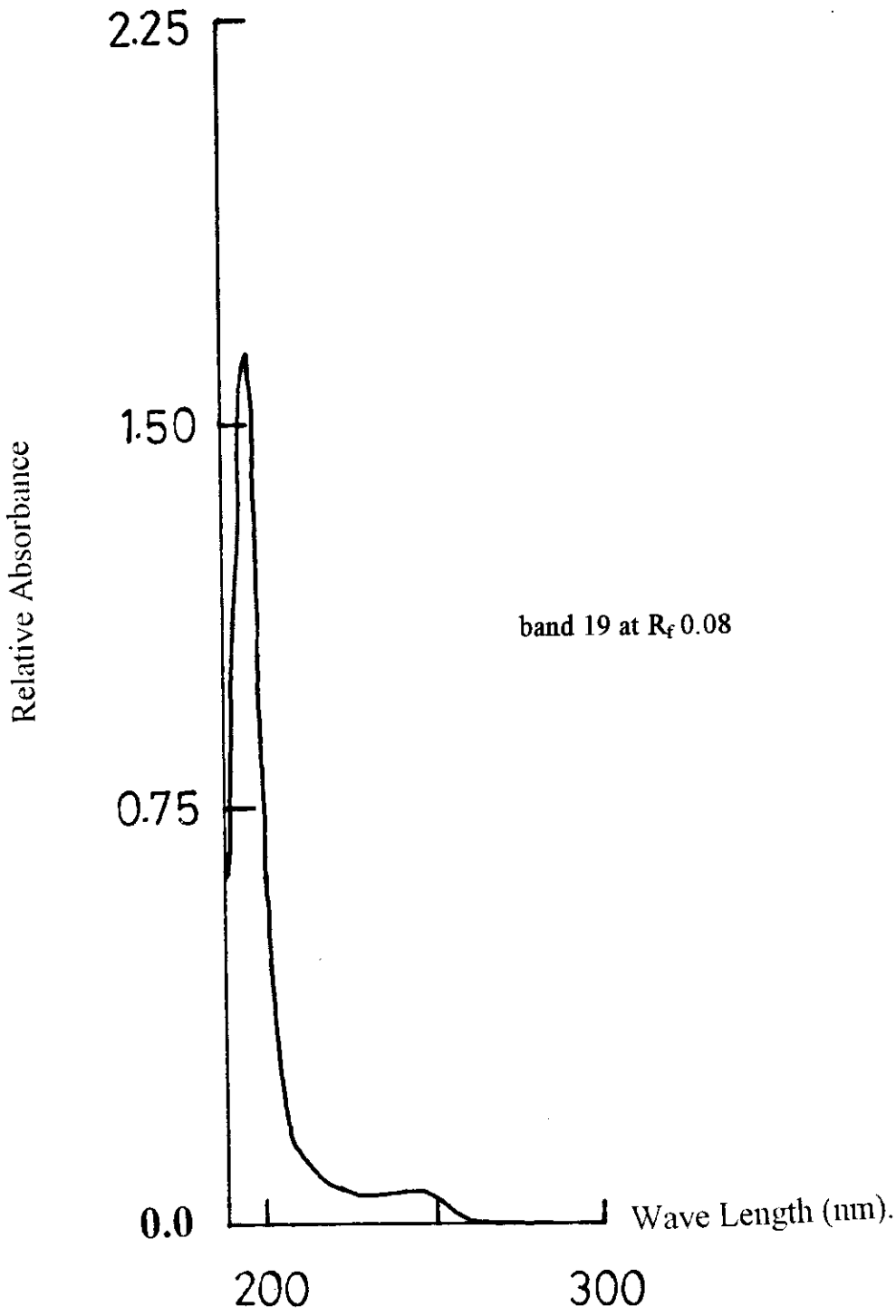


Fig. 26. U.V spectrum of phytoalexin compound of band 19 at R_f 0.08 from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with *F. solani* f.sp. *phaseoli* simultaneously with *B. subtilis*.

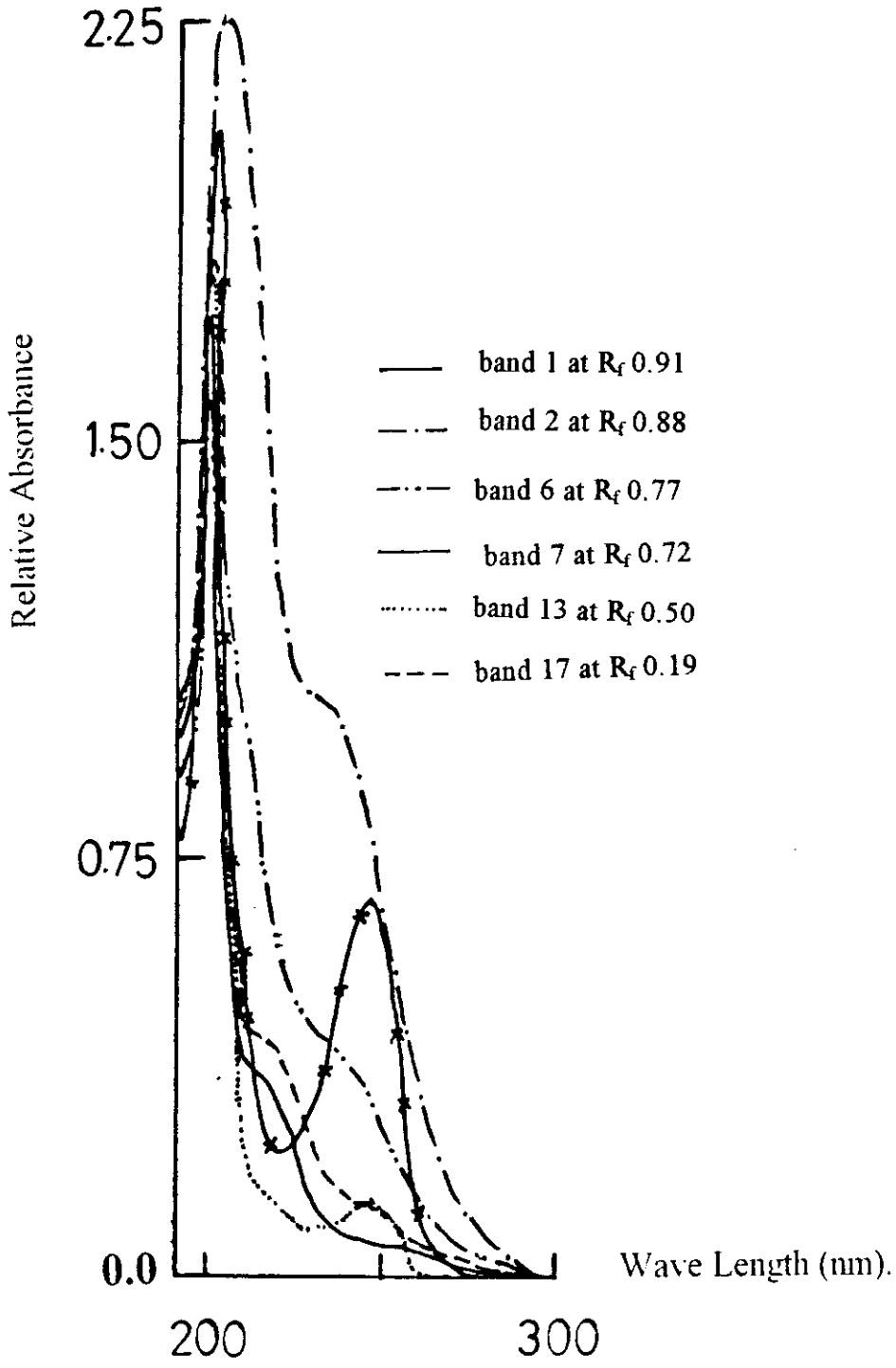


Fig. 27. U.V spectra of phytoalexin compounds of bands 1, 2, 6, 7, 13 and 17 at R_f 0.91, 0.88, 0.77, 0.72, 0.50 and 0.19, respectively, from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with *F. solani* f.sp. *phaseoli* simultaneously with *S. roseodiastaticus*.

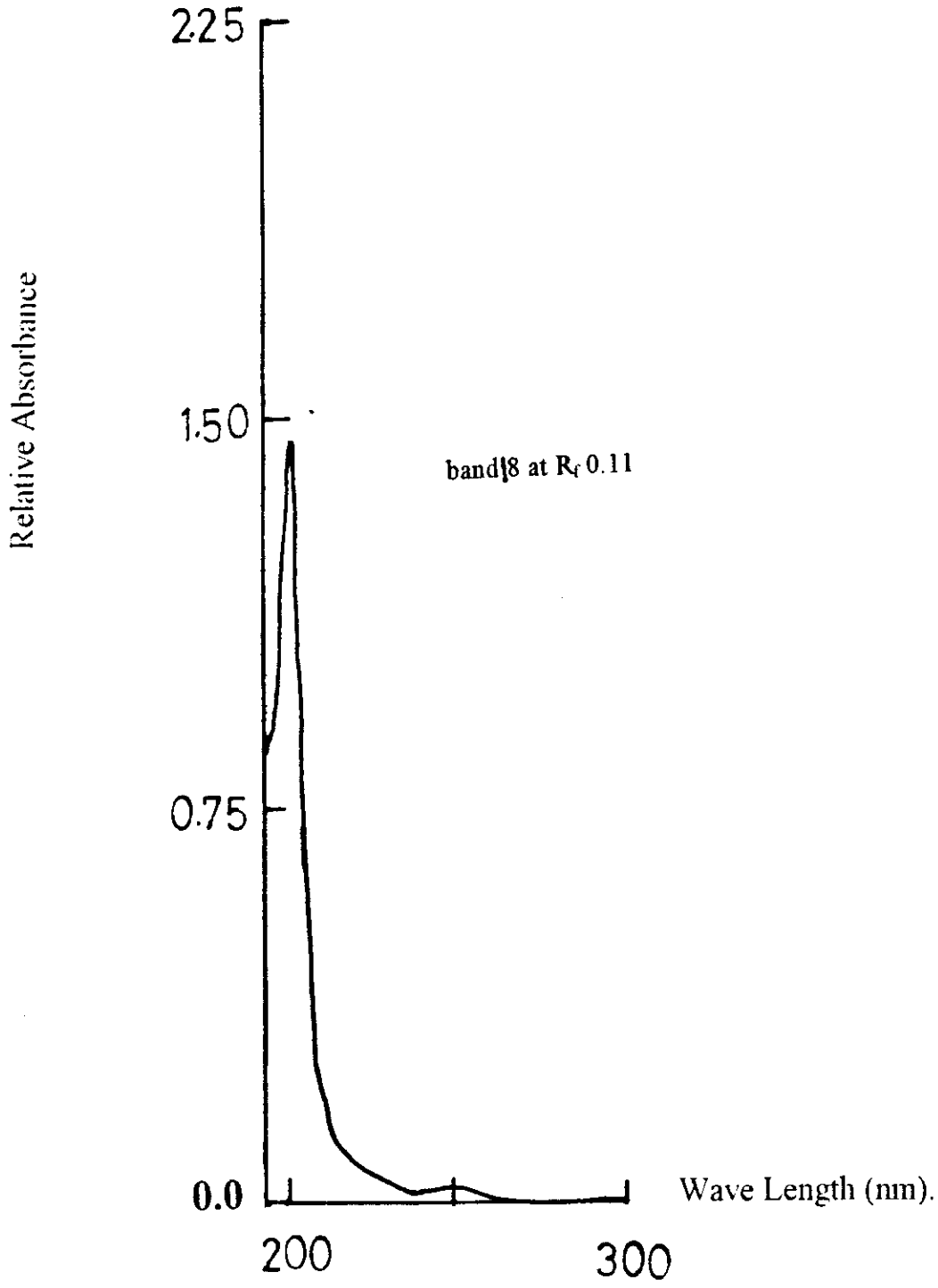


Fig. 28. U.V spectra of phytoalexin compound of band 18 at R_f 0.11 from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with *F. solani* f.sp. *phaseoli* simultaneously with *S. olivaceiscleroticus*.

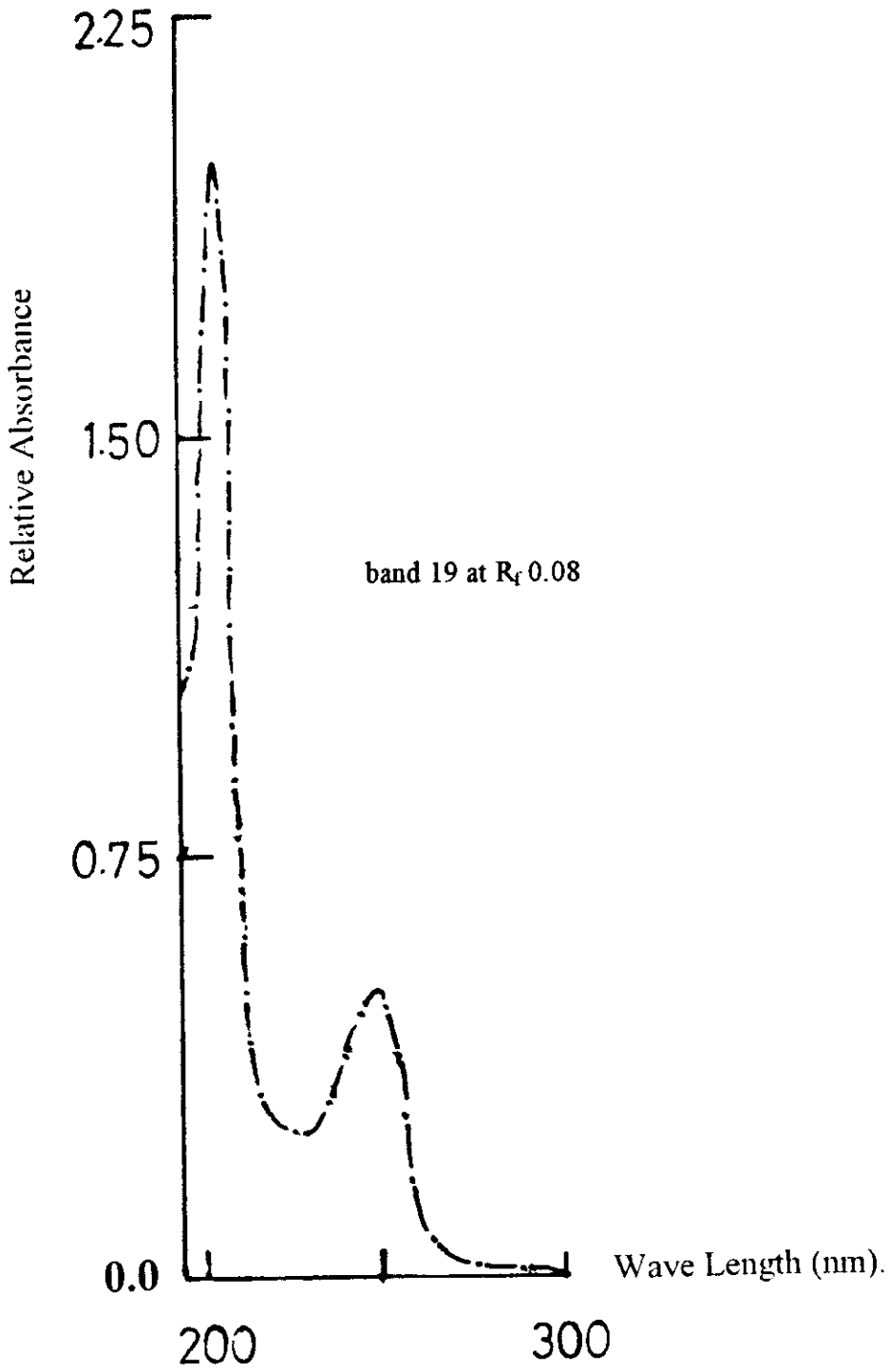


Fig. 29. U.V spectrum of phytoalexin compound of band 19 at R_f 0.08 from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old grown under greenhouse condition after inoculation with ***F. solani*** f.sp. ***phaseoli*** simultaneously with *T. harzianum*.

4.5.1.2 *Screening of Phytoalexins Production in Soybean (Leaves)-Pathogens/ Biocontrol Agents Interaction Systems in Greenhouse.*

Four constitutive compounds were detected in the control (untreated-uninoculated) plant tissue leaves at R_f 0.88, 0.77 and 0.72 and 0.05 (Table 23). The first one at R_f 0.88 was detected as dark red in the control leaves and it was similar to the compound which was detected with *S. roseodiastaticus* (isolate No. 112) at λ_{max} 396, 194 nm, see Fig. 33. The intensity of color decreased to red color in Macrophomina pathogen system, see Fig. 30 and Table 23 for its λ_{max} absorbance) and in all of the tested biocontrol agents interaction systems, e.g., *M/S. roseodiastaticus* (isolate No. 112), Fig. 32. The second constitutive compound at R_f 0.77, Fig. 33 and Table 21, was detected as red color in the control, M./*S. olivaceiscleroticus* (isolate No. 729) and M./*T. harzianum* interaction systems. The third and fourth constitutive compounds at R_f 0.72 and 0.05 were only detected in the control leaves, *B. subtilis* and *S. olivaceiscleroticus* (isolate No. 729) interaction systems as red color with the control leaves but the color was rose red with the *B. subtilis* and *S. olivaceiscleroticus* (isolate No. 729) interaction systems under the long U.V.

Six inducible compounds were detected in the Macrophomina pathogen leaves at R_f 0.91, 0.86, 0.83, 0.36, 0.27 and 0.027 (Table 23). The first inducible compound at R_f 0.91 was also detected at similar R_f in M/*B. subtilis* interaction system, see Fig. 31 for its U.V. spectrum,, and has a different characterization under the long U.V as it exhibited red and fluorescent colors. The second inducible compound at R_f 0.86 was detected as red color of λ_{max} 194 similar to that inducible compound which was present with the tissue of *T. harzianum* interaction system only, but it has with different characterization under the long U.V. as it exhibited dark red with both *B. subtilis* and *S. roseodiastaticus* (isolate No. 112), Fig. 33, interaction systems only. The third inducible compound at R_f 0.83 exhibited red color under the long U.V with λ_{max} 439, 274 and 240 nm with the *S. roseodiastaticus* (isolate No. 112) interaction system, Fig. 33. This compound was similar to the inducible compound which was

present with the Macrophomina pathogen only (Table 23), M./*B. subtilis* (Fig. 31)and M./*S. roseodiastaticus* (isolate No. 112) interaction systems. The fourth inducible compound at R_f 0.36 exhibited a red color under the long U.V. with the tissue of *T. harzianum*, Macrophomina pathogen, M./*B. subtilis* and M./*S. roseodiastaticus,* (isolate No. 112, Fig. 32) interaction systems (Table 23) with λ_{max} 314, 220 and 194 nm. This compound was of different characterization under the long U.V. with the tissue of *M/T. harzianum* interaction system (Table 23) as it exhibited a fluorescent color with λ_{max} 305, 194 nm, Table 23 and Fig. 35. The fifth inducible compound at R_f 0.27, Table 23, exhibited red color with the *M/B. subtilis* whereas with the *M/S. roseodiastaticus* (isolate No. 112), it exhibited a fluorescent color under the long U.V. Also, this compound was present with the *M/S. olivaceiscleroticus* (isolate No. 729) and *M/T. harzianum* interaction systems but it exhibited a bright fluorescent color under the long U.V, see Figs 31 and 34 for its U.V. spectrum.

The sixth inducible compound at R_f 0.027, which was detected in all of the interaction systems tested except for the two interaction systems that involved the *B. subtilis* and *S. olivaceiscleroticus* (isolate No. 729), exhibited red color under the long U.V., (Table 23),

There were only three specific inducible compounds in the *S. roseodiastaticus* (isolate No. 112) interaction system at R_f 0.63, 0.55, 0.38 (Table 23). The first specific one at R_f 0.63 differs in characterization with the compound at the same R_f that was detected in all the other interaction systems studied. It is exhibited dark red color of λ_{max} 400 and 194 nm, see Table 23 and Fig. 33, where with the other system (*B. subtilis)*, it exhibited a fluorescent color under the long UV. The second specific inducible compound at R_f 0.55, Fig. 33, of λ_{max} 394, 220 and 194 nm exhibited a red color under the long UV. While the third specific inducible compound at R_f 0.38 was of brown color under the long U.V.

Table 23. Screening of phytoalexins production in soybean- *M. phaseolina* / biocontrol agents interaction systems in greenhouse by TLC

No.of band	Soybean-leaves* /in gm fresh weight													
	Control (water) (3gm)			*B.subtilis* (2gm)			*S.roseodiasstaticus* (5.5gm)			*S.olivaceiscleroticus* (9gm)			*T.harzianum* (4gm)		
	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max
(1)a							0.88	dR^C	396	0.88	rR^C	N.E			
(2)a	0.88	dR^C	N.E						194						
(3)a				(0.86)	dr^I	N.E	(0.86)	dR^I	194				(0.86)	R^I	N.E
(4)a							(0.83)	R^I	439						
(5)a				0.80	rR^I	N.E			274 240	0.80	rR^I	N.E			
(6)a	(0.77)	R^C	N.E				(0.77)	r^C	334 194						
(7)a															
(8)a	0.72	R^C	N.E	0.72	rR^C	N.E				0.72	rR^C	N.E			
(9)a															
(10)a				0.63	F^I	N.E	0.63	dR^I	400 194						
(11)a										0.61	fF^I	N.E			
(12)a							0.55	R^I	394 220 194	0.55	bF^I	N.E			
[13]				0.52	fF^I	N.E				0.52	fF^I	N.E			
[14]															
[15]															
[16]							0.38	$brown^I$	N.E				0.36	R^I	N.E
(17)a															
(18)a															
(19)a	0.05	rR^C	N.E	0.05	dR^C	N.E				0.05	dR^C	N.E			
[20]							0.027	R^I	N.E				0.027	R^I	N.E

a represents the same suspected compounds in different treatments and also with the other tested host (sunflower) for the examined ones only.
[ ] refers to the specific compound for (soybean)
( ) refer to coresponding compound detected from excised hypocotyls previous work (Emara 1987)
* age of plant 18 day, grown in greenhous ** solvent system (ethyle acetate- hexan- methanol 60: 40: 1 (v/v/v)
*** observation of band under long UV 366 nm I inducible C constitutive
R red dR dark red bF bright fluorescent fF faint fluoresce r rose NE not examined

Cont.

Table 23. Screening of phytoalexins production in soybean- *M. phaseolina* / biocontrol agents interaction systems in greenhouse by TLC

No.of band	Soybean-leaves* /in gm fresh weight													
	M.phaseolina (4.6gm)			*M+B.subtilis* (12 gm)			*M + S.roseodiastaticus* (15gm)			*M+S.olivaceiscleroticus* (13gm)			*M+ T.harzianum* (18gm)		
	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max	R_f**	U.V***	λ (Et oH) max
(1)a	0.91	R^1	N.E	0.91	F^1	194									
(2)a	0.88	R^c	N.E	0.88	R^c	N.E	0.88	R^c	314 220 194	0.88	R^c	N.E	0.88	R^c	N.E
(3)a	(0.86)	R^1	194												
(4)a	(0.83)	R^1	N.E	(0.83)	R^1	194	(0.83)	R^1	N.E						
(5)a															
(6)a										(0.77)	R^c	N.E	(0.77)	R^c	N.E
(7)a													0.75	R^1	220
(8)a															
(9)a										0.66	dR^1	314 194	0.66	R^1	N.E
(10)a															
(11)a															
(12)a															
[13]										0.52	r^1	N.E			
[14]										0.50	r^1	314 200			
[15]										0.41	r^1	314 194			
[16]															
(17)a	0.36	R^1	N.E	0.36	R^1	N.E	0.36	R^1	314 220 194				0.36	F^1	305 194
(18)a	0.27	R^1	N.E	0.27	F^1	194	0.27	F^1	N.E	0.27	bF^1	314 194	0.27	bF^1	N.E
(19)a															
[20]	0.027	R^1	N.E	0.027	R^1	N.E	0.027	R^1	N.E	0.027	R^1	N.E	0.027	R^1	N.E

a represents the same suspected compounds in different.treatments and also with the other tested host (sunflower)for the examined ones only.
[ ] refers to the specific compound for(soybean)
( ) refer to coresponding compound detected from excised hypocotyls pr evious work (Emara 1987)
* age of plant 18 day, grown in greenhous ** solvent system (ethyle acetate- hexan- methanol 60: 40: 1 (v/v/v)
*** observation of band under long UV 366 nm I inducible C constitutive
R red dR dark red bF bright fluorescent fF faint fluoresce r rose NE not examined

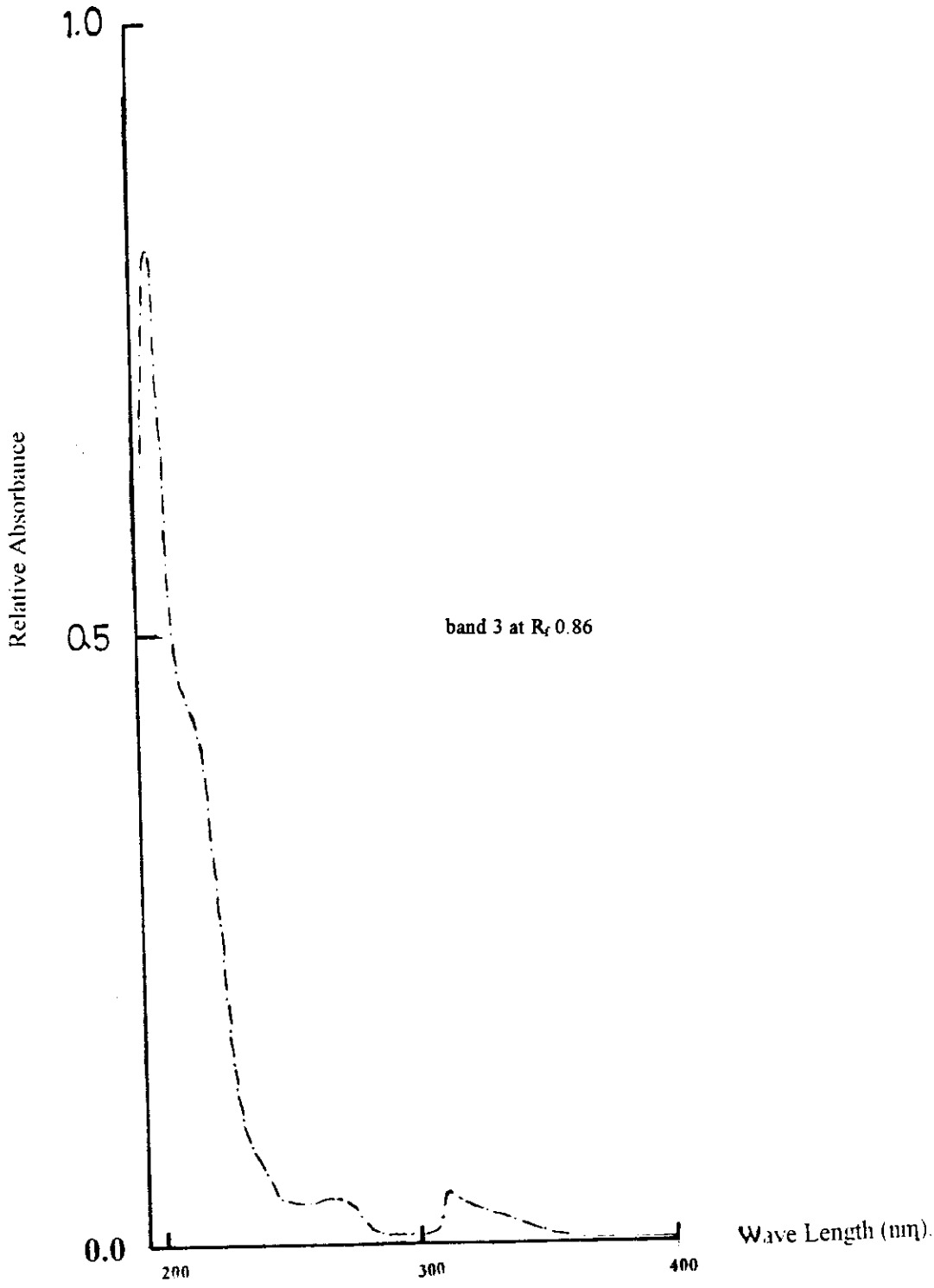


Fig. 30. U.V spectrum of phytoalexin compound of band 3 at R_f 0.86 from TLC plate of the extract of soybean (cv. Crowford) leaves of 18 days old grown under greenhouse condition after inoculation with *M. phaseolina.*

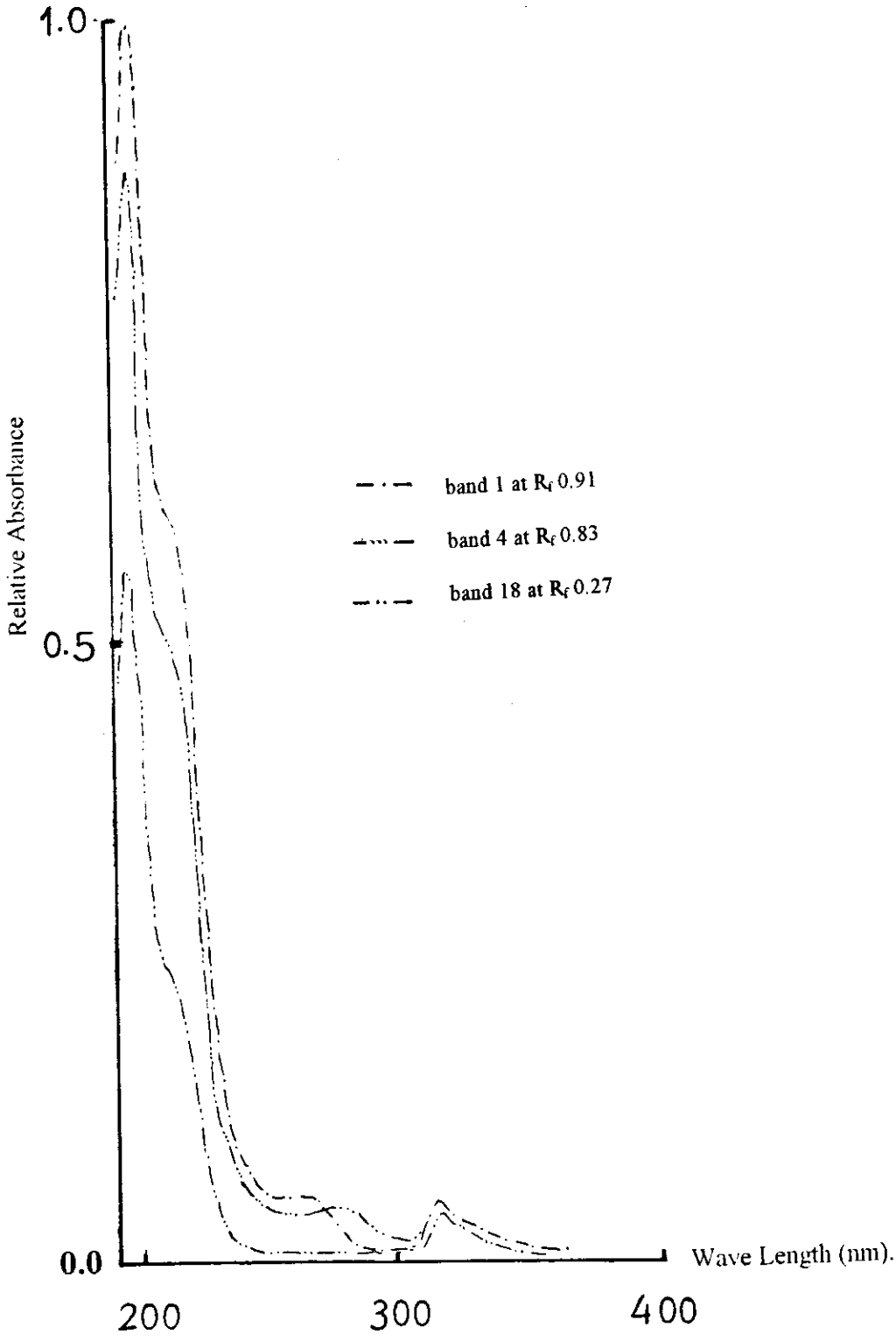


Fig. 31. U.V spectra of phytoalexin compounds of bands 1, 4 and 18 at R_f 0.91, 0.83 and 0.27, respectively, from TLC plate of the extractof soybean (cv. Crowford) leaves of 18 days old grown under greenhouse condition after inoculation with *M. phaseolina* simultaneously with *B. subtilis.*

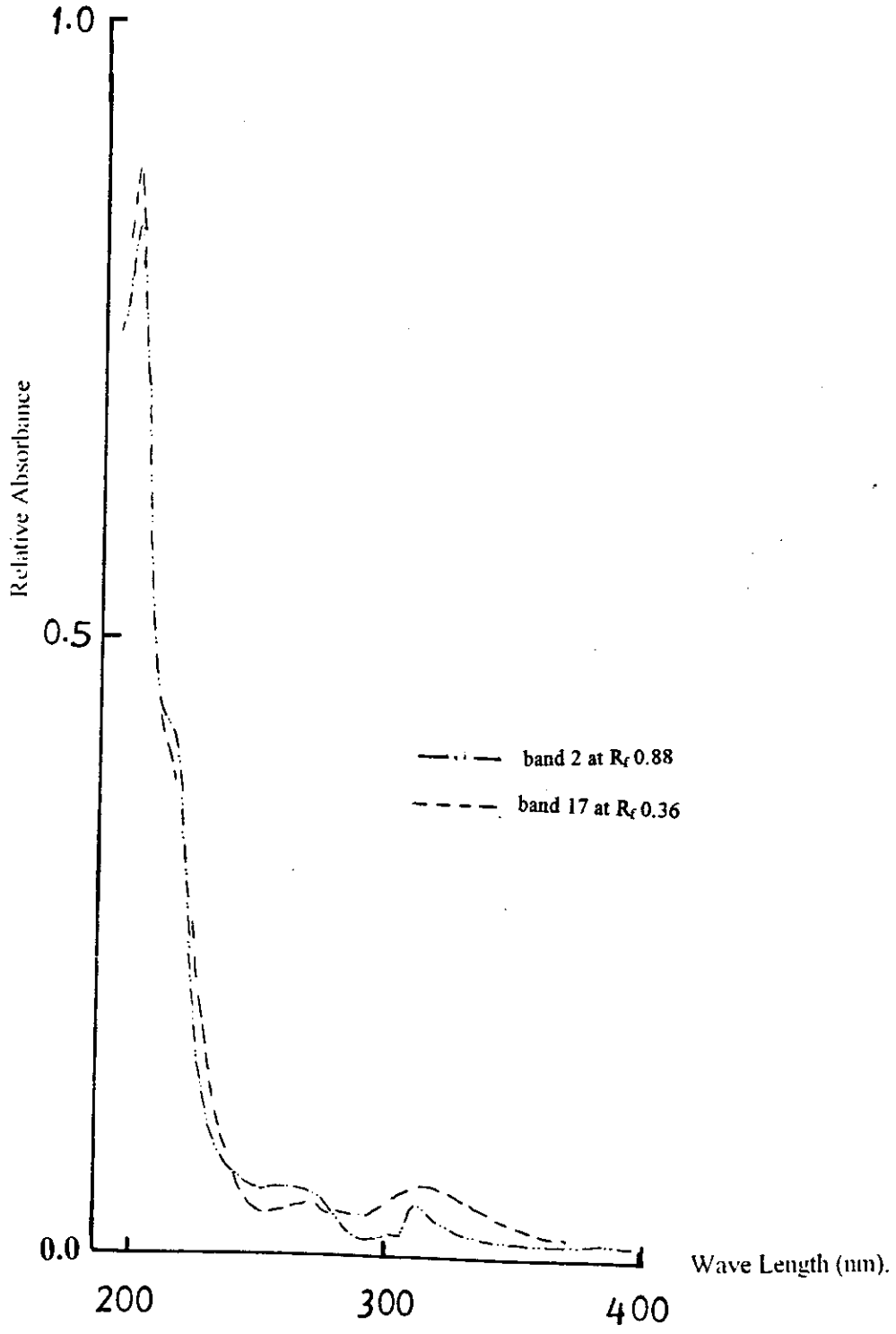


Fig. 32. U.V spectra of phytoalexin compounds of bands 2 and 17 at R_f 0.88 and 0.36, respectively, from TLC plate of the extract of soybean (cv. Crowford) leaves of 18 days old grown under greenhouse condition after inoculation with *M. phaseolina* simultaneously with *S. roseodiastaticus.*

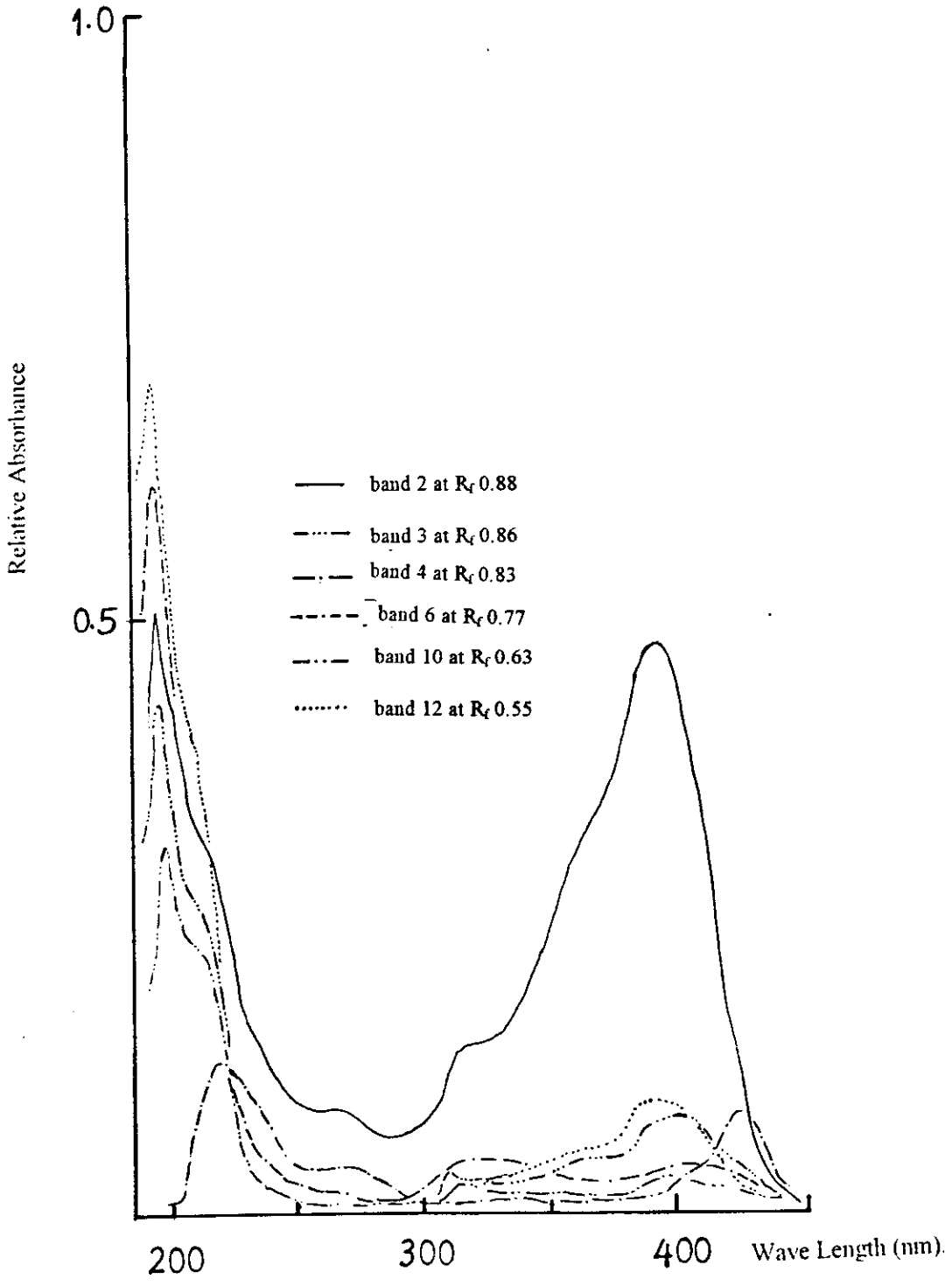


Fig. 33. U.V spectra of phytoalexin compounds of bands 2, 3, 4, 6, 10 and 12 at R_f 0.88, 0.86, 0.83, 0.77, 0.63 and 0.55, respectively, from TLC plate of the extract of soybean (cv. Crowford) leaves of 18 days old grown under greenhouse condition after inoculation with *S. roseodiastaticus*.

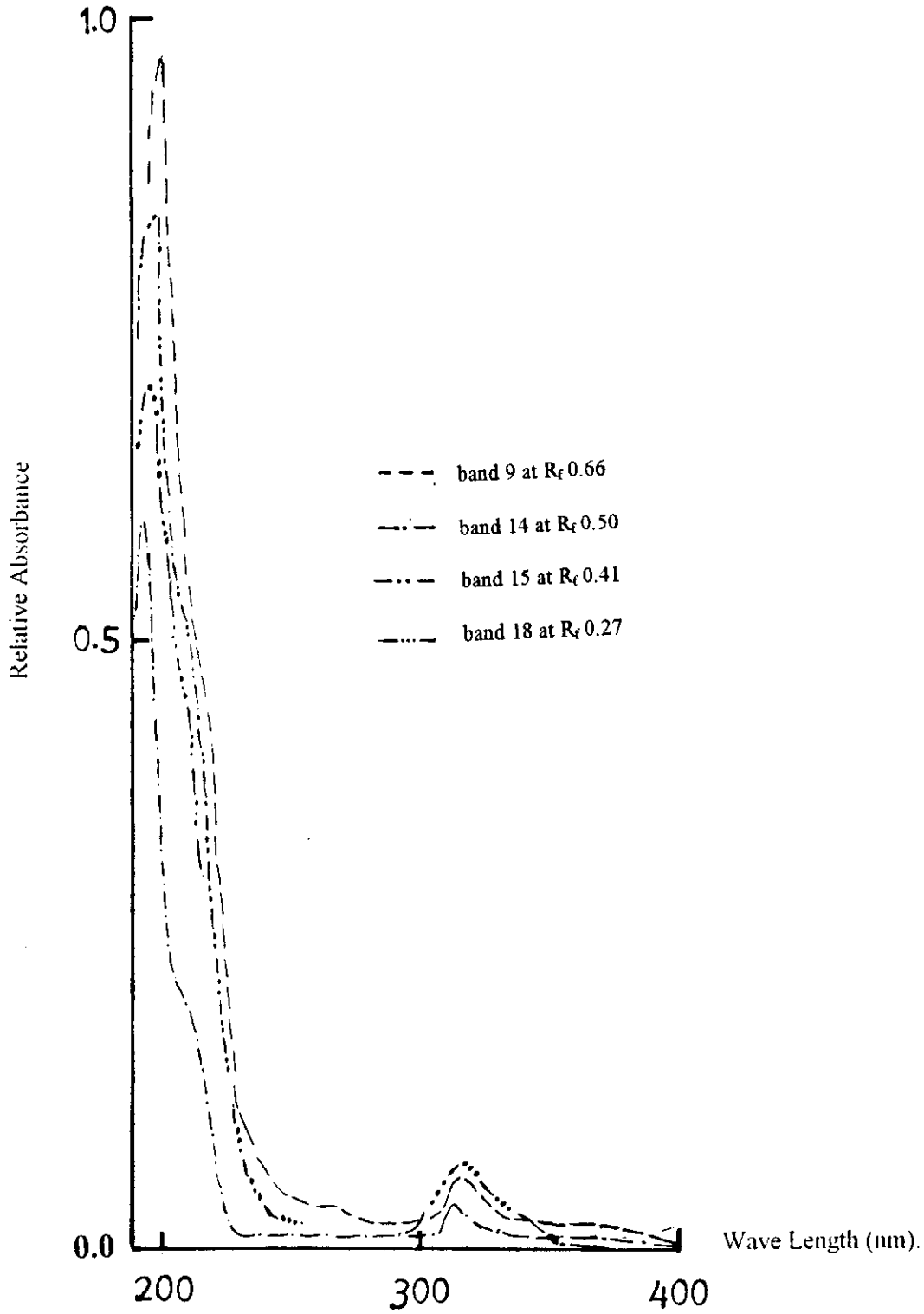


Fig. 34. U.V spectra of phytoalexin compounds of bands 9, 14, 15 and 18 at R_f 0.66, 0.5, 0.41 and 0.27, respectively, from TLC plate of the extract of soybean (cv. Crowford) leaves of 18 days old grown under greenhouse condition after inoculation with *M. phaseolina* simultaneously with *S. olivacescleroticus*.

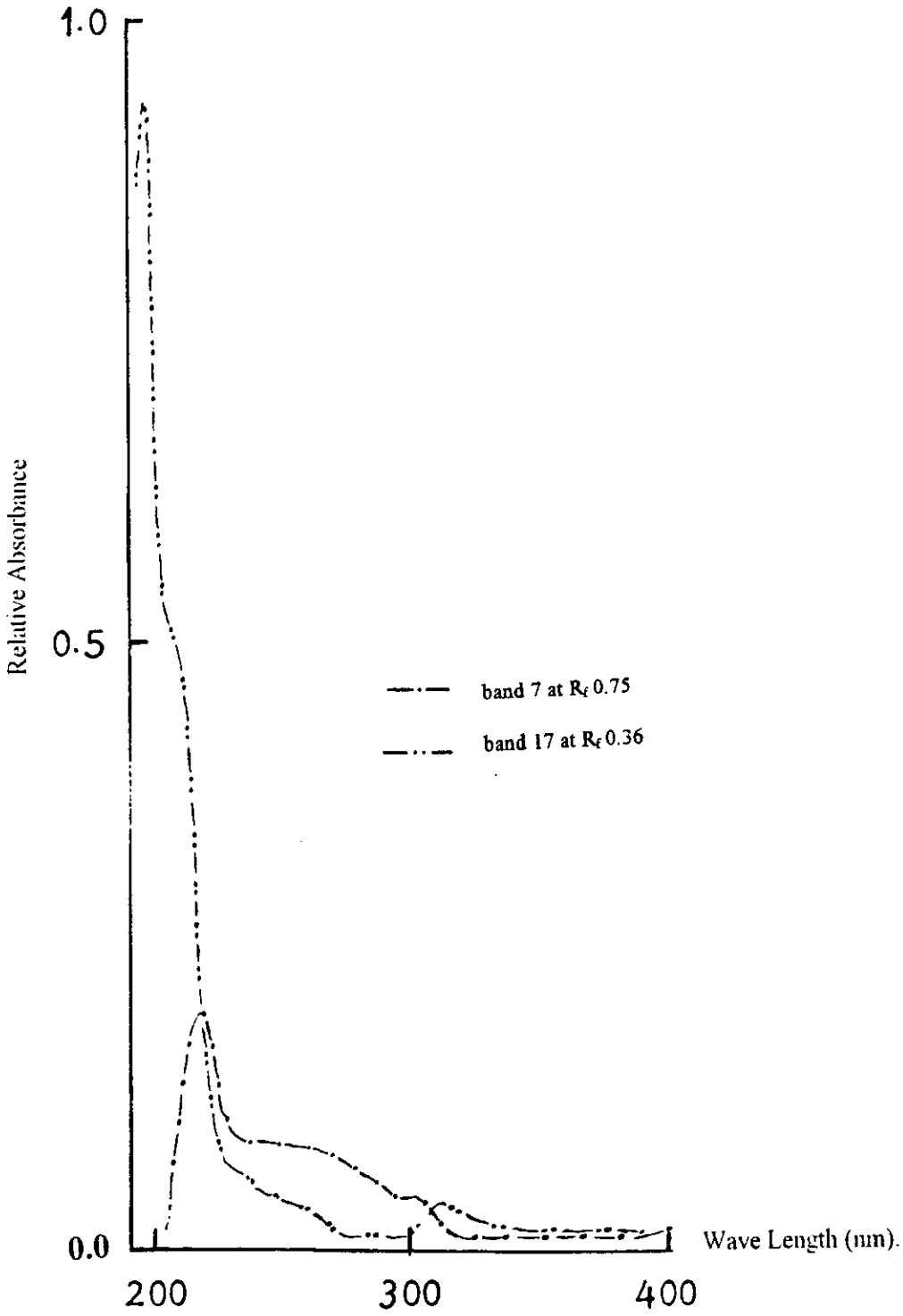


Fig. 35. U.V spectra of phytoalexin compounds of bands 7 and 17 at R_f 0.75 and 0.36, respectively, from TLC plate of the extract of soybean (cv. Crowford) leaves of 18 days old, grown under greenhouse condition after inoculation with *M. phaseolina* simultaneously with *T. harzianum*.

In the *M./S. olivaceiscleroticus* (isolate No. 729) tissue interaction systems there were three specific inducible compounds at R_f 0.52, 0.50 and 0.41 (Table 23). The first compound at R_f 0.52 differs in its characteristics under the long U.V. from the compound of similar R_f which was detected in the biocontrol agents interaction systems only (*B. subtilis* and *S. olivaceiscleroticus* (isolate No. 729), as it exhibited rose color whereas in the other systems it was of fluorescent color under the long U.V. The other two compounds at R_f 0.50 and 0.41 were not present with any of the other tested interaction systems. Both of these two compounds exhibited a rose color under the long U.V., see Fig. 34 for their U.V. spectra.

4.5.1.3 *Screening of Phytoalexins Production in Sunflower (Hypocotyl)-Pathogen/ Biocontrol Agents Interaction Systems in Greenhouse.*

There were two constitutive compounds that were detected in the control hypocotyls of sunflower plants (untreated-uninoculated) at R_f 0.91 and 0.75, see Table 24. These two compounds were detected with *S. roseodiastaticus* (isolate No. 112) tissue only, *M/S. olivaceiscleroticus* (isolate No. 729) and *M/T. harzianum* tissue systems and they have similar characterization as they exhibited a red color under the long U.V.

Five inducible phytoalexin compounds were detected in M/*B. subtilis* system at R_f 0.88, 0.83, 0.77, 0.72 and 0.05 (Table 24). The compound at R_f 0.88 was also detected with Macrophomina pathogen interaction system and it exhibited bright fluorescent color under the long U.V., while with *B. subtilis* interaction tissue it was detected as red color. The second compound at R_f 0.83 was detected as dark red color. Similar R_f compound was detected with *S. roseodiastaticus* (isolate No. 112), *S. olivaceiscleroticus* (isolate No. 729) and Macrophomina pathogen interaction systems but it exhibited a red color under the long U.V. It is of interest that these two compounds were also detected with the other tested Fusarium rot pathogen interaction systems, i.e., *F. solani* f. sp. *phaseoli*, F./*B. subtilis*, F./*S. roseodiastaticus* (isolate No. 112) and F./*S. olivaceiscleroticus*(isolate No. 729), see Table 25. The third phytoalexin compound at R_f 0.77 is specific for *M/B. subtilis* tissue system as it was detected

as dark red under the long U.V. This compound differs in its characteristic from the compound which was detected with the other tested interaction systems, involving the Fusarium rot pathogen (*F. solani* f.sp. *phaseoli*) only, F./*B. subtilis,* F./*S. roseodiastaticus* (isolate No. 112) and F./*S. olivaceiscleroticus* (isolate No. 729), as it was detected as red color. The fourth specific compound at R_f 0.72 was regarded a pronounced one as it exhibited a bright red color while the compound of the same band with *S. olivaceiscleroticus* (isolate No. 729) tissue was detected at lesser red color intensity, see Tables 24 and 25. It has similar characteristic as of the compound detected with the other tested system, i.e., with F. pathogen, F./*B. subtilis,* F./*S. roseodiastaticus* (isolate No. 112) and F./*S. olivaceiscleroticus* (isolate No. 729), see Table 25. The latter compound at R_f 0.05 is also regarded as a pronounced one compared to the same R_f compound detected with *B. subtilis* tissue. This compound exhibited a red color under long U.V.

There were two specific inducible phytoalexin compounds at R_{fs} 0.86 and 0.80 which were present in both, the *M/S. olivaceiscleroticus* (isolate No. 729) and *M/T. harzianum* interaction systems., see Table 24. These two compounds appeared as red color under the long U.V. (Table 24). Another specific inducible compound at R_f 0.11 was only present with *S. olivaceiscleroticus* (isolate No. 729) system and it appeared as flourescent color under the long U.V.

Four inducible phytoalexin compounds were detected in the Fusarium pathogen tissue interaction system at R_{fs} 0.83, 0.77, 0.72 and 0.33 (Table 25). Three of these inducible phytoalexins, i.e., 0.83, 0.77 and 0.72 were detected as red color. They exhibited similar characteristics as those detected at the same bands with F./*B. subtilis,* F./ *S. roseodiastaticus* and F./*S. olivaceiscleroticus* tissue interaction systems. The last one at R_f 0.33 was specific to F. pathogen but it was less pronounced than the compound detected with *F/B. subtilis* interaction system. This compound was characterized as red color under the long U.V.

Table 24. Screening of phytoalexins production in sunflower -*M. phaseolina* / biocontrol agents interaction systems in greenhouse by TLC

No. of band	Sunflower-hypocotyls*/in gm fresh weight									
	Control (water) (2.7gm)		*B.subtilis* (1.5gm)		*S.roseodiastaticus* (1.5gm)		*S.olivaceiscleroticus* (1.5gm)		*T..harzianum* (3gm)	
	R_{f**}	UV***	R_{f**}	UV***	R_{f**}	UV***	R_{f**}	UV***	R_{f**}	UV***
(1)a	0.91	fR^C			0.91	R^C				
(2)a			0.88	R^I						
(3)a										
(4)a					0.83	R^I	0.83	R^I		
(5)a										
(6)a										
[7]	0.75	R^C			0.75	R^C				
(8)a							0.72	R^I		
(9)a										
(10)a					0.55	R^I	0.55	R^I		
[11]										
[12]									0.44	R^I
(13)a										
(14)a										
[15]										
(16)a							0.11	F^I		
(17)a			0.05	dR^I						
(18)a									0.027	R^I

a represents the same suspected compounds in different.treatments and also with the other tested host (soybean) for the examined ones only.
[ ] refers to the specific compound for sunflower.
* age of plant 18 day, grown in greenhous ** solvent system (ethyle acetate- hexan- methanol 60: 40: 1 (v/v/v)
*** observation of band under long UV 366 nm
R red dR dark red bF bright flourescent fF faint flourescent
I inducible C constitutive

Cont.

Table 24. Screening of phytoalexins production in sunflower - *M. phaseolina* / biocontrol agents interaction systems in greenhouse by TLC

No. of band	sunflower-hypocotyls*/in gm fresh weight									
	M.phaseolina (1.6gm)		*M+B.subtilis* (1.5gm)		*M+S.roseodiastaticus* (3.5gm)		*M+S.olivaceiscleroticus* (6gm)		*M+T..harzianum* (5gm)	
	R_{f}**	UV***	R_{f}**	UV***	R_{f}**	UV***	R_{f}**	UV***	R_{f}**	UV***
(1)a					↑	↑	0.91	R^C	0.91	R^C
(2)a	0.88	bF^I	0.88	dR^I	↑	↑				
(3)a					↑	↑	0.86	R^I	0.86	R^I
(4)a	0.83	R^I	0.83	dR^I	↑	↑				
(5)a					↑	↑	0.80	R^I	0.80	R^I
(6)a			0.77	dR^I	↑	↑				
[7]					↑	↑	0.75	R^I	0.75	R^I
(8)a			0.72	bR^I	↑	↑				
(9)a					↑	↑				
(10)a					↑	↑				
[11]					↑	↑				
[12]					↑	↑				
(13)a					↑	↑				
(14)a					↑	↑				
[15]					↑	↑				
(16)a					↑	↑				
(17)a			0.05	R^I	↑	↑				
(18)a					↑	↑				

a represents the same suspected compounds in different.treatments and also with the other tested host (soybean) for the examined ones only.
[ ] refers to the specific compound for sunflower.
* age of plant 18 day, grown in greenhous ** solvent system (ethyle acetate- hexan- methanol 60: 40: 1 (v/v/v)
*** observation of band under long UV 366 nm
R red dR dark red bF bright fluorescent fF faint fluorescent
I inducible C constitutive ↑ fairly detected

25. Screening of phytoalexins production in sunflower - *F. solani* f.sp. *phaseoli* / biocontrol agents interaction systems in greenhouse by TLC

No. of band	sunflower-hypocotyls*/in gm fresh weight									
	F.solani f.sp.phaseoli (1.6gm)		*F+B.subtilis* (2gm)		*F+S.roseodiastaticus* (4.2gm)		*F+S.olivaceiscleroticus* (5gm)		*F+T..harzianum* (5.5gm)	
	R_{f**}	UV***	R_{f**}	UV***	R_{f**}	UV***	R_{f**}	UV***	R_{f**}	UV***
(1)a										
(2)a										
(3)a										
(4)a	0.83	R^I	0.83	R^I	0.83	R^I	0.83	R^I		
(5)a										
(6)a	0.77	R^I	0.77	R^I	0.77	R^I	0.77	R^I		
[7]										
(8)a	0.72	R^I	0.72	R^I	0.72	R^I	0.72	R^I		
(9)a			0.66	R^I	0.66	R^I	0.66	R^I		
(10)a			0.55	R^I	0.55	R^I	0.55	R^I		
[11]			0.50	R^I	0.50	R^I				
[12]										
(13)a	0.33	R^I							0.27	F^I
(14)a									0.22	F^I
[15]									0.11	F^I
(16)a										
(17)a							0.027	dR^I	0.027	dR^I
(18)a										

a represent the same suspected compounds in different. treatments and also with the other tested host (soybean) for the examined ones only.
[ ] refer to the specific compound for sunflower.
* age of plant 18 day, grown in greenhous ** solvent system (ethyle acetate- hexan- methanol 60: 40: 1 (v/v/v)
*** observation of band under long UV 366 nm
R red dR dark red bF bright fluorescent fF faint fluorescent I inducible C constitutive

Three major specific inducible phytoalexins were detected in the *F/B. subtilis* tissue system at R_{fs} 0.66, 0.55 and 0.50, see Table 25. The first compound at R_f 0.66 was specifically detected with F./*B. subtilis*, F./*S. roseodiastaticus* (isolate No. 112) and F./*S. olivaceiscleroticus* (isolate No. 729) interaction systems. It appeared as red color under the long U.V. The second compound at R_f 0.55 was also a specific one since it was only present with the biocontrol agents: *S. roseodiastaticus* (isolate No. 112) and *S. olivaceiscleroticus* (isolate No. 729) tissues, see Table 24, and it was also present with the F./*S. roseodiastaticus* (isolate No. 112) system in addition to the *S. olivaceiscleroticus* (isolate No. 729) tissue interaction systems, see Table 25. This compound exhibited a red color under the long U.V. The third specific inducible phytoalexin compound at R_f 0.50 was detected in F./*B. subtilis* and F./*S. roseodiastaticus* (isolate No. 112) interaction systems.

In the *F/T. harzianum* tissue interaction system, the TLC results (Table 25) revealed the presence of four inducible specific phytoalexins at R_{fs} 0.27, 0.22, 0.11 and 0.027. Two of these compounds of R_f 0.27 and 0.22 exhibited a fluorescent color under the long U.V. The compound from the band sixteen at R_f 0.11 was regarded as a pronounced one when compared with the same band detected with *S. olivaceiscleroticus* (isolate No. 729) tissue system, see Figs. 33 and 34. This inducible compound was of a flourescent color. The last compound at R_f 0.027, (R_{f5}), differed in its characteristics from the one detected at the same band , i.e., R_{f5}, in the *T. harzianum* tissue interaction system, see Figs. 33 and 34. It exhibited a dark red color (pronounced) while it was of lesser color intensity (major), red, under the long U.V. It should be noted that there was a specific inducible phytoalexin compound that was only detected at R_f 0.44 with *T. harzianum* tissue interaction system and it exhibited a red color under the long U.V., see Table 25

4.5.1.4 *Efficiency of TLC Used in This Study*

The efficiency of thin layer chromatography (TLC) technique, employed in this study, for isolation and purification of phytoalexins was examined as indicated below. Consistent separations were achieved by using the TLC plates which were from Merck, similar to those previously used by **Abdallah (1987a)** with excised soybean organs systems.

The peak area of a chromatographically pure sample of the band which had identical R_f value and physiochemical characteristics as that of the synthesized (Glyceollin) standard was determined for a sample of 20 µl injected into the HPLC. The separation of this band by HPLC was done as described in the materials and methods. The absorbence of the pure sample was then determined spectrophotometrically in ethanol. Fig. 36 illustrates one representative example showing the composition of the sixth band at R_f 0.77 from the TLC plate of the crude extract of the soybean hypocotyls, (untreated-uninoculated) tissue, when it was subjected to HPLC separation. It revealed the presence of seven compounds at retention times R_{ts} 22.8, 11.8, 9.7, 14.1, 16.4, 16.9 and 24.2 min. Each of these elution times refer to a compound and the first one mentioned is the highest in its level of detection and it is followed by the others in descending order of detection levels. Among these compounds, several ones were identified following **Graham (1991a) and Morris *et al.* (1991)** protocol for the identification of the aromatic secondary metabolite compounds, see Table 34. For example, the compounds at retention times 22.8, 16.4 and 24.2 were identified as **Isorahamantein, 6- malonyl daidzin** and **Formonentein** ,respectively, and they are indicated by arrows in Fig. 36. The other four compounds at the remaining retention times 11.8, 9.7, 14.1, and 16.9 are still unknown and they need further work. Fig 37 represents another HPLC example for the soybean/*S. roseodiastaticus* (isolate No. 112) hypocotyls tissue mixture. When the seventh band at R_f 0.72 from the TLC plate of this tested sample was subjected to HPLC separation, it revealed the presence of nine compounds at retention times 24.9, 26.1, 15.6, 12.7, 10.55, 1.57, 11.2, 29.02 and 17.9, see Fig. 37. Again, each of these elution times refers to a compound of which

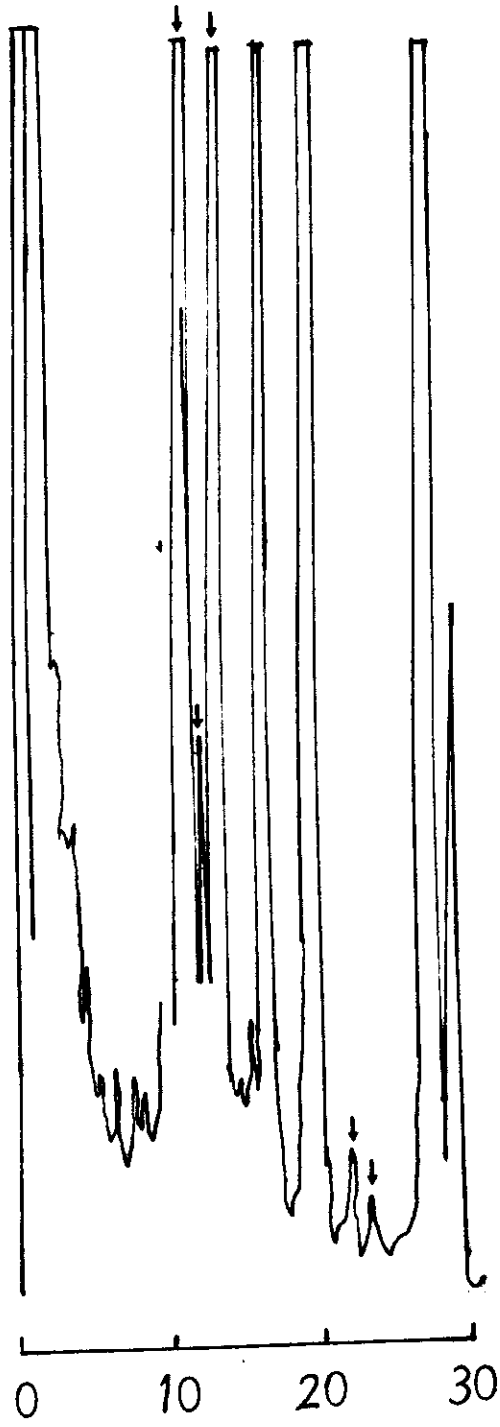


Fig. 36. HPLC profile of the sixth band at R_f 0.77 for aromatic metabolites from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old, grown under greenhouse (control) untreated - uninfected.Note: arrows point to known identified compounds.

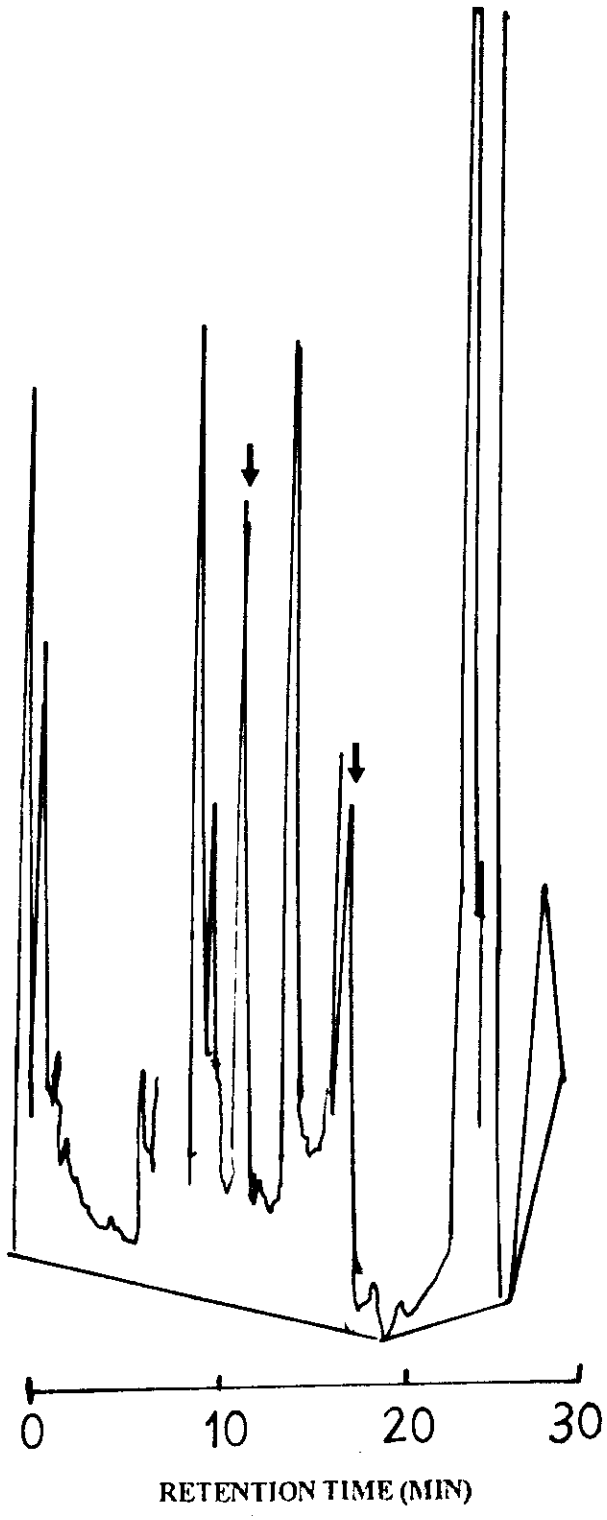


Fig. 37. HPLC profile of the seventh band at R_f 0.72 for aromatic metabolites from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old, grown under greenhouse after inoculation with *S. roseodiastaticus*. Note: arrows point to known identified compounds.

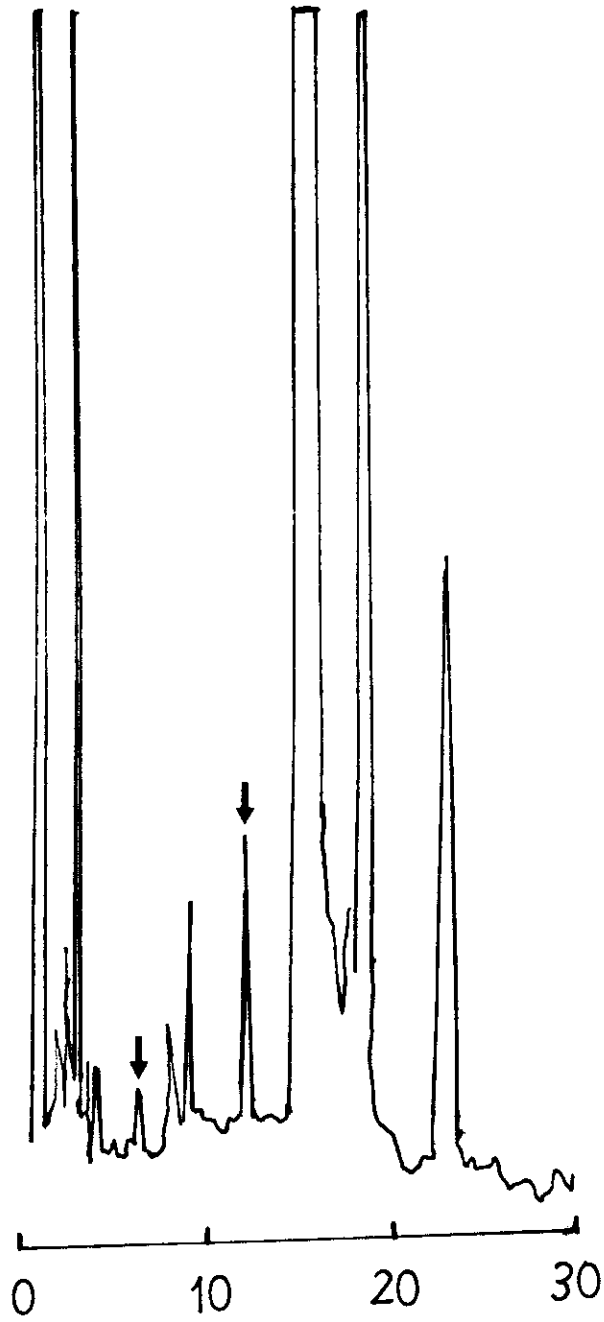


Fig. 38. HPLC profile of the sixth band at R_f 0.77 for aromatic metabolites from TLC plate of the extract of soybean (cv. Crowford) hypocotyls of 18 days old, grown under greenhouse after inoculation with *F. solani.* f.sp. *phaseoli* simultaneously with *S. roseodiastaticus*. Note: arrows point to known identified compounds.

retention times 15.6, 10.55, 1.57, 11.2 and 29.02 were not identified and therefore need further work .

Moreover, another examples was also included that represents the sixth band at R_f 0.77 from the TLC plate of the extract of soybean hypocotyls - *F/S. roseodiastaticus* (isolate No. 112) interaction tissue mixture, see Fig. 38. When it was subjected to HPLC separation, it revealed the presence of nine compounds at retention times 2.4, 4.7, 1.3, 2.8, 2.6, 8.8, 7.1, 14.3 and 12.4 min., see Fig. 38. These elution times are arranged ascendingly according to the compounds levels of detection. Again, following **Graham (1991a) and Morris et al. (1991)** protocol for the identification of aromatic secondary metabolite two compounds at retention times 7.1 and 12.4 were identified as **Phenylalanine** and **Daidzin** (they are marked by arrows in Fig. 38), respectively, see Table 34. The other seven compounds at the remaining retention times were not also identified and they need further work.

4.5.2 Phytoalexins Detection by HPLC

High performance liquid chromatography (HPLC) was used to separate and identify the phytoalexin components of the ethanol soluble tissue extracts as outlined in the materials and methods. Extraction from hypocotyl and leaf tissue was carried out as described previously. Individual components that absorbed at 236 nm (the setting of the HPLC detector) were collected from samples repetitively and injected into the HPLC to check for compound purity and retention times stability. A change in the method of extraction than was followed by other researchers, for HPLC analysis of the soybean phytoalexins **Graham (1991a)**, revealed the presence of a substantial amount of aromatic secondary metabolites compounds (isoflavonoids) and their conjugates that absorbed at 336 nm, the wave length used on the detector (Fig. 36 and Table 26). These compounds were found to accumulate in the tested hypocotyls and leaves of the two fungal pathogens interaction systems.

The compounds which have either identical or very close retention times (R_{ts}) as those of the known (previously reported) or artificially synthesized isoflavonoids phytoalexins and their conjugates will be referred to as identified compounds. These findings will be summarized later in this section (Table 34). While the other phytoalexin compounds that are not identified in this study, will be therefore referred to as unidentified ones., see Table 26 for their detailed distribution and elution times. The unknown compound(s) but of substantial amount, among the unidentified ones will be presented later in this section (Table 35).

The detailed distribution of these various isoflavonoids phytoalexins and their conjugates (identified or unidentified ones) of all the tested system and their elution times will be presented in the subsequent subsections (4.5.3.1-4.5.3.4). The level of detection, based on the area peak mesaurements, is classified into seven categories to simplify comparison between the different interaction systems. The seven catogeries are as follows :-

	Peak area range	**Classified as**
0	< peak area < 10,000	insignificant and was not considered in this study.
10,000	< peak area < 30,000	minor III denoted by ' in Tables 26-29.
30,000	< peak area < 70,000	minor II denoted by '' in Tables 26-29.
70,000	< peak area < 100,000	minor I denoted by ''' in Tables 26-29.
100,000	< peak area < 300,000	less prominent denoted by * in Tables 26-29.
300,000	< peak area < 700,000	prominent denoted by ** in Tables 26-29.
	< peak area	highly prominent (major) denoted by *** in Tables 26-29.

4.5.2.1 *Screening of Phytoalexins Production in Soybean (Hypocotyl)-M. phaseolina/Biocontrol Agents Interaction Systems in Greenhouse*

The data shown in Table 26 and Fig. 39 revealed the presence of twelve phytoalexin compounds in the soybean hypocotyl (untreated-uninoculated) extracts at retention times (R_{ts}) 12.42, 15.34, 17.58, 18.58, 23.36, 24.18, 25.12, 25.68, 25.7, 26.3, 26.6 and 27.4 min. Three

from these constitutive compounds were highly prominent at the R_{ts} 18.56, 23.36, and 24.18 min. Moreover, there were three compounds at the R_{ts} 25.12, 25.6.8 and 27.4 min. which were detected at less prominent levels. The highly prominent compounds will be referred to as the major ones. The remaining six compounds were detected at the R_{ts} 12.42, 15.34, 17.5, 25.7, 26.3 and 26.6 min., but at lesser levels, i.e., minor levels according to the previous classification. The identification of these compounds will be clarified later in (section 4.5.3.1).

Twenty-three phytoalexin compounds were detected in the soybean hypocotyl infected with Macrophomina pathogen, i.e., M-elicited tissue (susceptible), see Table 26. Twelve of these compounds were similar to those constitutive compounds detected with the control (untreated-uninoculated), but they were detected at higher levels. The last nine compounds at the R_{ts} 7.88, 13.3, 13.9, 19.28, 20.3, 21.33, 22.6, 28.03 and 28.8 min. were inducible ones. One from those inducible compounds was highly prominent at R_t 22.62, while three of them at the R_{ts} 20.3, 22.62 and 28.03 and min were of less prominent levels.. The last five compounds which were detected at the R_{ts} 7.88. 13.3, 13.9, 19.28 and 21.33 min. were of minor levels. These detected compounds (flavonoids and isoflavonoids) phytoalexins of the Macrophomina susceptible interaction tissue will be identified later in subsection 4.5.3.1.

However, in soybean hypocotyl extracts (biocontrol (s) elicited tissue) there were eighteen compounds which were detected in Bacillus (B) tissue elicited system (resistant). Eight of these compounds were constitutively present at R_{ts}. similar to those detected with the control (untreated-uninoculated), but they were detected at different levels. The other ten inducible phytoalexins were detected in the extract of the resistant tissue at similar R_{ts} as those detected in the inducible ones in the extract of the infected (susceptible) tissue. Five of these inducible compounds were detected at the R_{ts} 20.11, 21.6, 22.3, 22.6 and 28.6 min. Only one compound at the elution time (R_t) 20.1 min. among the five inducible ones was of prominent level. Whereas the other five phytoalexin compounds were detected with B. elicited tissue interaction system (resistant one) at the R_{ts} 1.44, 4.83, 5.58, 8.0 and 10.56 min. All of these

compounds were detected at less prominent levels with the exception of the compound at R_t 8.0 which was detected at a promiminent level (Fig. 39).

In the biocontrol elicited tissue system involving, *S. roseodiastaticus* (isolate No. 112), sixteen phytoalexin compounds were detected (Tables 26). Seven of these, were constitutively present at the R_{ts} 15.3, 17.1, 17.7, 18.3, 24.2, 24.6 and 27.7 min. and they were similar to those detected with the control tissue (untreated-uninoculated). Five of these phytoalexins compounds were detected at R_{ts} 13.5, 19.1, 20.9, 22.5 and 22.8 min., which were also similar to those detected with the (Macrophomina elicited tissue). The compounds at the R_{ts} 4.35, and 16.16, were detected at highly prominent and less prominent levels, respectively. The former at the R_t 4.35 min. was considered specific for *S. roseodiastaticus* (isolate No. 112) elicited tissue system, i.e., (resistant one).

In the other biocontrol elicited tissue, i.e., the *S. olivaceiscleroticus* (isolate No. 729) system (resistant) there were six detected compounds. Two compounds at the R_{ts} 18.9 and 25.77 min. were similar to those detected with the control (untreated- uninoculated), the latter compound at the R_t 25.77 was of less prominent level, while the former was of a minor level. The other four inducible phytoalexins were detected at the R_{ts} 4.96, 19.6, 21.7 and 22.6 min. The last three of these inducible phytoalexins in this extract of the resistant tissue were similar to those detected with the (Macrophomina elicited tissue). The phytoalexin compound at the R_t 4.96 was detected at a highly prominet level while it was also detected in the B. tissue interaction system but at a less prominent level.

In the *T. harzianum* tissue system (biocontrol elicited tissue), there were six phytoalexin compounds at the R_{ts} 4.2, 4.87, 6.42, 7.29, 7.87 and 15.35 min. Only the compound at the R_t 15.35 was similar to the one detected with the control (untreated-uninoculated). Two compounds which were detected at the R_{ts} 7.29 and 7.8 min. were also similar to the compounds detected with the Macrophomina pathogen system. The other three inducible compounds in this extract at the R_{ts} 4.2, 4.8 and 7.29 min. were also detected with

the *S. roseodiastaticus* (isolate No. 112) and *B. subtilis* tissue system. It should be noted that the inducible compound at the R_{ts} 4.2 min. was the only compound that was considered as a highly prominent one.

In the biocontrolled soybean hypocotyl protected tissue (pathogen-biocontrol elicited tissue); the M/*B. subtilis* tissue interaction system (biocontrolled protected), HPLC revealed the presence of thirty phytoalexin compounds (Table 26). Sixteen of these compounds were also found with the control (untreated-uninoculated), but with different levels. Nine inducible phytoalexin compounds of elution times similar to those compounds which have been detected with the Macrophomina elicited tissue were also found in the M/B system. Three of the nine inducible compounds at the R_{ts} 1.2, 1.4, and 1.9 min. were also found in the B. tissue system. While the inducible compound at the R_t 9.36 min. was only detected with the M/B system. The inducible compound at the R_t 16.47, in this tissue system, has been also detected in the *S. roseodiastaticus* (isolate No. 112) system.

The HPLC analysis of the M/*S. roseodiastaticus* (isolate No. 112) tissue interaction systems, revealed the presence of twenty five phytoalexin compounds. Twelve of these compounds were similar to those detected with the control (untreated-uninoculated), but they were detected at different levels. There were other seven phytoalexin compounds at the R_{ts} 7.95, 13.2, 13.39, 19.3, 20.0, 20.8 and 22.19 min. which were inducible ones. They were detected at similar R_{ts} as those compounds which have been detected with the Macrophomina pathogen elicited tissue. The last six compounds were inducible phytoalexins. They were detected at the R_{ts} 1.5, 1.9, 2.18, 2.88, 10.32 and 10.27 min. and were not identified in this study.

Table 26. Screening of phytoalexins production in soybean- *M. phaseolina*/biocontrol agents interaction systems in greenhouse by HPLC

Treatment	Soybean hypocotyls[a] Retention time														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control															
Water (only)												12.42"			15.34'
Biological agent															
B.subtilis	1.44*			4.83*	5.58*			8.0**		10.56*		12.6*			15.68"
S.rosodiastaticus				** *			** * 7.8**						13.5***		15.3*
S.olivaceiscleroticus				** *											
T.harzianum				4.2*** 4.8*		6.4*	7.2** 7.8'''								15.3"
Pathogen															
M.phaseolina							7.8'						13.3' 13.9'		15.3" 15.6'
Pathogen + Biological agent															
M+B.subtilis	1.2* 1.4** 1.9***						7.5'		9.36*				13.6'		15.4*
M+S.rosodiasticus	1.5" 1.9"	2.18''' 2.8'					7.9**			10.3** 10.7**		12.7**	13.2* 13.3*		15.6''' 15.9'''
M+S.olivaceiscleroticus	1.12*			4.2' 4.9*			7.2'		9.1***				13.8' 13.9*	14.9*	15.9'''
M+T.harzianum	1.19* 1.5"	2.16*					7.07* 7.8*	8.8**		10.7*			13.34* 13.9'''		

Age of plant 18 day, grown in greenhous

70,000 < peak area < 100,000 ** 100,000 < peak area < 300,000 *** 300,00 < peak area < 700,000

0 < peak area < 10,000 " 10,000 < peak area < 30,000 ''' 30,000 < peak area < 70,000

Compounds whose peah areas are greater than 70,000 are considered as major ones.

Cont.

26. Screening of phytoalexins production in soybean- *M.phaseolina*/bicontrol agents interaction systems in greenhouse by HPLC

Treatment		Soybean hypocotyls[a] Retention time														
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
D	*Control*															
	Water (only)		17.5'	** *					23.3***	24.1***	25.1* 25.6* 25.7'''	26.3' 26.6'	27.4*			
C	*Biological agent*															
c1	*B.subtilis*			18.03'		20.1**	21.6"	22.3" 22.6"	23.6*	24.5"		26.2** 26.5**	27.4"	28.6'		
c2	*S.roseodiastaticus.*	16.1*	17.15* 17.7'''	18.3*	19.1*	20.9**		22.5' 22.8'		24.2' 24.6'			27.7'			
c2	*S.olivaceiscleroticus*			18.9"	19.6*		21.7'	22.6'			25.7*					
c3	*T.harzianum*															
A	*Pathogen*															
	M.phaseolina		17.6* 17.9"	18.2" 18.5* 18.8'	19.2'	20.3*	21.3'''	** *	23.5***	24.6***	25.3** 25.4***	26.2** 26.9**	27.3*	28.03* 28.8*		
B	*Pathogen + Biological agent*															
B1	*M+B.subtilis*	16.4*	17.1* 17.7**	18.2* 18.6* 18.9"	19.4'	20.5' 20.6'	21.3'''	22.1' 22.8**	23.09* 23.6" 23.8*	24.1** 24.8**	25.6** 25.7*	26.3" 26.6'''	27.2**	28.9'		
B2	*M+S.roseodiastaticus.*		17.1" 17.4''' 17.7'''		19.2*	20.0" 20.8'''		** *	23.9**	24.4"	25.7**	26.0** 26.9*	** *			
B2\	*M+S.olivaceiscleroticus*		17.04* 17.2''' 17.6*	18.2" 18.6'''		20.1''' 20.4' 20.6"	21.3*	22.7* 22.8*	23.05* 23.7* 23.8*	24.1" 24.4' 24.7*	25.4** 25.9'	26.4'	27.2** 27.5'''			
B3	*M+T.harzianum*	16.4*				20.3'''		22.8'		24.5'	25.1' 25.7'	26.8'	27.11'			

a- Age of plant 18 day, grown in greenhous

* 70,000 < peak area < 100,000 ** 100,000 < peak area < 300,000 *** 300,00 < peak area < 700,000

' 0 < peak area < 10,000 " 10,000 < peak area < 30,000 ''' 30,000 < peak area < 70,000

Compounds whose peah areas are greater than 70,000 are considered as major ones.

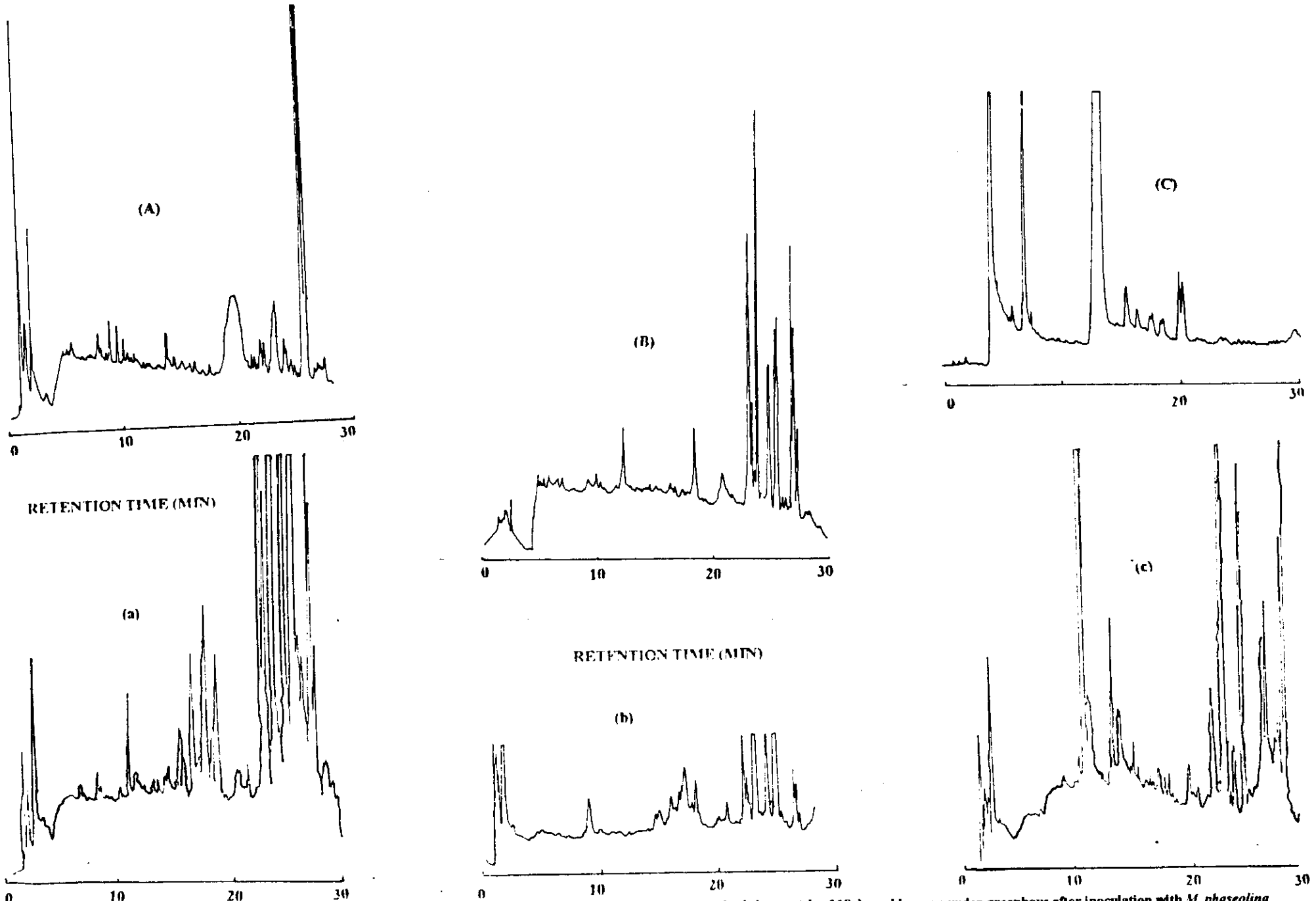
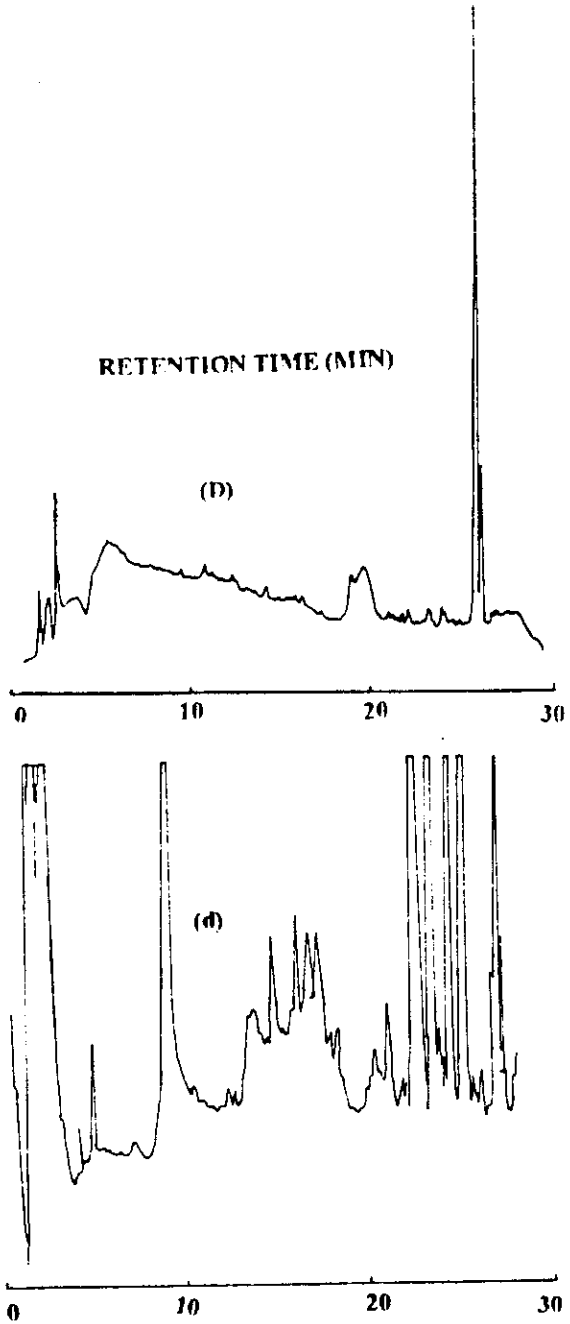


Fig. 39. HPLC profiles of aromatic metabolites from extracts of various treatments of soybean (cv. crowford) hypocotyls of 18 days old grown under greenhous after inoculation with *M. phaseolina* simultaneously with: (B), *B. subtilis*; (C) *S. roseodiasticus*; (D), *S. olivaceiscleroticus*; (E),*T. harzianium*. The other profiles represent the following treatments: (A), control pathogen; (a), uninfected untreatd (water); (b), *B. subtilis*; (c), *S. roseodiasticus*; (d), *S. olivaceiscleroticus*; (e),*T. harzianium*. See next page for continuation of this Fig.

Cont.



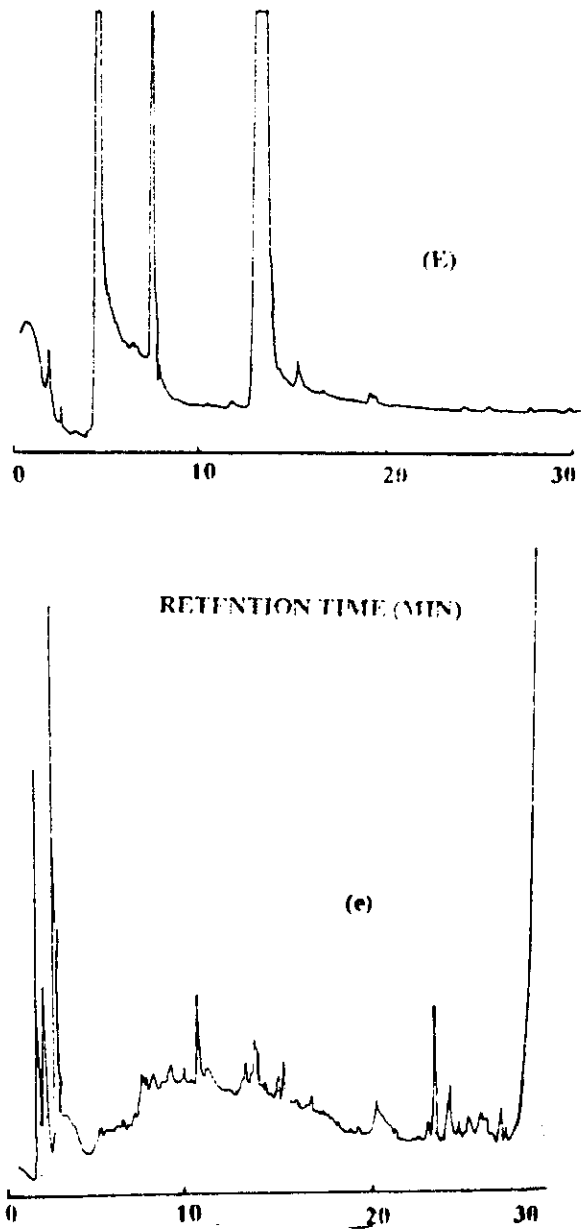


Fig. 39. HPLC profiles of aromatic metabolites from extracts of various treatments of soybean (cv. crowford) hypocotyls of 18 days old grown under greenhous after inoculation with *M. phaseolina* simultaneously with: (B), *B. subtilis*; (C) *S. roseodiasticus*; (D), *S. olivaceiscleroticus*; (E),*T. harzianium*. The other profiles represent the following treatments: (A), control pathogen; (a), uninfected untreatd (water); (b), *B. subtilis*; (c), *S. roseodiasticus*; (d), *S. olivaceiscleroticus*; (e),*T. harzianium*.

However, in the M/*S. olivaceiscleroticus* (isolate No. 729) tissue interaction systems the HPLC analysis revealed the presence of thirty three phytoalexin compounds (Table 26 and Fig. 39). Nineteen from these compounds were detected at similar R_{ts} to those constitutive compounds which have been detected with the control (untreated-uninoculated), but they were detected at different levels. Nine compounds were also detected at similar R_{ts} as those which have been detected with the M. elicited susceptible tissue. The elution times of these compound were 7.2, 13.8, 13.9, 20.18, 20.46, 20.64, 21.3, 22.7 and 22.8 min. There were other five inducible phytoalexins that were detected at the R_{ts} 1.2, 4.2, 4.9, 9.1, and 14.9 min. Only one of these inducible compounds detected at the R_t 4.9 was similar to the compound that was also detected with the *S. olivaceiscleroticus* (isolate No. 729) tissue, however, it was detected with lesser level. It should also be noted that the compound at the R_t 14.9 was considered as a specific compound to the biocontrolled protected tissue of the M/*S. olivaceiscleroticus* (isolate No. 729) system.

In the M/*T. harzianum* tissue interaction system, (Table 26 and Fig. 39), HPLC results revealed the presence of seventeen phytoalexin compounds. Five of these compounds were similar to those constitutive compounds detected with the control (untreated-uninoculated), but they were detected at different levels. However, six inducible compounds at the R_{ts} 7.07, 7.8, 13.34, 13.9, 20.3 and 22.8 min. were detected at similar R_{ts} as those detected with the M. elicited susceptible tissue. Two from these inducible compounds at the R_{ts} 7.07, 7.8 were also similar to those inducible ones detected in *T. harzianum* tissue and the latter one was detected at a less prominent level in this system while it was of minor leve with the *T. harzianum* system. The other six inducible phytoalexin compounds were at the R_{ts} 1.19, 1.5, 2.16, 8.8, 10.75 and 16.4 min.

4.5.2.2 *Screening of Phytoalexins Production in Soybean (Hypocotyl) F. solani. f.sp. phaseoli/ Biocontrol Agents Interaction Systems in Greenhouse.*

The data presented in Table 27 revealed the presence of twelve phytoalexin compounds in the soybean hypocotyl extracts (untreated-uninoculated) at the elution times R_{ts} 12.42, 15.34, 17.58, 18.58, 23.36, 24.18, 25.12, 25.68, 25.7, 26.6 and 27.4 min. Three of these constitutive compounds were highly prominent at the R_{ts} 18.56, 23.36 and 24.18 min. However, there were other three compounds of less prominent at the R_{ts} 25.12, 25.68 and 27.4 min. The remaining six compounds, at the R_{ts} 12.42, 15.34, 17.5, 25.7, 26.3 and 26.6 min. were detected at minor levels. These compounds will be identified in (subsection 4.5.3.2).

Seventeen phytoalexin compounds were detected in the soybean hypocotyls which were infected with the Fusarium rot pathogen. Eight of these compounds were similar to those compounds detected with the control (untreated-uninoculated), but they were detected at different levels. The last nine compounds at the R_{ts} 1.48, 2.07, 7.23, 16.7, 19.5, 20.22, 20.78, 21.6 and 22.6 min. were inducible ones. Seven of these inducible compounds at the R_{ts} 1.48, 2.07, 16.7, 19.5, 20.22, 20.78 and 22.6 were of different prominent levels (Table 27 and Fig. 40), min., while the two inducible compounds at the Rts 7.23 and 21.6 were of minor levels. The detected phytoalexins, i.e., the flavonoids and isoflavonoids compounds of the Fusarium elicited tissue (susceptible, infected system) will be identified in (subsection 4.5.3.2).

Furthermore, the screening of the phytoalexins, in soybean hypocotyl biocontrol/pathogen-elicited tissue system using the HPLC analysis (biocontrolled protected tissue) revealed the presence of two phytoalexin compounds in the biocontrolled F/*B. subtilis* tissue interaction systems (Table 27). None of these compounds were similar to those constitutive compounds detected with the control (untreated-uninoculated). These two compounds at the R_{ts} 2.94 and 21.9 min. were inducible ones. They were detected at similar the R_{ts} as those detected with Fusarium elicited tissue (susceptible). Those detected phytoalexin compounds of the Bacillus protected tissue will be identified in (subsection 4.5.3.2).

Table 27. Screening of phytoalexins production in soybean- *F.solani* f.sp.*phaseoli* / biocontrol agents interaction systems in greenhouse by HPLC

Treatment		Soybean hypocotyls[a] Retention time														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D	*Control*															
	Water (only)												12.42"			15.34'
C	*Biological agent*															
c1	*B.subtilis*	1.44*			4.83*	5.58*			8.0**		10.56*		12.6*			15.68"
c2	*S.roseodiastaticus*				** *			** * 7.8**						13.5***		15.3*
c2\	*S.olivaceiscleroticus*				** *											
c3	*T.harzianum*				4.2*** 4.8*		6.4*	7.2** 7.8"								15.3"
A	*Pathogen*															
	F.solani f.sp.phaseoli	1.4***	2.07*					7.2"								15.3*
B	*Pathogen + Biological agent*															
B1	*F+B.subtilis*		2.9*													
B2	*F+S. roseodiastaticus*	1.4**	2.6*		4.4***										14.09**	
B2\	*F+S.olivaceiscleroticus*					5.1*										
B3	*F+T.harzianum*	1.18* 1.5*	2.1*					7.7'				11.2*		13.3" 13.7'		15.6"

a- Age of plant 18 day, grown in greenhous

* 70,000 < peak area < 100,000 ** 100,000 < peak area < 300,000 *** 300,00 < peak area < 700,000

' 0 < peak area < 10,000 " 10,000 < peak area < 30,000 "' 30,000 < peak area < 70,000

Compounds whose peah areas are greater than 70,000 are considered as major ones.

Cont.

Table 27. Screening of phytoalexins production in soybean- *F.solani* f.sp. *phaseoli* / biocontrol agents interaction systems in greenhouse by HPLC

Treatment		Soybean hypocotyls[a] Retention time														
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
D	*Control* *Water (only)*		17.5'	** *					23.3***	24.1***	25.1* 25.6* 25.7'''	26.3' 26.6'	27.4*			'
C	*Biological agent*															
c1	*B.subtilis*			18.03'		20.1**	21.6''	22.3'' 22.6''	23.6*	24.5''		26.2** 26.5**	27.4''	28.6'		
c2	*S.roseodiastaticus*	16.1*	17.15* 17.7'''	18.3*	19.1*	20.9**		22.5' 22.8'		24.2' 24.6'			27.7'			
c2	*S.olivaceiscleroticus*			18.9''	19.6*		21.7'	22.6'			25.7*					
c3	*T.harzianum*															
A	*Pathogen*															
	F.solani f.sp. phaseoli	16.7**	17.2**	18.08**	19.58*	20.2* 20.7*	21.6'''	22.6*	23.5**	24.5**	25.7*	26.3*** 26.7'''				
B	*Pathogen + Biological agent*															
B1	*F+B.subtilis*						21.9*									
B2	*F+S.roseodiastaticus*					20.1**				24.8*	25.1*	26.8'				
B2\	*F+S.olivaceiscleroticus*															
B3	*F+T.harzianum*		17.3''' 17.9*	18.4'' 18.8'''	19.2''	20.3*		22.0* 22.4* 22.9'''	23.3**	24.8***	25.4' 25.7'''	26.4* 26.8*	27.6*	28.3*		

a- Age of plant 18 day, grown in greenhous

* 70,000 < peak area < 100,000 ** 100,000 < peak area < 300,000 *** 300,00 < peak area < 700,000

' 0 < peak area < 10,000 '' 10,000 < peak area < 30,000 ''' 30,000 < peak area < 70,000

Compounds whose peah areas are greater than 70,000 are considered as major ones.

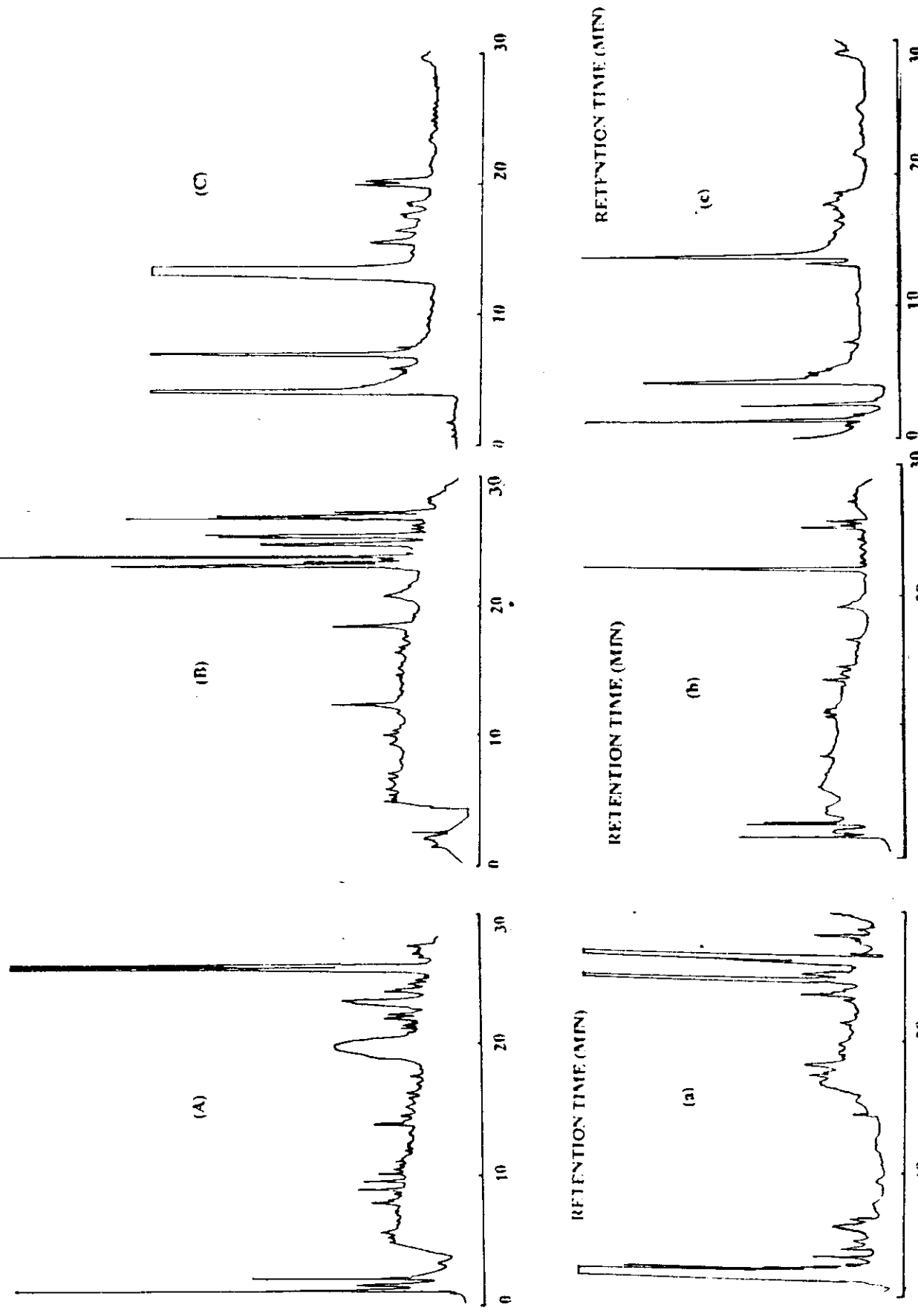


Fig. 40. HPLC profiles of aromatic metabolites from extracts of various treatments of soybean (cv. crowford) hypocotyls of 18 days old grown under greenhous after inoculation with *F. solani.* f.sp. *phaseoli* simultaneously with *(B). B subtilis; (C) S. roseodiasticus*: (D), *S. olivaceiscleroticus*: (E),*T. harzianus*.The other profiles represent the following treatments: *(A). control pathogen* ; (a). untreatd - uninfected (water); *(b). B. subtilis*; (c) *S. roseodiasticus*; (d), *S. olivaceiscleroticus*; (e),*T. harzianum*. See next page for the continuation of this Figure.

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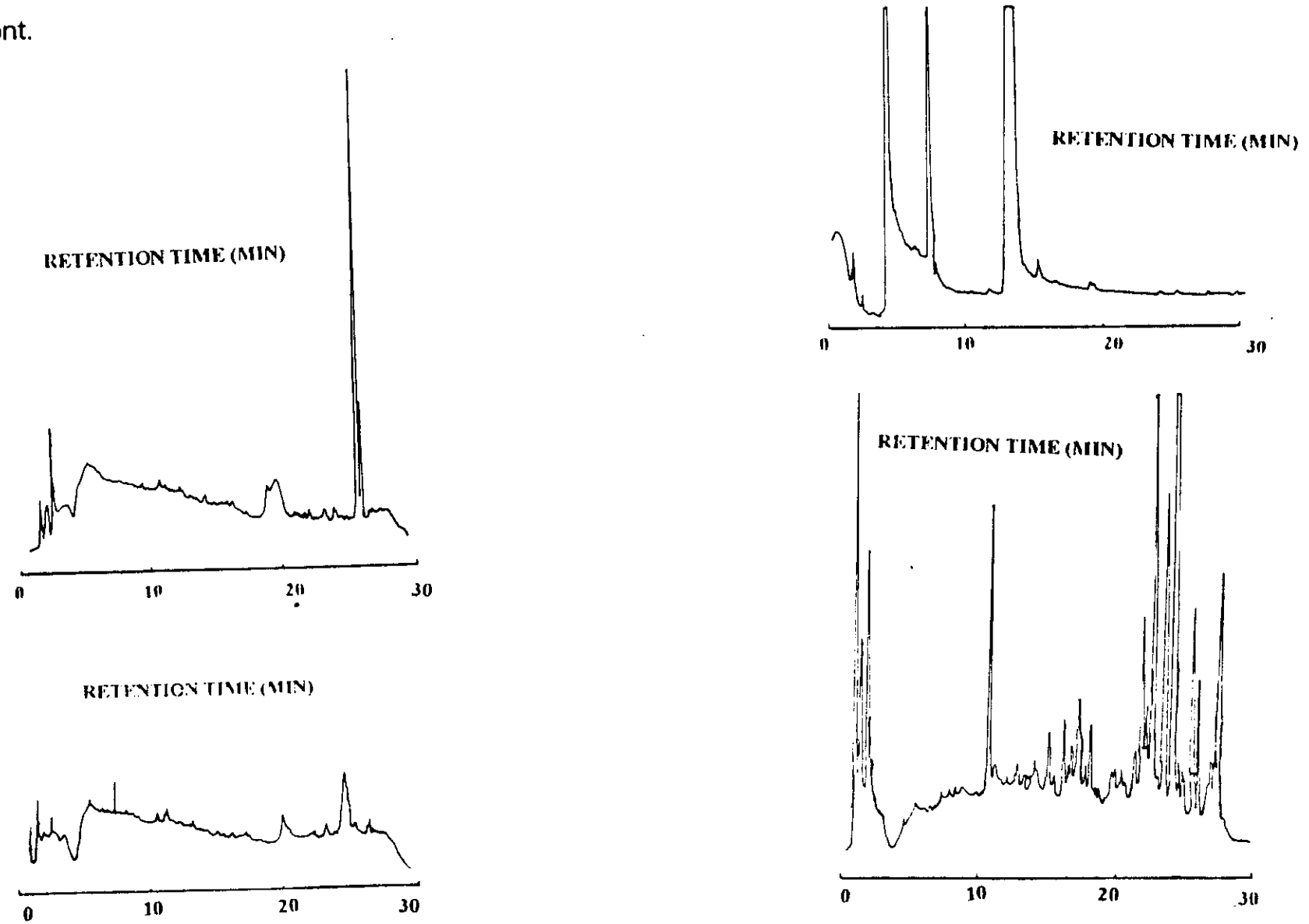

Fig. 40. HPLC profiles of aromatic metabolites from extracts of various treatments of soybean (cv crowford) hypocotyls of 18 days old grown under greenhous after inoculation with *F. solani* f sp *phaseoli* simultaneously with (B). B. subtilis. (C) S. roseodiasticus; (D). S. olivaceiscleroticus; (E),T. harzianius.The other profiles represent the following treatments: (A), control pathogen , (a), untreatd - uninfected (water); (b), B. subtilis; (c) S. roseodiasticus, (d). S. olivaceiscleroticus; (e).T harzianium

Secondly, in the F/*S. roseodiastaticus* (isolate No. 112) soybean hypocotyls tissue interaction system (protected) (Table 27), the HPLC analysis revealed the presence of four phytoalexin compounds. None of these compounds were similar to those constitutive compounds detected with the control (untreated-uninoculated). These compounds were detected at similar R_{ts} as those detected with the F. elicited susceptible tissue but they were of lesser levels. Two from those inducible phytoalexin ones which were detected at higly prominent and prominent levels in the protected tissue extract at the R_{ts} 4.42 and 14.09 min., respectively, and they were considered to be specific for this biocontrolled protected tissue.

In the *F/S. olivaceiscleroticus* soybean hypocotyl tissue interaction systems, (Table 27 and Fig. 40) revealed the presence of five phytoalexin compounds. Three of these compounds at the R_{ts} 24.86, 25.1 and 26.8 min. were similar to those constitutive compounds detected with the control (untreated-uninoculated), but they were detected at different levels. However, the other compound at the R_t 20.14 min. was detected at the same R_t as that of the F. elicited tissue (susceptible). The inducible phytoalexin compound at the R_t 5.18 min. was specific to this protected tissue and was detected at less prominent level.

Moreover, when the HPLC analysis was performed with the fungal biocontrol elicited system(s); the *F/T. harzianum* soybean hypocotyl tissue interaction systems (Table 27), it revealed the presence of twenty five phytoalexin compounds. Twelve of these compounds were similar to that detected with the control (untreated-uninoculated), but they were detected at different levels. Nine compounds at the R_{ts} 1.18, 1.59, 2.16, 7.77, 19.2, 20.35, 22.0, 22.4 and 22.9 min. were detected at similar R_{ts} as those detected with the F. elicited tissue susceptible. The other four inducible phytoalexin compounds at the Rts 11.28, 13.3, 13.7 and 28.32 min. were also detected in the *T. harzianum* elicited tissue system.

4.5.2.3 *Screening of Phytoalexins Production in Soybean (Leaves) M. phaseolina/ Biocontrol Agents Interaction Systems in Greenhouse.*

The data given in Table 28 and Fig. 41, revealed the presence of thirteen phytoalexin compounds in the soybean leaves extracts (untreated-uninoculated) at the retention times (R_{ts}) 1.23, 1.6, 2.25, 9.3, 13.5, 14.0, 14.3, 18.9, 22.11, 24.11, 22.8, and 27.2 min. Four of these constitutive compounds were detected at prominent levels at the R_{ts} 1.23, 9.34, 14.0 and 14.3 min. However, there were three compounds at the R_{ts} 1.6, 2.25 and 13.5 min. of less prominent levels (major). While there were five compounds that were detected at minor levels. Their elution times were 18.9, 22.11, 24.11, 25.1, 26.8 and 27.2 min. The identification of these compounds will be clarified in (subsection 4.5.3.3).

Nineteen phytoalexin compounds were detected in the soybean leaves infected with the Macrophomina pathogen (Macrophomina-elicited tissue). Eight of these compounds were similar to those constitutive compounds detected with the control (untreated-uninoculated), but they were detected at different levels. The eleven compounds at the R_{ts} 5.87, 7.2, 8.8, 15.6, 17.1, 17.5, 20.8, 23.01, 23.28, 28.3 and 29.04 min. were inducible ones. Five of these inducible compounds were less prominent, i.e., at the R_{ts} 7.2, 8.8, 15.6, 23.01 and 23.28 min. It should be noted that only compound at the R_t 5.87 was a prominent one. These detected flavonoids and isoflavonoids phytoalexin compounds will be identified (subsection 4.5.3.3).

However, in soybean leaves extract (biocontrol (s) elicited tissue) there were twenty one compounds which were detected in the B. tissue elicited system (resistant). Ten of these compound were constitutively present at the R_{ts} 2.54, 13.4, 13.9, 18.6, 18.9, 22.5, 24.8, 25.8, 27.5 and 27.6 min. They were similar to those compounds detected with the control (untreated-uninoculated), but they were detected at different levels. Eleven inducible phytoalexins were found similar in their elution times to those inducible compounds detected in the Macrophomina susceptible tissue. They were at the R_{ts} 7.4, 7.9, 15.6, 17.18, 19.3, 20.4, 20.9, 21.5, 21.7, 28.8 and 29.69 min. Six of these compounds were detected at similar R_{ts} 7.4, 7.9, 15.6, 17.18, 20.4

and 20.9 min. as those detected with the elicited Macrophomina susceptible tissue at very low level. Only one compound at the elution time the R_t 19.3 min. was detected at less considerable level. While two of the just previously mentioned phytoalexin compounds which were detected at the R_{ts} 21.5, 21.7 were detected at less prominent levels and another two compounds at the R_{ts} 28.8 and 29.69 min. can be considered as prominent compounds (Fig. 41).

In the *S. roseodiastaticus* (isolate No. 112) elicited tissue system (biocontrol elicited tissue), there were sixteen detected phytoalexin compounds (Table 28). Seven of these compound were constitutively present at the R_{ts} 2.05, 22.4, 22.9, 25.5, 25.8, 27.6, and 27.8 min. They were similar to those detected with the control tissue (untreated-uninoculated) but with different levels. Eight of the sixteen phytoalexin compounds were detected at the R_{ts} 5.3, 15.4, 20.7, 23.4, 28.5, 28.7, 28.9, and 29.9 min., and were similar to those detected with the Macrophomina elicited tissue. The compound at the R_t 19.9, was the only one detected at a minor level.

Screening of the phytoalexins in the *S. olivaceiscleroticus* (isolate No. 729) elicited tissue system (biocontrol elicited tissue), revealed the presence of twenty seven compounds. Nine of these, compounds were detected at the R_{ts} 1.99, 13.5 , 14.8, 14.9, 18.56, 24.3, 25.3, 25.9 and 27.9 min. They were similar to those detected with the control (untreated-uninoculated). The other eighteen inducible phytoalexins were inducible ones, eleven of these inducible phytoalexins were similar to those detected with the Macrophomina pathogen elicited tissue, i.e., (susceptible) at the R_{ts} 5.07, 7. 7, 15.2, 15.9, 17.4, 17.8, 17.9, 20.3, 20.7, 23.7 and 28.14 min. Two of these just mentioned compounds were highly prominents at the R_t 15.2, and 15.9 min., while the others were considered lesser prominent compounds. The detected phytoalexin compounds at the R_t 10.3, 10.86, 12.99 were specific for this biocontrolled protected tissue as a result of using *S. olivaceiscleroticus* (isolate No. 729) as biocontrol agent. The other four compounds were similar to those detected with the other biocontrol agents systems involving the biocontrols, i.e., *B. subtilis* and *S. roseodiastaticus* (isolate No. 112).

Table 28. Screening of phytoalexins production in soybean- *M.phaseolina* / biocontrol agents interaction systems in green house by HPLC

Treatment		Soybean leaves[a] Retention time														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D	*Control*															
	Water (only)	1.23** 1.6*	2.2*							9.3**				13.5*	14.0** 14.3**	
C	*Biological agent*															
c1	*B.subtilis*		2.5*					7.4' 7.9'						13.9* 13.4"		15.6'
c2	*S.roseodiastaticus*		2.05*			5.3**										15.4'
c2	*S.olivaceiscleroticus*	1.9**				5.07*	6.5*	7.7*			10.3** 10.8**		12.9**	13.5**	14.8*** 14.9**	** * ** *
c3	*T.harzianum*				4.3***			7.5'						13.9***	14.9**	15.6'"
A	*Pathogen*															
	M.phaseolina					5.8**		7.2*	8.8*							15.6*
B	*Pathogen + Biological agent*															
B1	*M+B.subtilis*		2.04*			5.9***			8.9**			11.1*		13.04* 13.7*	14.4*	15.5*
B2	*M+ S.roseodiastaticus*	1.04*														
B2\	*M+S.olivaceiscleroticus*	1.9*		3.1**			6.8***					11.4*		13.8**		
B3	*M+T.harzianum*		2.08*			5.12***								13.9'	14.9**	15.5*

a- Age of plant 18 day, grown in greenhous

* 70,000 < peak area < 100,000 ** 100,000 < peak area < 300,000 *** 300,00 < peak area < 700,000

' 0 < peak area < 10,000 " 10,000 < peak area < 30,000 '" 30,000 < peak area < 70,000

Compounds whose peah areas are greater than 70,000 are considered as major ones.

Cont.

Table 28. Screening of phytoalexins production in soybean- *M. phaseolina* / biocontrol agents interaction systems in greenhouse by HPLC

Treatment		Soybean leaves[a] Retention time														
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
D	*Control Water (only)*			18.9'				22.1"		24.1"	25.1"	26.8'	27.2'			'
C	*Biological agent*															
c1	*B.subtilis*		17.1'	18.6" 18.9'	19.3"'	20.4' 20.9"	21.5* 21.7*	22.5*		24.8*	25.8**		27.5* 27.6"'	28.8**	29.6**	
c2	*S.roseodiastaticus*				19.9'	20.7"		22.4" 22.9'	23.4'		25.5* 25.8**		27.6** 27.8*	28.5' 28.7"' 28.9"'	29.09*	
c2	*S.olivaceiscleroticus*	16.9**	17.4* 17.8*	18.5**	19.1* 19.8*	20.3* 20.7*	21.1*		23.7*	24.3*	25.3* 25.9"'		** *	28.1**		
c3	*T.harzianum*		17.4* 17.9"	18.3* 18.8**	19.3*	20.9* 20.5*		22.4* 22.8"'	23.3"' 23.8"	24.4' 24.9"		26.3"	27.9'			
A	*Pathogen M. phaseolina*		17.1' 17.5			20.8"		22.14"	23.1* 23.2*	24.7" 24.9"'	25.6'	26.0" 26.7'	27.3" 27.9'	28.3'	29.04'	
B	*Pathogen + Biological agent*															
B1	*M+B.subtilis*			18.9*		20.1" 20.8**	21.8"'	22.2' 22.8*		24.5*	25.1* 25.6"	26.8'	27.1"	28.3***		
B2	*M+S.roseodiastaticus*	16.16**	17.8**		19.7"	20.4*		22.3*		24.1* 24.7*	25.5*	26.2* 26.9**	27.1* 27.5"	28.4* 28.03"'	29.07** 29.5*	
B2\	*M+S.olivaceiscleroticus*		17.2**			20.7***	21.7***			24.8***			27.4**			
B3	*M+T.harzianum*				19.5'	20.1"		22.1"	23.8*		25.1"' 25.8"	26.4*	27.4"	28.3*		

a- Age of plant 18 day, grown in greenhous

* 70,000 < peak area < 100,000 ** 100,000 < peak area < 300,000 *** 300,00 < peak area < 700,000

' 0 < peak area < 10,000 " 10,000 < peak area < 30,000 "' 30,000 < peak area < 70,000

Compounds whose peah areas are greater than 70,000 are considered as major ones.

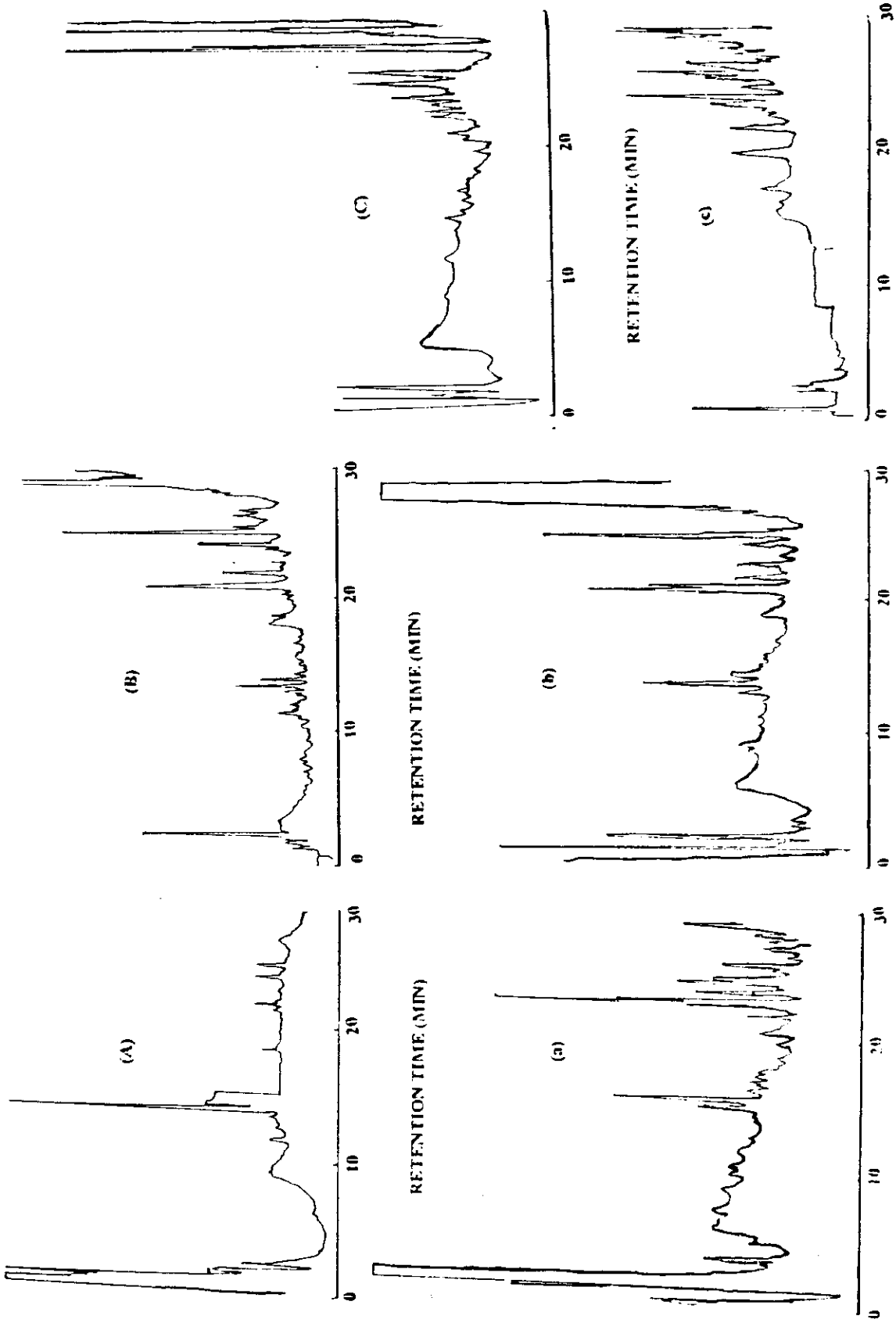

Fig. (41) HPLC profiles of aromatic metabolites from extracts of various treatment of soybean (cv. crowford leaves of 18 day old grown under greenhous after inoculation with *M.phaseolina* simultaneous with (B), *B.subtilis*; (C) *S.roseodiasticus*; (D), *S.olivaceiscleroticus*; (E)T.harzianium, (A), Control pathogen compared to control (a) untreatd (water); (b), *B.subtilis*; (c) *S.roseodiasticus*; (d), *S.olivaceiscleroticus*; (e),T.harzianium, see the continued of Fig (41) in next page.

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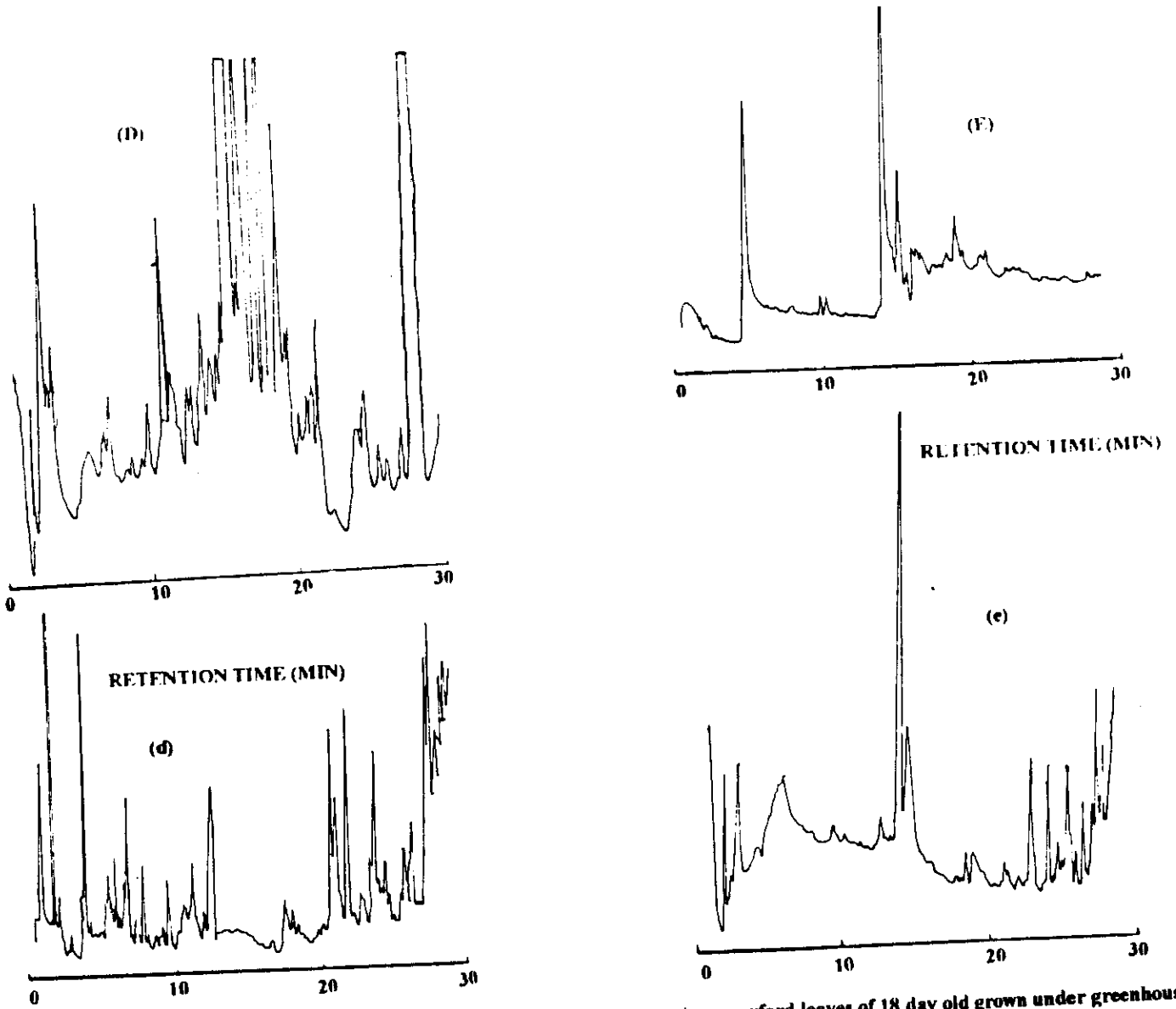

Fig. (41) HPLC profiles of aromatic metabolites from extracts of various treatment of soybean (cv. crowford leaves of 18 day old grown under greenhous after inoculation with *M.phaseolina* simultaneous with (B), *B.subtilis*; (C) *S.roseodiasticus*; (D), *S.olivaceiscleroticus*; (E)T.harzianium, (A), Control pathogen compared to control (a) untreatd (water); (b), *B.subtilis*; (c) *S.roseodiasticus*; (d), *S.olivaceiscleroticus*; (e),T.harzianium.

In the *T. harzianum* tissue system (fungal biocontrol elicited tissue), there were twenty phytoalexin compounds; ten of these compounds were at the R_{ts} 13.9, 14.9, 18.3, 18.8, 22.4, 22.8, 24.4, 24.9, 26.3, and 27.9 min. The compound at R_t 15.35 was similar to the one detected with the control (untreated-uninoculated). Also, eight compounds at the R_{ts} 7.5, 15.6, 17.4, 17.9, 20.5, 20.9, 23.3 and 23.8 min. were similar to those detected with the Macrophomina pathogen system (susceptible). The inducible compound at the R_t 19.3 min was similar to the one detected with the *S. roseodiastaticus* (isolate No. 112) and *B. subtilis* tissue systems. It should be noted that the compound, at the R_t 4.3 min. can be also considered as a highly prominent compound, specific for the Trichoderma elicited system.

In the biocontrolled soybean leaves (protected tissue), i.e., the biocontrol pathogen-elicited tissue. M/*B. subtilis* tissue interaction system, the HPLC analysis revealed the presence of twenty phytoalexin compounds in this protected tissue (Table 28). Twelve of these compounds were detected at similar elution times as those detected with the control (untreated-uninfected), but they were with different levels. Six inducible phytoalexin compounds at the R_{ts} 5.95, 8.96, 15.5, 20.1, 20.8 and 28.3 min. were similarly as those detected with the Macrophomina pathogen elicited tissue. Two inducible phytoalexins compounds were detected at the R_t 11.18, and 21.8 min. The latter compound at the R_t 21.8 min. was similar to the compound which was detected with the B. elicited tissue system while the former compound at the R_t 11.18 was similar to the compound at the elution time 11.18 min. which was detected with the M/*S. olivaceiscleroticus* (isolate No. 729) interaction system (biocontrolled, protected tissue).

The HPLC analysis of the M/*S. roseodiastaticus* (isolate No. 112) elicited tissue interaction systems, revealed the presence of seventeen phytoalexins compounds. Nine of these compounds were detected at the R_{ts} 1.04, 22.3, 24.1, 24.7, 25.5, 26.24, 26.9, 27.15 and 27.5 min. They were similar to those compounds detected with the control (untreated-uninoculated), but they were detected at different levels. Six phytoalexin compounds at the R_{ts} 17.8, 20.4, 28.03, 28.4, 29.07 and 29.5 min. were similar to these compound detected with the

Macrophomina pathogen elicited tissue. The two compounds which were detected at the R_{ts} 16.16 and 19.7 min. were inducible ones. The former was a prominent compound while the latter was a minor one.

Moreover, in the M/*S. olivaceiscleroticus* (isolate No. 729) elicited tissue interaction systems (Table 28 and Fig. 41), the HPLC analysis revealed the presence of ten phytoalexin compounds. Four of them were detected at the elution times (R_{ts})1.92, 13.88, 24.8, and 27.4 min., similar to the constitutive compounds detected with the control (untreated-uninoculated), but they were detected at different levels. Two compounds were detected at similar R_{ts} 17.18 and 20.7 min. as those detected with the M. elicited susceptible tissue but with higher levels (prominent and highly prominent levels, respectively). The other four inducible phytoalexin ones were detected at the R_{ts} 3.15, 6.8, 11.47 and 21.7 min. Two from these inducible compound detected at the R_t 6.8 and 21.7 were similar to the compounds which were detected with the *S. olivaceiscleroticus* (isolate No. 729) elicited tissue, but they were detected with highly prominent levels. While the two compounds at the R_{ts} 3.15, and 11.47 min. were detected at prominent and less prominent levels, respectively. It should be noted that these two compounds were not detected with either the *M.* pathogen or the *S. olivaceiscleroticus* systems and in particular the compound at Rt 3.15 is specific for this system.

In the M/*T. harzianum* elicited tissue interaction system (Table 28 and Fig. 41), the HPLC analysis revealed the presence of fourteen phytoalexin compounds(Table 28 and Fig. 41). Eight of these compounds were similar to those constitutive compounds detected with the control (untreated-uninoculated), but they were detected at different levels. Another five compounds at the R_{ts} 5.12, 15.5, 20.1, 23.8 and 28.38 min. were found to be similar to those detected with the M. elicited susceptible tissue. An inducible phytoalexin compound at the R_t 19.5 min. was detected at similar elution time as that of the inducible one detected in the *T. harzianum* elicited tissue, however, it was of minor level.

4.5.2.4 *Screening of Phytoalexins Production in Soybean (Leaves) F. solani f.sp. phaseoli/ Biocontrol Agents Interaction System in Greenhouse.*

The Data shown in Table 29 and Fig. 41 revealed the presence of thirteen phytoalexin compounds in the soybean leaves extracts (untreated-uninoculated) at retention times (R_{ts}) 1.23, 1.6, 2.25, 9.3, 13.5, 14.0, 14.3, 18.9, 22.11, 24.11, 25.1, 26.8, and 27.2 min. Four of these constitutive compounds were prominent ones at the R_{ts} 1.23, 9.34, 14.0 and 14.3 min. However, there were three compounds at the R_{ts} 1.6, 2.25 and 13.5 min. of less prominent levels (major). While there were five compounds that were detected at minor levels. Their elution times were at the R_{ts} 18.9, 22.11, 24.11, 25.1, 26.8 and 27.2 min. The identification of these compounds will be clarified in subsection 4.5.3.4.

Eleven phytoalexin compounds were detected in the soybean leaves infected with the Fusarium root rot pathogen (susceptible). Five of these compounds were similar to those constitutive compounds detected with the control (untreated-uninoculated), but they were detected at different levels. The other six compounds at the R_{ts} 4.16, 4.74, 12.5, 15.8, 16.9, and 29.9 min. were inducible ones. Two of these inducible compounds at the R_{ts} 4.16, and 4.74 were highly prominent compounds (Table 29 and Fig. 42). It should be noted that the four compounds which were detected at the Rts 12.5, 15.8, 16.9, and 29.9 min. were of prominent levels. These detected phytoalexins compounds (flavonoids and isoflavonoids) of the Fusarium susceptible interaction system will be identified in subsection 4.5.3.4.

However, in the soybean leaves biocontrols/pathogen-elicited tissue system (biocontrolled protected tissue), the HPLC analysis revealed that: Firstly, in the F/*B. subtilis* tissue system, seven phytoalexin compounds were detected (Table 29). Three of these compounds at the R_{ts} 2.24, 25.5 and 26.28 min. were found similar to those constitutive compounds detected with the control (untreated-uninoculated). Moreover, another two compounds at the R_{ts} 15.4 and 16.56 min. were inducible ones. Their elution times (Rt_S) were similar to those detected with the Fusarium elicited susceptible tissue. With

respect to the other two phytoalexin compounds, detected at the R_{ts} 5.34 and 23.75 min., they were similar to those detected with the F/*S. roseodiastaticus* (isolate No. 112) tissue interaction system. The identification of the phytoalexin compounds of the biocontrolled protected tissue will be identified in subsection 4.5.3.4.

Secondly, in the F/*S. roseodiastaticus* (isolate No. 112) soybean leaves tissue interaction system ,the HPLC analysis revealed the presence of seventeen phytoalexin compounds (Table 29). Seven of these compounds were similar to those constitutive compounds detected with the control (untreated-unidentified). Two compounds were detected at similar elution times, i.e. at 15.8 and 29.9 min., as those detected with the Fusarium elicited susceptible tissue but they were with lesser levels. Eight inducible phytoalexin compounds were detected at the R_{ts} 5.12, 7.02, 7.28, 17.9, 20.7, 23.13, 23.4 and 28.7 min. Two of these inducible compounds at the R_{ts} 5.12 and 23.13 min. were detected with prominent levels, while the other ones at the elution times 7.2, 7.28, 17.9, 20.7, 23.4, and 28.7 min. were detected with lesser (minor) levels.

Thirdly, in the F/S. olivaceiscleroticus soybean leaves tissue interaction systems, the HPLC analysis revealed the presence of seventeen phytoalexin compounds (Table 29 and Fig. 42). Ten of these compounds at the Rts 1.1, 1.4, 1.9, 2.04, 24.4, 24.8, 25.1, 25.7, 26.7 and 27.42 min. were similar to those constitutive compounds detected with the control (untreated-uninoculated), but they were detected at different levels. However, the two compounds at the Rts 15.6 and 29.7 min. were similar to those detected with Fusarium elicited susceptible tissue. The other inducible phytoalexin compounds at the R_{ts} 7.28, 17.5, 20.9, 23.6, and 28.7 min., were also detected with the S. olivasceiscleroticus elicited tissue system.

Finally, in the *F/T. harzianum* soybean leaves tissue interaction system, the HPLC analysis revealed the presence of 18 phytoalexin compounds (Table 29). Nine of these compounds were similar to the constitutive compounds detected with the control (untreated-uninoculated), but they were also detected at different levels. Four compounds at the R_{ts} 4.88,

15.4, 15.9, and 29.9 min. were found similar to those detected with the Fusarium elicited susceptible tissue. Five inducible phytoalexin compounds were detected at the R_{ts} 5.14, 20.4, 28.5, 28.8 and, 17.6, min, the first four compounds were present at less prominent levels while the last one was detected at minor level. Two from those inducible ones were also detected in the T. harzianum elicited tissue system at the elution times R_{ts} 17.6 and 20.4 min.

Table 29. Screening of phytoalexins production in soybean- *F.solani* f.sp.*phaseoli* / biocontrol agents interacion systems in greenhouse by HPLC

Treatment		Soybean leaves[a] Retention time														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D	*Control*															
	Water (only)	1.23** 1.6*	2.2*							9.3**				13.5*	14.0** 14.3**	
C	*Biological agent*															
c1	*B.subtilis*		2.5*					7.4' 7.9'						13.9* 13.4"		15.6'
c2	*S. roseodiastaticus*		2.05*			5.3**										15.4'
c2	*S.olivaceiscleroticus*	1.9**				5.07*	6.5*	7.7*			10.3** 10.8**		12.9**	13.5**	14.8*** 14.9**	** * ** *
c3	*T.harzianum*				4.3***			7.5'						13.9***	14.9**	15.6"
A	*Pathogen*															
	F.solani f.sp.phaseoli	1.03** 1.3**			4.1*** 4.7***					9.9***			12.5**			15.8*
B	*Pathogen + Biological agent*															
B1	*F+B.subtilis*		2.2*			5.3**										15.1'
B2	*F+S. roseodiastaticus*	1.9*				5.1**		7.02" 7.2'						13.2'		15.8'
B2\	*F+S.olivaceiscleroticus*	1.1* 1.4** 1.9*	2.04**					7.2'								15.6"
B3	*F+T.harzianum*	1.2*	2.3*		4.8**	5.1*										15.4' 15.9"

a- Age of plant 18 day, grown in greenhous

* 70,000 < peak area < 100,000 ** 100,000 < peak area < 300,000 *** 300,00 < peak area < 700,000

' 0 < peak area < 10,000 " 10,000 < peak area < 30,000 " 30,000 < peak area < 70,000

Compounds whose peah areas are greater than 70,000 are considered as major ones.

Cont.

Table 29. Screening of phytoalexins production in soybean- *F.solani* f.sp.*phaseoli* / biocontrol agents interactionsystems in greenhouse by HPLC

Treatment		Soybean leaves[a] Retention time														
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
D	*Control Water (only)*			18.9'				22.1"		24.1"	25.1"	26.8'	27.2'			'
C	*Biological agent*															
c1	*B.subtilis*		17.1'	18.6"' 18.9'	19.3"'	20.4' 20.9"	21.5* 21.7*	22.5*		24.8*	25.8**		27.5* 27.6"'	28.8**	29.6**	
c2	*S. roseodiastaticus*				19.9'	20.7"		22.4" 22.9'	23.4'		25.5* 25.8**		27.6** 27.8*	28.5' 28.7"' 28.9"'	29.09*	
c2	*S.olivaceiscleroticus*	16.9**	17.4* 17.8*	18.5**	19.1* 19.8*	20.3* 20.7*	21.1*		23.7*	24.3*	25.3* 25.9"'		** *	28.1**		
c3	*T.harzianum*		17.4* 17.9"	18.3* 18.8**	19.3*	20.9* 20.5*		22.4* 22.8"'	23.3"' 23.8"	24.4' 24.9"		26.3"	27.9'			
A	*Pathogen*															
	F.solani f.sp. phaseoli	16.9**		18.9**				22.04*							29.9**	
B	*Pathogen + Biological agent*															
B1	*F+B.subtilis*	16.5*							23.7*		25.5*	26.2*				
B2	*F+S. roseodiastaticus*		17.9'			20.7'			23.1** 23.4*	24.8*	25.1"' 25.8"	26.5"	27.6'	28.7*	29.9"	
B2\	*F+S.olivaceiscleroticus*		17.5'			20.9"'			23.6*	24.4"' 24.8'	25.1" 25.7'	26.7"	27.4**	28.7"'	29.7"'	
B3	*F+T.harzianum*		17.6'			20.4*		22.5*		24.8"	25.5"	26.2" 26.5'	27.1"' 27.6'	28.5* 28.8*	29.9***	

a- Age of plant 18 day, grown in greenhous

* 70,000 < peak area < 100,000 ** 100,000 < peak area < 300,000 *** 300,00 < peak area < 700,000

' 0 < peak area < 10,000 " 10,000 < peak area < 30,000 "' 30,000 < peak area < 70,000

Compounds whose peah areas are greate than 70,000 are considered as major one

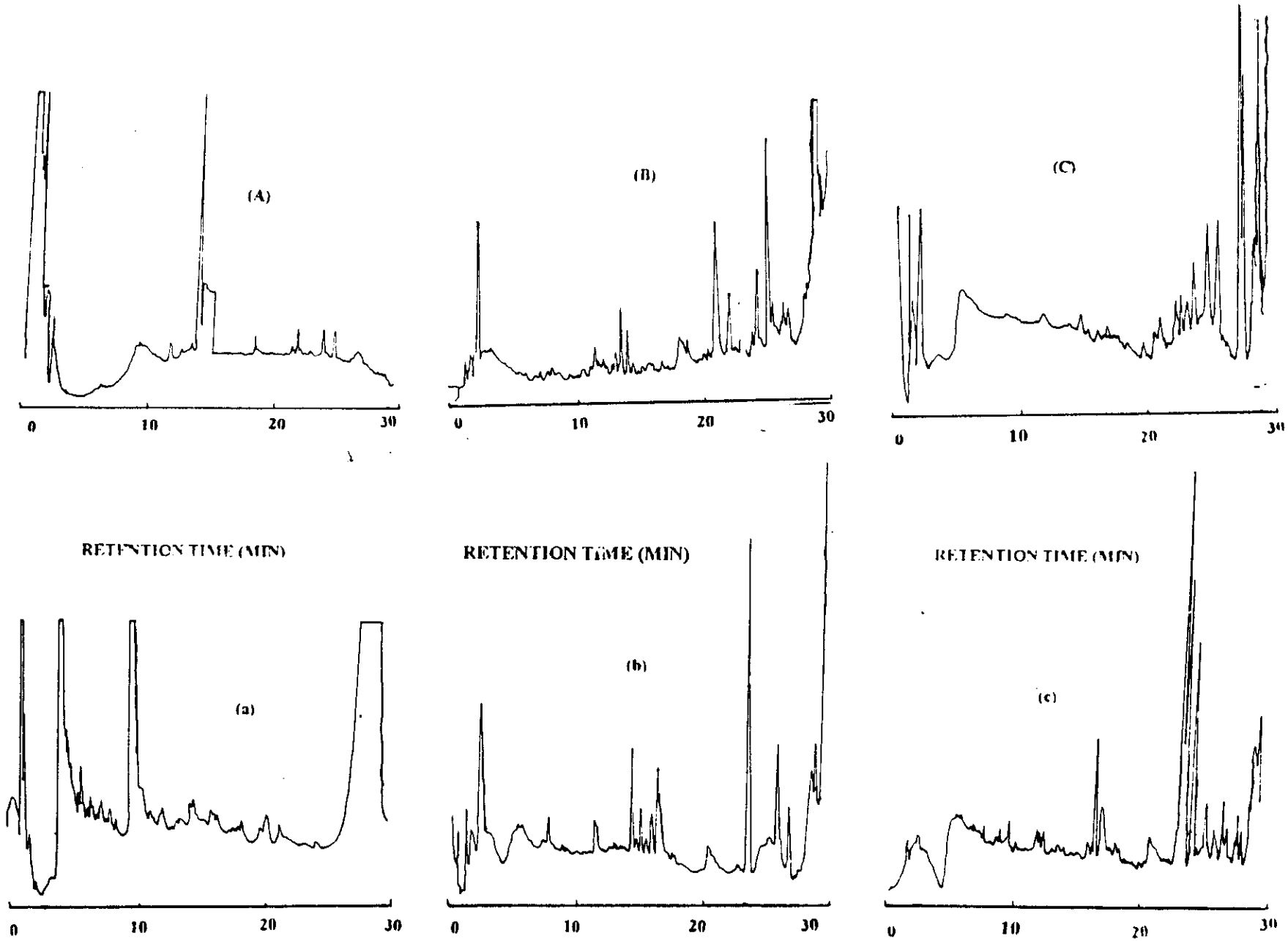

Fig. 42. HPLC profiles of aromatic metabolites from extracts of various treatments of soybean (cv. crowford) leaves of 18 days old grown under greenhous after inoculation with *F. solani* f.sp. *phaseoli* simultaneously with: (B). *B. subtilis*; (C) *S. roseodiasticus*; (D). *S. olivaceiscleroticus*; (E), *T. harzianum*. The other profiles represent the following treatments: (A), control pathogen; (a), untreatd - uninfected (water); (b), *B. subtilis*; (c) *S. roseodiasticus*; (d), *S. olivaceiscleroticus*; (e), *T. harzianum*. See next page for the continuation of this Figure.

Cont.

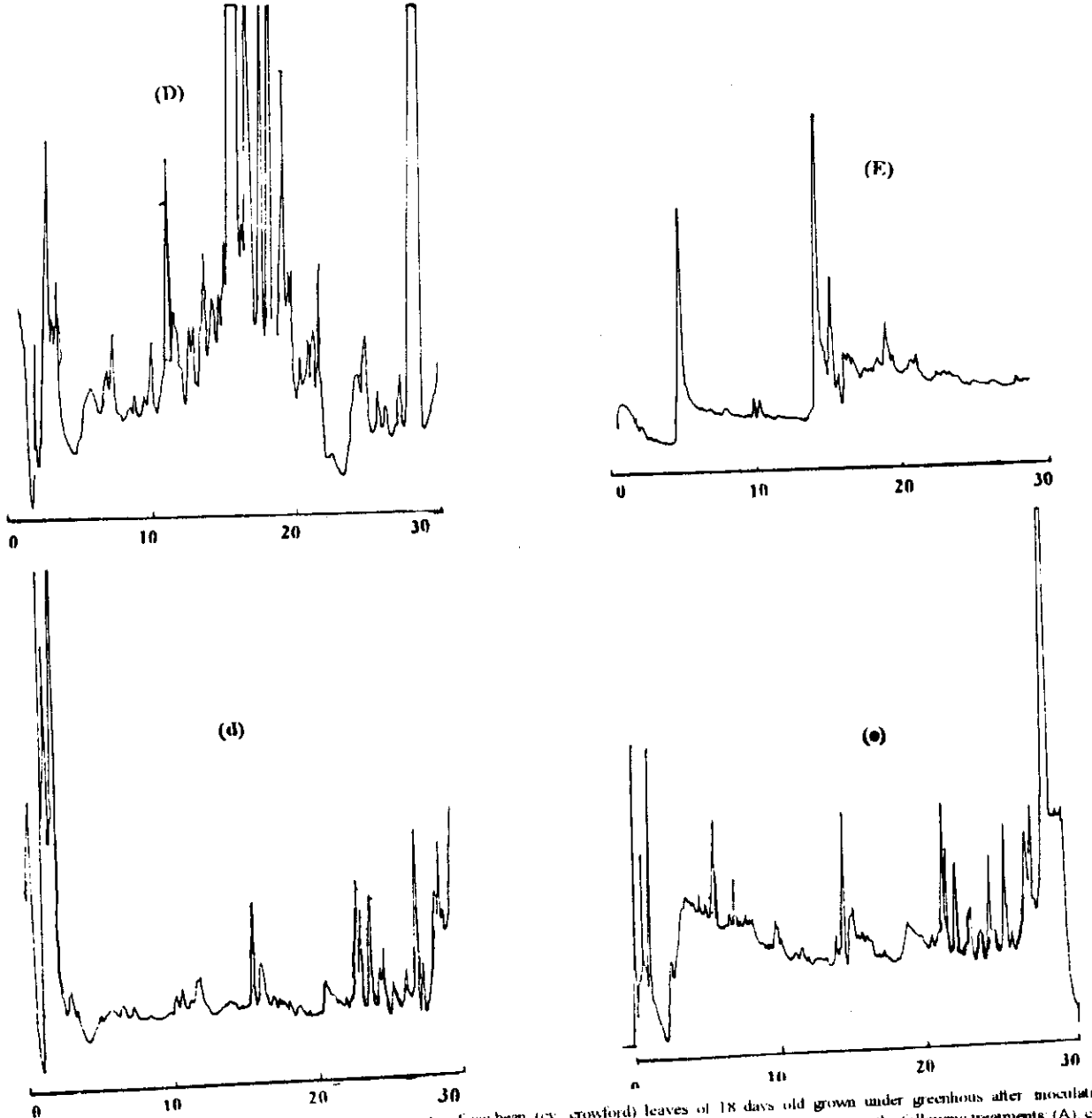

Fig. 42. HPLC profiles of aromatic metabolites from extracts of various treatments of soybean (cv. crowford) leaves of 18 days old grown under greenhous after inoculation with *F. solani* f.sp. *phaseoli* simultaneously with: (B), *B. subtilis*; (C) *S. roseodiasticus*; (D), *S. olivaceiscleroticus*; (E); *T. harzianum*. The other profiles represent the following treatments. (A), control pathogen; (a), untreated - uninfected (water); (b), *B. subtilis*. (c) *S. roseodiasticus*. (d), *S. olivaceiscleroticus*. (e), *T. harzianum*.

4.5.3 *Characterization, Distribution and Identification of Phytoalexins Produced in Soybean Hypocotyls and Leaves - Pathogen(s)/ Biocontrol Agents Interaction Systems in Greenhouse*

In what follows, the detailed distribution and characterization of the the different detected isoflavonoids aromatic secondary compounds (phytoalexins) and their conjugates (identified and unidentified), in addition, to simple flavonoids and other isoflavonoids, at different elution times are given for the soybean pathogens interaction systems tested in this study. These compounds are the ones detected within the elution times of 0.5 minute range starting from 7 min., assuming that variability within half a minute is due to measurement inconsistency and not due to having different compounds in this rather small range.The compounds which have retention times either identical or very close to the previously reported identified phytoalexins, in particular those reported by **Graham (1991a),** are referred to as identified compounds, otherwise, the compounds are referred to as unidentified ones.

4.5.3.1 *Characterization, Distribution and Identification of Phytoalexins Produced in Soybean (Hypocotyls)* - **M. phaseolina/*Biocontrol Agents Interaction Systems in Greenhouse***

Table 30 shows the retention times of all the detected aromatic secodary metabolites compounds in the soybean (hypocotyls) - M. phaseolina biocontrol agents interaction systems. Descrpitions of all of these metabolites and their detected levels are given below:

A- Compounds at the Elution Times (7-8): Phenylalanin (7.6)

The first compound detected was at the R_t 7.0-7.5. It is an inducible, unidentified compound which is accumulated in the *S. roseodiastaticus* (isolate No. 112) tissue at a higher level than with the *T. harzianum* tissue system only and at about 5 fold of the level in the M /*T. harzianum* tissue system. This indicates that this compound is specifically produced by the biocontrol agent elicitation. Another compound was detected at the R 7.6-8.0. It is also an

inducible, identified compound which was detected in the *S. roseodiastaticus* (isolate No. 112) tissue at, a higher level than with the M/*S. roseodiastaticus* (isolate No. 112) tissue system, three and half fold of the *T. harzianum* tissue system, about four fold of the M/*T. harzianum* system and about eight and half fold of the Macrophomina pathogen. This compound is regarded as inducible one since it is not present in the healthy control (untreated-uninoculated) plants.

B- Compounds at the Elution Times (13-14): Free daidzin (13.6)

The first compound was detected at the R_t 13.0-13.5. It is an inducible unidentified compound which was only detected in the M/*S. roseodiastaticus* (isolate No. 112) and the M/ *T. harzianum* systems. This compound is neither produced with the biocontrol agents (*S. roseodiastaticus* (isolate No. 112) or *T. harzianum*) nor with the *Macrophomina pathogen.* While the other compound detected at the R_t 13.5-14.0 is an inducible identified compound which was produced more extensively in the *S. roseodiastaticus* (isolate No. 112) tissue system at level about $3x10^3$ times the level detected the M/*S. roseodiastaticus* (isolate No. 112) system. This compound was also present at very small amount (> 100,000) with the M/B and M/*T. harzianum* tissue systems.

C- Compounds at the Elution Times (15-16): Genistin (15.5)

The first compound was detected at the R_t 15.0-15.5. It is a constitutive compound which was detected in the M/B system at: two and half times the level in the *S. roseodiastaticus* (isolate No. 112) system , three and half times the level in the Macrophomina pathogen tissue, four and half times the level in the M/*S. roseodiastaticus* (isolate No. 112), nine fold the level in the *T. harzianum* system tissue and sixteen times the level in the healthy control plants (untreated - uninoculated)

D- Compounds at the Elution Times (15-16): 6 malonyl daidzin (15.7)

While the second inducible compound in the range 15-16 min. was detected at the R_t 15.5-16. It was detected in the *S. roseodiastaticus* (isolate No. 112), tissue system at: four and half times the level in the t M/*S. olivaceiscleroticus* (isolate No. 729) systems, six fold of the B system level, eight fold the level in the M/*S. roseodiastaticus* (isolate No. 112) system and at nine fold of the Macrophomina pathogen system level.

E- Compounds at the Elution Times (17-18): 6 malonyl genistin (17.3)

The first detected compound was at the R_t 17.0-17.5. It is an inducible, identified compound which was detected in the M/B system at: twofold the *S. roseodiastaticus* (isolate No. 112) tissue system, fourfold the M/*S. olivaceiscleroticus* (isolate No. 729) systems and sevenfold the M/*S. roseodiastaticus* (isolate No. 112) system. While the other compound detected at the R_t 17.5-18 is an constitutive unidentified compound. It was detected in the M/B system at a level: two and half times the level in the Macrophomina pathogen system, five and half times the M/*S. olivaceiscleroticus* (isolate No. 729) tissue system, sixteen times the *S. roseodiastaticus* (isolate No. 112) tissue system, and twenty one times the M/*S. roseodiastaticus* (isolate No. 112) tissue system.

F- Compounds at the Elution Times (18-19): Daidzein (18.9)

The first detected compound was at the R_t 18.0-18.5. It is an inducible, unidentified compound which was detected in the Macrophomina pathogen at the same level as in the *S. roseodiastaticus* (isolate No. 112) system and at higher level than the M/B tissue system, but at three and half times the level in the M/*S. olivaceiscleroticus* (isolate No. 729) tissues system. The other compound was detected at the R_t 18.5-19.0. It is a constitutive, identified compound (de novo synthesis) which was detected at very small amount (> 100,000) in healthy plants (untreated-uninoculated), of the *T. harzianum*, Macrophomina pathogen, M/B and M/*S. olivaceiscleroticus* (isolate No. 729) tissue systems.

G- Compounds at the Elution Times (19-20): Glycetin 7, 0-β- glucoside (19.6)

The first compound was detected at the R_t 19.0- 19.5. It is an inducible identified compound which was produced in the *S. roseodiastaticus* (isolate No. 112), tissue system at one and half times the level in the M/*S. roseodiastaticus* (isolate No. 112) tissue system. It was also present at a very low concentration (which is regarded to be of little importance) in the *T. harzianum*, Macrophomina pathogen and the M/B tissue systems.

H- Compounds at the Elution Times (19-20): Cinnamic Acid (19.9)

The second inducible identified compound of pronounced level was detected at the R_t 19.5-20. It was detected in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a very high level (pronounced), whereas in the *T. harzianum* tissue system it was detected at a level below the limit of consideration.

I- Compounds at the Elution Times (20-21): Luteolin (20.2)

The first compound was detected at the R_t 20.0-20.5. It is an inducible identified compound which was produced by the B. tissue system at fourfold the level of either the *S. roseodiastaticus* (isolate No. 112), tissue system or the Macrophomina pathogen system. Also, this compound which was present in the M/B, M/*S. olivaceiscleroticus* (isolate No. 729), M/ *S. roseodiastaticus* (isolate No. 112), and *M/T. harzianum* tissue systems but at very low of concentration (> 100,000). While there was another compound detected at the R_t 20.5-21.0. It is an inducible unidentified compound which was detected in the *S. roseodiastaticus*, (isolate No. 112), tissue system at eight fold the level in the M/ *S. roseodiastaticus*, (isolate No. 112), tissue systems. Further, this compound was also present in the M/B and M/*S. olivaceiscleroticus* (isolate No. 729) systems but at very low concentration.

J- Compounds at the Elution Times (21-22): Genistein (21.6)

The first compound was detected at the R_t 21.0-21.5. It is an inducible identified compound which was detected at a very small amount (>100,000) in the Bacteria *S. olivaceiscleroticus* (isolate No. 729) and Macrophomina pathogen tissue systems and also in the M/B and M/*S. olivaceiscleroticus* (isolate No. 729) tissue systems.

K- Compounds at the Elution Times (21-22): Kampferol (21.8)

Another, inducible identified compound which was present at the R_t 21.5-22. in the *S. roseodiastaticus* (isolate No. 112) tissue system, M/B and M/*S. olivaceiscleroticus* (isolate No. 729) systems at very small amount (>100,000).

L- Compounds at the Elution Times (22-23): Isorhamnetin (22.5)

The first compound was detected at the R_t 22.0-22.5. It is an inducible identified compound which was produced with M/ *S. roseodiastaticus* (isolate No. 112) system at .high level .while it was present in the Bacteria and *S. roseodiastaticus* (isolate No. 112), tissue systems at small concentrations which are regarded below the limit of consideration. There was another compound that was detected at the R_t 22.5-23.0. It is also an inducible unidentified compound which was produced in the Macrophomina pathogen tissue system at a level which is one and half times the level in the M/B tissue system and about seven and half times the level in the M/*S. olivaceiscleroticus* (isolate No. 729) tissue system.

M- Compounds at the Elution Times (23-24): Unknown compound

There were two compounds that were detected. The first compound was detected at the R_t 23.0-23.5. It is an inducible unidentified compound which was produced with the Macrophomina pathogen tissue system at high level which was about 5 thousands times the levels attained in either the healthy plants (untreated - uninoculated) or the M/*S. olivaceiscleroticus* (isolate No. 729) tissue systems and around two and half thousand times

the level in the M/B tissue system. The second compound was detected at the R_t (23.6-24.0). It is also an inducible unidentified compound which was detected in a large amount with the M/B tissue system at, threefold of the M/*S. olivaceiscleroticus*, (isolate No. 729), system, four fold of the M/*S. roseodiastaticus* (isolate No. 112) tissue system and fivefold of the Bacillus tissue system.

N- Compounds at the Elution Times (24-25): Formononetin (24.5)

The first compound was detected at the R_t 24.0-24.5. It is a constitutive identified compound which was detected with high concentration in healthy plants (untreated-uninoculated). This compound which was detected in very small amount (>100,000), regarded to be below the limit of consideration, with *B. subtilis* tissue system. While the other compound was detected at the R_t 24.5-25.0. It is also a constitutive unidentified compound. it was present in the Macrophomina pathogen system at a level of concentration higher than the M/B tissue system and at threefold the level in the M/*S. olivaceiscleroticus* (isolate No. 729) tissue system.

O- Compounds at the Elution Times (25-26): Glyceollin (25.8)

The first compound was detected at the R_t 25.0-25.5. It is a constitutive unidentified compound which produced de novo in healthy plants (untreated-uninoculated). It was detected in the M/B tissue system at, higher level than in the *Macrophomina pathogen* system, twofold of the M/*S. olivaceiscleroticus* (isolate No. 729) system and, fivefold than the level in the healthy plants. This compound was present at very small amount (100,000), which is regarded to be below the limit of consideration, with the Bacillus tissue system, the *S. roseodiastaticus* (isolate No. 112) tissue system and the M/*T. harzianum* tissue systems. The second compound was detected at the R_t 25.5-26.0. It is also another constitutive identified compound which was detected with the M/B tissue system at two times the level in the M/*S. roseodiastaticus* (isolate No. 112) tissue system and at threefold the level in either the healthy plants (untreated-uninoculated) or the *S. olivaceiscleroticus* (isolate No. 729) tissue system. This compound was also present at very small concentration, which is regarded below the limit of

consideration, with the Bacillus tissue, the *S. roseodiastaticus* (isolate No. 112), the M/*S. olivaceiscleroticus* (isolate No. 729) and the M/*T. harzianum* tissue systems.

P- Compounds at the Elution Times (26-27): Chrysin (26.4)

The first compound was detected at the R_t 26.0-26.5. It is a constitutive identified compound which was detected in the B tissue system at a level higher than that of *Macrophomina pathogen* system and at fivefold the level in the M/B tissue systems. While the other compound was detected at the R_t 26.-27.0. It is also a constitutive unidentified compound which was detected in the *Macrophomina pathogen* system at five times the level in the M/*S. olivaceiscleroticus* (isolate No. 729) tissue systems and at six fold the level in the M/B tissue system. Further, this compound was present at very small concentration with the *S. olivaceiscleroticus* (isolate No. 729) tissue and the M/*T. harzianum* tissue systems; which is regarded to be below the limit of concentration.

Q- Compounds at the Elution Times (27-28): Biochanin A (27.7)

The first compound was detected at the R_t 27.0-27.5. It is a constitutive unidentified compound that was detected in the M/B tissue system at a level which was, one and half times the level in the *Macrophomina pathogen* system. Moreover, it was detected at a higher level in the B tissue system than that in the *Macrophomina pathogen* system which was twofold the level in the M/*S. olivaceiscleroticus* (isolate No. 729) system, while it was two to four times the level in the healthy plants (untreated - uninoculated). T second compound was detected at the R_t 27.5-28.0. It is also an inducible identified compound which was detected in the M/*S. roseodiastaticus* (isolate No. 112), system. It was detected at higher level, i.e., four and half times than that in the *B. subtilis* tissue system and at fivefold than that in the M/*S. olivaceiscleroticus* (isolate No. 729) tissue system.

Table 30. Summarized results from Table 26 of aromatic secondary metabolites compounds in soybean - *M.phaseolina* /biological agents interaction systems: hypocotyls.

	Treatment	In gm fresh weight	R_t of identified[a] compound from hypocotyls													
			7.6(I)		13.6(I)		15.5(I)		17.3(c)		18.9(c)		19.6(I)		20.1(I)	
			(ui)	(i)	(ui)	(i)	(i)	(i)	(i)	(ui)	(ui)	(i)	(ui)	(i)	(i)	(ui)
D	*Control*															
	Water (only)	1.5					11			5		64				
C	*Biological agent*															
c1	*B.subtilis*	1.8						43			5				474	
c2	*S.roseodiastaticus*	2.6	781	302		2.559	127	451	210	78	219		211		178	432
c2	*S.olivaceiscleroticus*	4										46		128		
c3	*T.harzianum*	2.5	708	85			32						12	11		
A	*Pathogen*															
	M.phaseolina	1.5		22			50	29		295 56	57 146	26	5	111	111	
B	*Pathogen + Biological agent*															
B1	*M+B.subtilis*	1	9			24	121	2	164 126	492	104	233 54	14	16	16	18
B2	*M+S.roseodiastaticus*	3.5		308	143 293	98	88	78	61 90	83			202	67	67	76
B2\	*M+S.olivaceiscleroticus*	2.7	10			101		97	117 89	224	67	82		75 12 87	75 12 78	65
B3	*M+T.harzianum*	2.5	142	120	167	79										

a- refers to constitutive (c) or inducible (I) compounds based on the work of Graham *et al.* (1990) and Morris *et al.* (1991)
(i) identified (ui) unidentified.

cont.

Table 30. Summarized result from Table 26 of aromatic secondary metabolites compounds in soybean - *M. phaseolina* /biocontrol agents interaction systems: hypocotyls.

Treatment		In gm fresh weight	R_t of identified[a] compound from hypocotyls													
			21.6(I)		22.5(I)		23(UI)		24.4(c)		25.8(I)		26.4(I)		27.4(c)	
			(i)	(i)	(i)	(ui)	(ui)	(ui)	(i)	(ui)	(ui)	(i)	(i)	(ui)	(ui)	(i)
D	*Control*															
	Water (only)	1.5					168		349	3	142	118	8	8	120	
C	*Biological agent*															
c1	*B.subtilis*	1.8	34		35	36		185	57		15	19	474 316		160	57
c2	*S.roseodiastaticus*	2.6		14	6	5			28	22	6	11			45	
c2\	*S.olivaceiscleroticus*	4	41			7						243	60	51		6
c3	*T.harzianum*	2.5							4			4				5
A	*Pathogen*															
	M.phaseolina	1.5	90			923	1021			800	461 727		337	631	196	
B	*Pathogen + Biological agent*															
B1	*M+B.subtilis*	1	81	18	18	345	204	490 217	59	550	569	204	42	78	191	
B2	*M+S.roseodiastaticus*	3.5			701			411	51			344		314 230		600
2	*M+S.olivaceiscleroticus*	2.7	94	11		238	272	368	47	442	566	24	25		311	80
\						189		213	24							
B3	*M+T.harzianum*	2.5				8			5		10	5		10	4	

a- refers to constitutive (c) or inducible (I) compounds based on the work of Graham *et al.* (1990) and Morris *et al.* (1991)
(i) identified (ui) unidentified.

4.5.3.2 *Characterization, Distribution and Identification of Phytoalexins In Soybean Hypocotyls* - F. solani *f.sp.* phaseoli/*Biocontrol Agents Interaction Systems in Greenhouse.*

Table 31 shows the retention times of all the detected aromatic secodary metabolites compounds in the soybean (hypocotyls) - *F. solani* f.sp. *phaseoli* biocontrol agents interaction systems. Descrpitions of all of these metabolites and their detected levels are given below:

A- Compounds at the Elution Times (7-8): Phenylalanin (7.6)

The first compound detected was at the R_t 7.0-7.5. It is an inducible unidentified compound which was detected in the *S. roseodiastaticus* (isolate No. 112) tissue system at a level higher than with the *T. harzianum* tissue system and at tenfold of the level in the Fusarium pathogen system. The second compound was detected at the R_t 7.5-8.0. It is also another inducible identified compound which was present in the *S. roseodiastaticus* (isolate No. 112) system at three and half times the level in the *T. harzianum* tissue system and at 30 fold the level in the F/*T. harzianum* tissue systems.

B- Compounds at the Elution Times (13-14): Free daidzin (13.6)

The first compound detected was at the R_t 13.0-13.5. It is an inducible unidentified compound which was present in the F/*T. harzianum* tissue system at a level that was 20 times that of the F/ *S. olivaceiscleroticus* tissue systems. Moreover, it was detected in very small amount (>100,000) in both systems. There was another compound that was detected at the R_t 13.5-14.0. It is also an inducible identified compound which was present as major one in the *S. roseodiastaticus* (isolate No. 112) tissue system. This compound was also present in the F/*T. harzianum* system but at half the level of the first compound and with very low level of concentration which can be regarded as below the limit of consideration.

C- Compounds at the Elution Times (15-16):Genestin (15.5)

The first compound was detected at the R_t 15.0-15.5. It is a constitutive identified compound which was present in the Fusarium pathogen tissue system at, twice the level in the *S. roseodiastaticus* (isolate No. 112) tissue system, 7 fold the level in the *T. harzianum* tissue system and, 13 times the level in the healthy plants (untreated-uninoculated).

D- Compounds at the Elution Times (15-16): 6 malonyl daidzin (15.7)

The second inducible identified compound was present at the R_t 15.6-16.0 in the *S. roseodiastaticus* tissue system at a level, six and half times as in the Bacillus tissue system, nine fold of the level in the F/*T. harzianum* tissue system and 30 times of the level in the F/*S. roseodiastaticus* (isolate No. 112) tissue system.

E- Compounds at the Elution Times (17-18): 6 Malonyl genistin (17.3)

The first compound was detected at the R_t 17.0-17.5. It is an inducible identified compound, which was present in the Fusarium pathogen tissue at twofold than with the *S. roseodiastaticus* (isolate No. 112) tissue system and at six fold than with the F/*T. harzianum* tissue system. The second compound was detected at the R_t 17.5-18.0. It is a constitutive unidentified compound which was present in the F/*T. harzianum* tissue system at a level, which was almost twofold the level in the *S. roseodiastaticus* (isolate No. 112) tissue system and twenty times the level in the healthy (untreated - uninoculated) tissue.

F- Compounds at the Elution Times (18-19):Diadzein (18.9)

The first compound was detected at the R_t 18.0-18.5. It is an inducible unidentified compound which was present in the Fusarium pathogen at, twofold the level in the *S. roseodiastaticus* (isolate No. 112) system and twelve times the level in the F/*T. harzianum* tissue systems. The second compound was detected at the R_t 18.5-19.0. It is a constitutive

identified compound which was produced in the healthy plants (untreated-uninoculated) at a higher level than in the F/*T. harzianum* tissue system and at six times the level in the *S. olivaceiscleroticus* (isolate No. 729) tissue system.

G- Compounds at the Elution Times (19-20):Glycetin 7-0-β-glycoside(19.6)

The first compound was detected at the R_t 19.0-19.6. It is an inducible identified compound which was present with the *S. roseodiastaticus* (isolate No. 112) tissue system at fourfold more the level in the F/*T. harzianum* tissue system and at seventeen times the level in the *T. harzianum* tissue system.

H- Compounds at the Elution Times (19-20): Cinnamic acid (19.9)

The second inducible identified compound was detected at the R_t 19.6-20.0. It was present in the Fusarium pathogen system a t fourfold the level in the *S. olivaceiscleroticus* (isolate No. 729) tissue system and at thirty fold the level in the *T. harzianum* tissue system.

I- Compounds at the Elution Times (20-21):Luteolin (20.2)

The first compound was detected at the R_t 20.0-20.5. It is an inducible identified compound which was present in the Bacillus tissue system at a level, one and half times that of the F/*S. olivaceiscleroticus* (isolate No. 729) tissue systems , threefold the level in the Fusarium pathogen system, fourfold the level in the *S. roseodiastaticus* (isolate No. 112) tissue system and six fold the level in the F/*T. harzianum* tissue systems. The second compound was detected at the R_t 20.5-21.0. It is also an inducible unidentified compound which was present in the *S. roseodiastaticus* (isolate No. 112) tissue system at two and half times the level in the Fusarium pathogen tissue system.

J- Compounds at the Elution Times (21-22):Genistein (21.6)

The first compound was detected at the R_t 21.0-21.6. It is an inducible identified compound that was detected in the Bacillus tissue system at seventeen fold the level in the *S. olivaceiscleroticus* (isolate No. 729) tissue system.

K- Compounds at the Elution Times (21-22):Kampferol (21.8)

The second inducible identified compound was detected at the R_t 21.5-22.0. It was present in the Fusarium pathogen system at a higher level than in the F/*T. harzianum* tissue system, and this level was twice as that of the F/*S. roseodiastaticus* (isolate No. 112) tissue system and ten times more than in the *S. roseodiastaticus* (isolate No. 112) tissue system.

L- Compounds at the Elution Times (22-23):Isorhamantein (22.4)

The first compound was detected at the R_t 22.0-22.5. It is an inducible identified compound which was present in the F/*T. harzianum* tissue system at a high level. This compound was also present in the Bacillus tissue and *S. roseodiastaticus* (isolate No. 112) tissue systems, however, It has a very low concentration, (below the limit of consideration). The second compound was detected at the R_t 22.5-23.0. It is also an inducible unidentified compound which was present in the Fusarium pathogen tissue system at a level, twofold that of the F/*T. harzianum* tissue system and threefold that of the Bacillus tissue system. It was also present with the *S. roseodiastaticus* (isolate No. 112) and the *S. olivaceiscleroticus* (isolate No. 729) tissue systems but at a very low concentration.

M- Compounds at the Elution Times (23):Unknown compounds

The first compound was detected at the R_t 23.0-23.5. It is a constitutive unidentified compound which was present in the F/*T. harzianum* tissue system at a higher level than in the Fusarium pathogen tissue system and which was twice as that in the healthy plants (untreated -

uninoculated). The second compound was detected at the R_t 23.5-24.0. It is an inducible unidentified compound which was specifically present in the Bacillus tissue system. It was produced at a high concentration, hence, it is regarded as a pronounced compound.

N- Compounds at the Elution Times (24-25): Formononetin (24.5)

The first compound was detected at the R_t 24.0-24.5. It is a constitutive identified compound which was present in the Fusarium pathogen tissue at a level, twofold that of the healthy plants (untreated-uninoculated) tissue, threefold that of the F/*T. harzianum* tissue systems, twelve fold that of the Bacillus tissue system and forty fold that of the *S. roseodiastaticus* (isolate No. 112) tissue system. The second compound was detected at the R_t 24.5-25.0. It is also a constitutive unidentified compound which was present in the F/*T. harzianum* system at a level which was eleven fold that of the F/*S. olivaceiscleroticus* (isolate No. 729) tissue system.

O- Compounds at the Elution Times (25-26):Glyceollin (25.8)

The first compound was detected at the R_t 25.0-25.5. It is a constitutive unidentified compound which produced de novo in the healthy plants (untreated- uninoculated) tissue at a high level. Therefore it is regarded as a pronounced one. This compound was also present in the Bacillus tissue, *S. roseodiastaticus* (isolate No. 112) tissue, F/*S. olivaceiscleroticus* (isolate No. 729) tissue, and F/*T. harzianum* tissue systems, though with very low level of concentrations. The other compound was detected at the R_t 25.5-26.0. It is also a constitutive identified compound which was present in the Fusarium pathogen tissue system at a level, twofold that of either the healthy plants (untreated-uninoculated) or the *S. olivaceiscleroticus* (isolate No. 729) tissue system and six fold that of the F/*T. harzianum* **tissue system.**

P- Compounds at the Elution Times (26-27):Chrysin (26.4)

The first compound was detected at the R_t 26.0-26.5. It is a constitutive identified compound which was present at a high level .in the Fusarium pathogen tissue system. This level is twice that of the Bacillus tissue system and nine and half times that of the F/*T. harzianum* tissue system. This compound was also present in the *S. olivaceiscleroticus* (isolate No. 729) tissue system but at a level which is regarded to be insignificant in this study. The

second compound was detected at the R_t 26.5-27.0. It is also a constitutive unidentified compound which was present at a very low concentration (>100,000) with the healthy plants (untreated-uninoculated), the *S. olivaceiscleroticus* (isolate No. 729) tissue, the Fusarium pathogen tissue, the F/*S. olivaceiscleroticus* (isolate No. 729) tissue and the F/*T. harzianum* tissue systems.

Q- Compounds at the Elution Times (27-28): Biochanin A (27.7)

The first compound was detected at the R_t (27.0-27.5). It is a constitutive unidentified compound which was detected in the Bacillus tissue system at a higher level than in the healthy plants (untreated- uninoculated) and this was fivefold that of the *S. roseodiastaticus* (isolate No. 112) tissue. The second compound was detected at the R_t (27.5-28.0). It is an inducible identified compound which was detected in the F/*T. harzianum* tissue system at a higher level than with the Bacillus tissue system. (>100,000) It was also present in the *S. olivaceiscleroticus* (isolate No. 729) and *T. harzianum* tissue systems but at a negligible concentration.

Table 31. Summarized results from Table 27 of aromatic secondary metabolites compounds in soybean - *F.solani* f. sp. *phaseoli* /biocontrol agents interaction systems: hypocotyls.

	Treatment	In gm fresh weight	Rt of identified[a] compounds from hypocotyls													
			7.6(I)		13.6(I)		15.5(I)		17.3(c)		18.9(c)		19.6(I)		20.1(I)	
			(ui)	(i)	(ui)	(i)	(i)	(i)	(i)	(ui)	(ui)	(i)	(ui)	(i)	(i)	(ui)
D	*Control*															
	Water (only)	1.5					11			5		64				
C	*Biological agent*															
c1	*B.subtilis*	1.8						43			5				447	
c2	*S.roseodiastaticus*	2.6	781	302		2.559	127	451	210	78	219		211		178	432
c2	*S.olivaceiscleroticus*	4										46		128		
c3	*T.harzianum*	2.5	708	85			32						12	11		
A	*Pathogen*															
	F.saloni f.sp.phaseoli	1.5	42				147		303		336			205	124	109
B	*Pathogen + Biological agent*															
B1	*F+B.subtilis*	0.7														
B2	*F+S.roseodiastaticus*	1.6						93								
B2\	*F+S.olivaceiscleroticus*	2.3	2		3				4						448	
B3	*F+T.harzianum*	2.5		9	64	33		85	91	168	40	90	44		125	

a- refers to constitutive (c) or inducible (I) compounds based on the work of Graham *et al.* (1990) and Morris *et al.* (1991)
(i) identified (ui) unidentified.

cont.

Table 31. Summarized results from Table 27 of aromatic secondary metabolites compounds in soybean - *F.saloni* f. sp. *phaseoli* / biocontrol agents interaction systems: hypocotyls

Treatment		In gm fresh weight	R_t of identified[a] compound from hypocotyls													
			21.6(I)		22.5(I)		23(ui)		24.4(c)		25.8(I)		26.4(I)		27.4(c)	
			(i)	(i)	(i)	(ui)	(ui)	(ui)	(i)	(ui)	(ui)	(i)	(i)	(ui)	(ui)	(i)
D	*Control*															
	Water (only)	1.5					168		349	3	142	118	8	8	120	
C	*Biological agent*															
c1	*B.subtilis*	1.8	34		35	36		185	57		15	19	474		160	57
													316			
c2	*S.roseodiastaticus*	2.6		14	6	5			28	22	6	11			45	
c2\	*S.olivaceiscleroticus*	4	41			7						243	60	51		6
c3	*T.harzianum*	2.5							4			4				5
A	*Pathogen*															
	F.saloni f.sp.phaseoli	1.5		92		108	329		686			276	973	63		
B	*Pathogen + Biological agent*															
B1	*F+B.subtilis*	0.7		42												
B2	*F+S.roseodiastaticus*	1.6								65	26			11		
B2\	*F+S.olivaceiscleroticus*	2.3		116	116	77	429		351	758	25	79	160	105		129
B3	*F+T.harzianum*	2.5			252											

a- refers to constitutive (c) or inducible (I) compounds based on the work of Graham *et al.* (1990) and Morris *et al.* (1991)
(i) identified (ui) unidentified.

4.5.3.3 *Characterization, Distribution And Identification of Phytoalexins In Soybean (Leaves)- M. phaseolina/Biocontrol Agents Interaction Systems In Greenhouse*

Table 32 shows the retention times of all the detected aromatic secodary metabolites compounds in the soybean (leaves) - M. phaseolina biocontrol agents interaction systems. Descrpitions of all of these metabolites and their detected levels are given below:

A- Compounds at the Elution Times (7-8): phenylalanin (7.6)

The first compound was detected at the R_t 7.0-7.5. It is an inducible unidentified compound which was present in the *Macrophomina* pathogen tissue system at a pronounced level. It was present at very small concentrations with the Bacillus tissue and *T. harzianum* systems; levels which are regarded to be below the limit of consideration. While the second compound was detected at the R_t 7.5-8.0. It is an also inducible identified compound, specifically, produced in the Bacillus tissue system, but at very low concentration which is also regarded as below the limit of consideration.

B- Compounds at the Elution Times (13-14): Free daidzin (13.6)

The first compound was detected at the R_t 13.0-13.5. It is an inducible unidentified, compound which was present in the Bacillus tissue system at a level higher than the M/B tissue system, but its concentration is below the limit of consideration. The second compound was detected at the R_t 13.5-14.0. It is also an inducible identified compound which was present in the *T. harzianum* tissue system at a level, fourfold that of the Bacillus tissue system, six and half fold times the level in the *S. olivaceiscleroticus* (isolate No. 729) tissue system and eleven and half times that of the M/B tissue system.

C- Compounds at the Elution Times (15-16): Free genistin (15.5)

The first compound was detected at the R_t 15.0-15.5. It is an inducible identified compound which was present in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a level, three and half times as that of either *the . harzianum* tissue or *the /T. harzianum* tissues systems, about seven and half times more than with the M/B tissue system, tenfold that of the Bacillus tissue system and forty six times more than with *the S. roseodiastaticus* (isolate No. 112) tissue system.

D- Compounds at the Elution Times (15-16): 6 malonyl daidzin (15.7)

Whereas the second inducible identified compound was present at the R_t 15.5-16.0 with the *S. olivaceiscleroticus* (isolate No. 729) tissue system at two and half times more than with the *T. harzianum* tissue system.

E- Compounds at the Elution Times (17-18): 6 malonyl genistin (17.3)

The first compound was detected at the R_t 17.0-17.5. It is an inducible identified compound which was present in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a level, twofold more that of the *T. harzianum* tissue system, nine fold that of the Bacillus tissue system and about fifteen fold more than that of the *Macrophomina* pathogen system. While the second compound was at the R_t 17.5-18.0. It is also an inducible unidentified compound which was present in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a level, slightly higher than that of the *M/S. roseodiastaticus* (isolate No. 112) tissue system and one and half times more than that of the *T. harzianum* tissue system.

F- Compounds at the Elution Times (18-19): Daidzein (18.9)

The first compound was detected at the R_t 18.0-18.5. It is an inducible unidentified compound which was present with the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a level which is one and half times more than with the *T. harzianum* issue system. While

the second compound was detected at the R_t 18.5-19.0. It is a constitutive identified compound which was present in the *T. harzianum* tissue system at a level, higher than that of the control plant (untreated-uninoculated) tissue system, and eight fold that of the M/B tissue system. Further, this compound was present in the *M/T. harzianum* tissue system at low concentration, which is regarded as below the limit of consideration.

G- Compounds at the Elution Times (19-20): glycitein 7-0-β-glucoside (19.3)

The first compound was detected at the R_t 19.0-19.5. It is an inducible identified compound which was present in the *T. harzianum* tissue system at a level, higher than that of the *S. olivaceiscleroticus* (isolate No. 729) tissue system and eleven fold the level of the Bacillus tissue system.

H- Compounds at the Elution Times (19-20): Cinnamic acid (19.9)

The second inducible identified compound which was present at the R_t 19.5-20.0 with the *S. olivaceiscleroticus* (isolate No. 729) tissue system at high level and hence it is regarded as a pronounced one. However, it was present in the *M/T. harzianum* at a very low concentration which is below the limit of consideration.

I- Compounds at the Elution Times (20-21): luteolin (20.1)

The first compound was detected at the R_t 20.0-20.5. It is an inducible identified compound which was present in the *T. harzianum* tissue system at a level, one and half times as that of the M/B tissue system, two and half fold times as that of either the *S. olivaceiscleroticus* (isolate No. 729) tissue or the *M/S. roseodiastaticus* (isolate No. 112) tissue systems, four and half as that of the Bacillus tissue systems and six and half times as that of the *Macrophomina* pathogen system. While, the second compound was detected at the R_t 20.5-21.0. It is an inducible unidentified compound which was present in the *T. harzianum* tissue system at a level which is, three times more than with the *S. olivaceiscleroticus* system,

four times more than with the Bacillus tissue system, six times ore than with the *Macrophomina* pathogen tissue system and nine times more than with the *S. roseodiastaticus* (isolate No. 112) tissue system.

J- Compounds at the Elution Times (21-22):Genistein (21.6)

The first compound was detected at the R_t 21.0-21.6. It is a constitutive identified compound which was particularly present in the Bacillus tissue system at great concentration level (pronounced). It was detected at a level which is eight fold as that of the control plants (untreated, uninoculated) tissue.

K- Compounds at the Elution Times (21-22): Kampferol (21.8)

The second constitutive identified compound was present at the R_t 21.7-22.0 with the Bacillus tissue at a high level (considerable concentration), while in the cases of, the control plants tissue (untreated, uninoculated), the *T. harzianum* tissue, the Macrophomina pathogen and M/B tissue systems, it was detected at very low of concentrations which are regarded as below the limit of consideration.

L- Compounds at the Elution Times (22-23): Isorhamantein (22.4)

The first compound was detected at the R_t 22.0-22.5. It is a constitutive identified compound which was present in the Bacillus system at a level which is, two and half times as that of the *T. harzianum* tissue system, four times as that of the M/B tissue system. whereas this compound was present at very low concentrations in, the control plants (untreated, uninoculated), the *S. roseodiastaticus* (isolate No. 112) tissue, the Macrophomina pathogen and the M/*T. harzianum* tissue systems. The second compound was detected at the R_t 22.5-23.0. It is an inducible unidentified compound which was present at very low concentrations (>100,000) in the, *S. roseodiastaticus* (isolate No. 112) tissue, the *S. olivaceiscleroticus* (isolate No. 729) tissue, the *T. harzianum* tissue, and the M/B tissue systems.

M- Compounds at the Elution Times (23-24): Unknown compounds

The first compound was detected at the R_t 23.0-23.5. It is an inducible unidentified compound which was present in the Macrophomina pathogen tissue system at a concentration level which is, three times as that of the *T. harzianum* tissue system and fourteen times as that of the *S. roseodiastaticus* (isolate No. 112) tissue system. The second compound was detected at the R_t 23.5-24.0. It is also an inducible unidentified compound which was present in the Macrophomina pathogen system at a higher level than in the *S. olivaceiscleroticus* (isolate No. 729) tissue system and threefold more than in the M/*T. harzianum* tissue system.

N- Compounds at the Elution Times (24-25): Formononetein (24.5)

The first compound was detected at the R_t 24.0-24.5. It is a constitutive identified compound which was detected in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a level which is, one and half times more than that in both the Bacillus tissue and the M/B tissue systems and twofold more than that of the control plants (untreated, uninoculated). The second compound was detected at the R_t 24.5-25.0. It is an inducible unidentified compound which was present in the Bacillus tissue system at threefold more than with the M/B tissue system. Also, this compound was present at very low concentrations in the *T. harzianum* tissue and the Macrophomina pathogen tissue systems.

O- Compounds at the Elution Times (25-26): Glyceollin (25.8)

The first compound was detected at the R_t 25.0-25.5. It is a constitutive unidentified compound which was present with the control tissue (untreated, uninoculated), de novo synthesis. It was detected in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a higher level than in either the *S. roseodiastaticus* (isolate No. 112) tissue system or M/B tissue system. The second compound was detected at the R_t 25.5-26.0. It is an inducible identified compound which was present in, the *S. roseodiastaticus* (isolate No. 112) tissue, the *S.*

olivaceiscleroticus (isolate No. 729) tissue system, the Macrophomina pathogen tissue, the M/B tissue and the M/*T. harzianum* tissue systems at very low concentrations.

P- Compounds at the Elution Times (26-27):Chrysin (26.4)

The first compound was detected at the R_t 26.0-26.5. It is an inducible identified compound which was detected in the Bacillus tissue system at level which is, three and half times as that with the M/*S. roseodiastaticus* (isolate No. 112) tissue systems and five and half times as that with the M/*T. harzianum* tissue system. The second compound was detected at the R_t 26.5-27.0. It is an inducible unidentified compound which was present at very low concentration which is regarded to be below the limit of consideration with the Bacillus tissue system, Macrophomina pathogen tissue system and M/B tissue systems.

Q- Compounds at the Elution Times (27-28): Biochanin A (27.7)

The first compound was detected at the R_t 27.0-27.5. It is an inducible unidentified compound which was detected in the Bacillus tissue system at four and half times more than in the M/B tissue systems. However its concentration is considered below the limit of consideration. The second compound was detected at the R_t 27.5-28.0. It is a constitutive identified compound which was present at high levels in the biocontrol agents [Bacillus, *S. roseodiastaticus* (isolate No. 112) and *S. olivaceiscleroticus* (isolate No. 729)] tissue systems, i.e., biocontrol agents enhanced its accumulation.

Table 32. Summarized results from Table 28 of aromatic secondary metabolites compounds in soybean - *M.phaseolina* /biocontrol agents interaction systems: leaves.

	Treatment	In gm fresh weight	R_t of identified[a] compound from leaves													
			7.6(I)		13.6(I)		15.5(I)		17.3(c)		18.9(c)		19.6(I)		20.1(I)	
			(ui)	(i)	(ui)	(i)	(i)	(i)	(i)	(ui)	(ui)	(i)	(ui)	(i)	(i)	(ui)
D	*Control*															
	Water (only)	3			100							8				
C	*Biological agent*															
c1	*B.subtilis*	2	14	10	33	102	16		17		2	105	8		18	30
												54			23	
c2	*S.roseodiastaticus*	5.5					82							10		34
c2	*S.olivaceiscleroticus*	9				317	739	777	686	231	686		242	168	181	182
c3	*T.harzianum*	4	23			968	90		152	66	197	277	194		200	239
A	*Pathogen*															
	M.phaseolina	4.6	299					140	18						16	39
									22						35	
B	*Pathogen + Biological agent*															
B1	*M+B.subtilis*	12			140	251	128					20			371	
												9				
B2	*M+S.roseodiastaticus*	15								313				44	299	
B2\	*M+S.olivaceiscleroticus*	13														
B3	*M+T.harzianum*	10				22	215					7	20		35	

a- refers to constitutive (c) or inducible (I) compounds based on the work of Graham *et al.* (1990) and Morris *et al.* (1991)
(i) identified (ui) unidentified.

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Cont.

Table 32. Summarized results from Table 28 of aromatic secondary metabolites compounds in soybean -*M. phaseolina* / biocontrol agents interaction systems: leaves

Treatment		In gm fresh weight	R_t of identified[a] compound from leaves													
			21.6(I)		22.5(I)		23(UI)		24.4(c)		25.8(I)		26.4(I)		27.4(c)	
			(i)	(i)	(i)	(ui)	(ui)	(ui)	(i)	(ui)	(ui)	(i)	(i)	(ui)	(ui)	(i)
D	*Control*															
	Water (only)	3	49	31	31				38		35					15
									6							
C	*Biological agent*															
c1	*B.subtilis*	2	243	142	147				39	190	4		147	36	187	881
c2	*S.roseodiastaticus*	5.5	4		38	82	22	5			130	147		0.02		395
						28										210
c2\	*S.olivaceiscleroticus*	9		36		36		242	243		100	7				983
c3	*T.harzianum*	4			110	73	87	57	21	32			32			12
A	*Pathogen*															
	M.phaseolina	4.6		33	33		267	151		69		12	53	17	45	33
							84			80						
B	*Pathogen + Biological agent*															
B1	*M+B.subtilis*	12		83	17	103			99		245	17		11	24	
B2	*M+S.roseodiastaticus*	15			209				294	382	124		160		237	
B2\	*M+S.olivaceiscleroticus*	13											314		39	
B3	*M+T.harzianum*	10			39			123			83	47	133		44	

a- refers to constitutive (c) or inducible (I) compounds based on the work of Graham *et al.* (1990) and Morris *et al.* (1991)
(i) identified (ui) unidentified.

4.5.3.4 *Characterization, Distribution And Identification of Phytoalexins Production in Soybean (Leaves)-F. solani* f. sp. *phaseoli/Biocontrol Agents Interaction Systems in Greenhouse*

Table 33 shows the retention times of all the detected aromatic secodary metabolites compounds in the soybean (leaves) - *F. solani* f. sp. *phaseoli* biocontrol agents interaction systems. Descrpitions of all of these metabolites and their detected levels are given below:

A- Compounds at the Elution Times (7-8): Phenylalanin (7.6)

The first compound was detected at the R_t 7.0-7.5. It is an inducible unidentified compound which was present at very low concentrations, which is regarded to be below the limit of consideration, in the Bacillus tissue, *T. harzianum* tissue, F/*S. roseodiastaticus* (isolate No. 112) and F/*S. olivaceiscleroticus* (isolate No. 729) tissue systems. The second compound was detected at the R_t 7.5-8.0. It is an inducible identified compound which was also present at very low concentrations in the Bacillus tissue system and F/*T. harzianum* tissue systems.

B- Compounds at the Elution Times (13-14): Free daidzin (13.6)

The first compound was detected at the R_t 13.0-13.5. It is a constitutive unidentified compound (de novo synthesis) which was detected in the control plants (untreated, uninoculated) at a considerable concentration (about 100,000), while in the Bacillus tissue and F/*S. roseodiastaticus* (isolate No. 112) tissue systems, this compound was present at very low concentrations; which are regarded to be below the limit of consideration. The second compound was detected at the R_t 13.5-14.0. It is an inducible identified compound which was present in the *T. harzianum* tissue system at level which is, fourfold that of the Bacillus tissue system and sevenfold that of the *S. olivaceiscleroticus* (isolate No. 729) tissue system.

C- Compounds at the Elution Times (15-16): Genistin (15.5)

The first compound was detected at the R_t 15.0-15.5. It is an inducible identified compound. It was detected at the highest level in the *S. olivaceiscleroticus* (isolate No. 729) tissue system. This level was, threefold that of the *T. harzianum* tissue system, nine fold that of the Bacillus tissue, sixteen fold that of the F/B tissue system, twenty one times that of the F/*T. harzianum* tissue system and forty four times that of the *S. roseodiastaticus* (isolate No. 112) tissue system.

D- Compounds at the Elution Times (15-16): 6 malonyl daidzin (15.7)

The second inducible identified compound was detected at the R_t 15.5-16.0. It was present at a very high level with the *S. olivaceiscleroticus* (isolate No. 729) tissue system. This level was, higher than the one detected in the Fusarium pathogen and six fold that of the F/*T.harzianum* tissue system. However, this compound was present at very low concentrations, of insignificant consideration, in the F/Bacillus, F/*S. roseodiastaticus* (isolate No. 112) and F/*S. olivaceiscleroticus* (isolate No. 729) systems.

E- Compounds at the Elution Times (17-18): 6 malonyl genistin (17.3)

The first compound was detected at the R_t 17.0-17.5. It is an inducible identified compound that was present with *S. olivaceiscleroticus* (isolate No. 729) tissue system at a level which was two and half times that of the *T. harzianum* tissue system. Also, this compound was present in the, Bacillus tissue, F/*S. olivaceiscleroticus* (isolate No. 729) and F/*T. harzianum* tissue systems, however, at very low concentration, i.e. below the limit of consideration. The second compound was detected at the R_t 17.5-18.0. It is an inducible unidentified compound which was present in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a level which was more than one and half times as that with the *T. harzianum* tissue system. Further, it was present in the F/*S. roseodiastaticus* (isolate No. 112) and F/*T. harzianum* tissue systems, but at very low concentration which is regarded as below the limit of consideration.

F- Compounds at the Elution Times (18-19): Diadzein (18.9)

The first compound was detected at the R_t 18.0-18.5. It is an inducible unidentified compound which was present in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a level which was twofold that of the *T. harzianum* tissue system. This compound was also present in the Bacillus tissue and F/*S. olivaceiscleroticus* (isolate No. 729) tissue systems at very small concentration which is regarded to be below the limit of consideration. The second compound was detected at the R_t 18.5-19.0. It is a constitutive identified compound which was present in the Fusarium pathogen tissue system at a higher level than in either the *T. harzianum* tissue system or the Bacillus tissue system. Moreover, it was detected in the F/*T. harzianum* tissue system but at a very low concentration.

G- Compounds at the Elution Times (19-20): Glycetin 7-0-β-glycoside (19.6)

The first compound was detected at the R_t (19.0-19.5). It is an inducible identified compound which was present in the *T. harzianum* tissue system at a level higher than that in the *S. olivaceiscleroticus* (isolate No. 729) tissue system.

H- Compounds at the Elution Times (19-20): Cinnamic Acid (19.9)

The second compound that was detected at the R_t 19.7-20.0 is an inducible identified compound. It was detected in the *S. olivaceiscleroticus* (isolate No. 729) tissue system at a considerable concentration. It was also present in the F/*S. olivaceiscleroticus* (isolate No. 729) tissue system but at very low concentration.

I- Compounds at the Elution Times (20-21): Luteoln (20.2)

The first compound was detected at the R_t 20.0-20.5. It is an inducible identified compound which was present in the *T. harzianum* tissue system at a level which was, one and half times that of the F/*T. harzianum* tissue system and two times that of the *S. olivaceiscleroticus* (isolate No. 729) tissue system. The second compound was detected at the

R_t 20.5-21.0. It is an inducible unidentified compound which was present in the *T. harzianum* tissue system at a level, two and half times that of the *S. olivaceiscleroticus* (isolate No. 729) tissue system and eight fold that of the F/*S. olivaceiscleroticus* (isolate No. 729) tissue system. Moreover, this compound was present in the Bacillus tissue, *S. roseodiastaticus* (isolate No. 112) tissue and F/*S. roseodiastaticus* (isolate No. 112) tissue systems but at very low and insignificant concentration consideration.

J- Compounds at the Elution Times (21-22): Genistein (21.6)

The first compound was detected at the R_t 21.0-21.6. It is a constitutive identified compound which was present at a pronounced level in the Bacillus tissue system. However it was present in the control plants (untreated-uninoculated) tissue and the *S. roseodiastaticus* (isolate No. 112) tissue system at very low and insignificant concentration.

K-Compounds at the Elution Times (21-22): Kampferol (21.8)

The second constitutive identified compound was at the R_t 21.7-22.0. It was present in the Bacillus tissue system at a level higher than with the Fusarium pathogen tissue system. Also, this compound was present in the control plant tissue (untreated-uninoculated), *S. olivaceiscleroticus* (isolate No. 729) tissue and F/*T. harzianum* tissue systems but at very low and insignificant concentration.

L- Compounds at the Elution Times (22-23): Isorhamantein (22.4)

The first compound was detected at the R_t 22.0-22.5. It is a constitutive identified compound which was present in the B tissue system at a level which was, two and half times as that of the *T. harzianum* tissue system, about seven times as that of the control plants (untreated-uninoculated) and nine times as that of the *S. roseodiastaticus* (isolate No. 112). Also, this compound was present at very low and insignificant concentration in the F/*S. roseodiastaticus* (isolate No. 112), F/*S. olivaceiscleroticus* (isolate No. 729) and F/*T.*

harzianum systems. The second compound was detected at the R_t 22.5-23.0. It is an inducible unidentified compound which was present at very low concentrations (>100,000) in the *S. roseodiastaticus* (isolate No. 112), *S. olivaceiscleroticus* (isolate No. 729), *T. harzianum*, F/*S. olivaceiscleroticus* (isolate No. 729) and F/*T. harzianum* tissue systems, which are also regarded as below the limit of consideration.

M- Compounds at the Elution Times (23-24): Unknown compounds

The first compound was detected at the R_t 23.0-23.5. It is an inducible unidentified compound which was present in the F/*S. roseodiastaticus* (isolate No. 112) tissue system at, two and half times as that in the F/*S. olivaceiscleroticus* (isolate No. 729) tissue system, four times as in the Bacillus tissue system and fivefold that of the *S. olivaceiscleroticus* (isolate No. 729) tissue system. The other compound was detected at the R_t 23.5-24.0. It is also an inducible unidentified compound which was present in the F/B tissue system at, a higher level than with the *S. roseodiastaticus* (isolate No. 112) and eight fold that of the *S. olivaceiscleroticus* (isolate No. 729) tissue system.

N- Compounds at the Elution Times (24-25): Formononetein (24.5)

The first compound was detected at the R_t 24.0-24.5. It is a constitutive identified compound which was detected at very low concentrations with the control plant (untreated-uninoculated), Bacillus, *S. olivaceiscleroticus* (isolate No. 729) and *T. harzianum* tissue systems, in addition to F/*S. roseodiastaticus* (isolate No. 112), F/*S. olivaceiscleroticus* (isolate No. 729) and F/*T. harzianum* tissue systems. The other compound was detected at the R_t 24.5-25.0. It is an inducible unidentified compound which was present in the Bacillus tissue system at three and half times as that in the F/*S. roseodiastaticus* (isolate No. 112) tissue system. Also, this compound was present in the *T. harzianum* tissue, F/*S. olivaceiscleroticus* (isolate No. 729) and F/*T. harzianum* tissue systems, however, it was produced at very low and insignificant concentrations.

O- Compounds at the Elution Times (25-26): Glyceollin (25.8)

The first compound was detected at the R_t 25.0-25.5. It is a constitutive unidentified compound which was present in the, control tissue (untreated-uninoculated), Bacillus tissue, *S. roseodiastaticus* tissue (isolate No. 112), *S. olivaceiscleroticus* tissue (isolate No. 729), F/*S. olivaceiscleroticus* (isolate No. 729) and F/T. h*arzianum* tissue systems at very low concentrations which are regarded as below the limit of consideration. .The second compound was detected at the R_t 25.5-26.0. It is an inducible identified compound which was present in the Bacillus tissue system at a level which was tenfold as that of the *S. roseodiastaticus* (isolate No. 112) tissue system. Further, this compound was present in the *S. roseodiastaticus* (isolate No. 112) and F/*S. olivaceiscleroticus* (isolate No. 729) tissue systems at very low and insignificant concentrations.

P- Compounds at the Elution Times (26-27): Chrysin (26.4)

The first compound was detected at the R_t 26.0-26.5. It is an inducible identified compound which was present in the Bacillus tissue system at a level fourfold that of the F/B tissue system. Also, this compound was present in, the *T. harzianum* tissue, the F/*S. roseodiastaticus* (isolate No. 112) and the F/*T. harzianum* tissue systems at low concentrations which are regarded as below the limit of consideration. The second compound was detected at the R_t 26.5-27.0. It is a constitutive unidentified compound which was present at very low and insignificant concentrations with the control plants tissue (untreated, uninoculated), the Bacillus, the *S. roseodiastaticus* (isolate No. 112), the F/*S. roseodiastaticus* (isolate No. 112) and the F/*S. olivaceiscleroticus* (isolate No. 729) tissue systems.

Q- Compounds at the Elution Times (27-28): Biochanin A (27.7)

The first compound was detected at the R_t 27.0-27.5. It is an inducible unidentified compound which was present in the Bacillus tissue system at a level which was, fivefold as that in either in the F/*S. olivaceiscleroticus* (isolate No. 729) tissue system or the F/*T. harzianum*

tissue systems. The second compound was detected at the R_t 27.5-28.0. It is a constitutive identified compound which was present in the Bacillus tissue system at a level which was, fourfold as that in the *S. olivaceiscleroticus* (isolate No. 729) system and eight fold as that in the *S. roseodiastaticus* (isolate No. 112) tissue system. Also, this compound which was present at very low concentrations in, the F/*S. roseodiastaticus* (isolate No. 112), the F/*S. olivaceiscleroticus* (isolate No. 729) and the F/*T. harzianum* tissue systems which are regarded as below the limit of consideration.

Table 33. Summarized results from Table 29 of aromatic secondary metabolites compounds in soybean - *F.solani* f. sp. *phaseoli* /biological agents interaction systems: leaves.

	Treatment	In gm fresh weight	R_t of identified[a] compound from leaves													
			7.6(I)		13.6(I)		15.5(I)		17.3(c)		18.9(c)		19.6(I)		20.1(I)	
			(ui)	(i)	(ui)	(i)	(i)	(i)	(i)	(ui)	(ui)	(i)	(ui)	(i)	(i)	(ui)
D	*Control*															
	Water (only)	3			100							8				
C	*Biological agent*															
c1	*B.subtilis*	2	14	10	33	102	16		17		2	105	8		18	30
												54			23	
c2	*S.roseodiastaticus*	5.5					82							10		34
c2\	*S.olivaceiscleroticus*	9				317	739	777	686	231	686		242	168	181	182
c3	*T.harzianum*	4	23			968	90		152	66	197	277	194		200	239
A	*Pathogen*															
	F.saloni f.sp.phaseoli	3.2						238								
B	*Pathogen + Biological agent*															
B1	*F+B.subtilis*	4.9					25	9								
B2	*F+S.roseodiastaticus*	8.5	35		10			118		24					5	20
			21												3	
B2\	*F+S.olivaceiscleroticus*	6	28					77	8		6			9		78
									12							
B3	*F+T.harzianum*	5		5		0.04	17	87	4	11		3			141	
										13						

a- refers to constitutive (c) or inducible (I) compounds based on the work of Graham *et al.* (1990) and Morris *et al.* (1991)
(i) identified (ui) unidentified.

cont.

Table 33. Summarized results from Table 29 of aromatic secondary metabolites compounds in soybean - *F.saloni* f. sp. *phaseoli* /biocontrol agents interaction systems: leaves.

Treatment		In gm fresh weight	R_t of identified[a] compound from leaves													
			21.6(I)		22.5(I)		23(UI)		24.4(c)		25.8(I)		26.4(I)		27.4(c)	
			(ui)	(i)	(i)	(ui)	(ui)	(ui)	(i)	(ui)	(ui)	(i)	(i)	(ui)	(ui)	(i)
D	*Control*															
	Water (only)	3	49	31	31				38 6		35					15
C	*Biological agent*															
c1	*B.subtilis*	2	243	142	147				39	190	4		147	36	187	881
c2	*S.roseodiastaticus*	5.5	4		38	82 28	22	5			130	147		0.02		395 210
c2\	*S.olivaceiscleroticus*	9		36		36		242	243		100	7				983
c3	*T.harzianum*	4			110	73	87	57	21	32			32			12
A	*Pathogen*															
	F.saloni f.sp.phaseoli	3.2		181												
B	*Pathogen + Biological agent*															
B1	*F+B.subtilis*	4.9						252			167		121			
B2	*F+S.roseodiastaticus*	8.5			2		340 250		9	201	66	39	36	20		28
B2\	*F+S.olivaceiscleroticus*	6			10	8	101		87	24	61	14		38	114	25
B3	*F+T.harzianum*	5		15	15 9	117			7	48	3		61 18		90	10

a- refers to constitutive (c) or inducible (I) compounds based on the work of Graham *et al.* (1990) and Morris *et al.* (1991)
(i) identified (ui) unidentified.

The preceding subsections illustrated the retention times of standard compounds, which have been reported by **Graham(1991b),** representing the range of aromatic classes of metabolites, i.e., phenyl propanoid derivatives including simple phenyl propaionic acids, flavanoids, and their glucosides and malonyl glucosides. The criterion used for compounds identification was the requirement that the compound elution time to be either similar or very close (due to possible measurement variability) to the elution time of a known or artificially synthesized compound that was previously reported in the literature, see Table 34. Furthermore, compounds whose elution times did not correspond to elution times of known compounds were referred to as unidentified compounds, see Table 35.

4.5.4 *Confirmation of the Identity of one of the Major Known Phytoalexin Compounds*

The compounds having minor peaks in Figs. 39, 40, 41 and 42 are not subjected to further identification. To test the identification of compounds based on their reported elution times, it was necessary to closely examine one of the known phytoalexin compounds that has one of the major peak, namely, the formononetin compound at the elution time 24.5. Firstly when the desired eluted compound was injected into the HPLC , the result showed the presence (or release) of two or three compounds that have λ_{max} at 236 nm (Tables 26, 27, 28, 29). The major compound as represented by the largest peaks in Figs.39-42, when chromatographed into the HPLC again it revealed its presence at the same, previously detected, elution time. This confirms its purity and identity. However, it should be noted that the identity of a given peak is highly tentative if based solely on retention time.

Table 34. HPLC analysis of identified isoflavonoids and isoflavonoids glucosides phytoalexins from the various soybean - pathogens / biocontrols interaction systems tested in this study and also in other[a] soybean - pathogen interaction systems, which did not involve biocontrol agents.

Identified compound	HPLC R_f[b](mn)	soybean organs						Reference	Class
		Seed [a]	Root[a]	Hypocotyl*		Leaves*			
				NI	I	NI	I		
					M/F		M/F		
Daidzin	13.6	-	-	-	(+)	+ +[M]	+ (+)	Graham et al. (1990)[I]	Neutral glucoside
Genistin	15.5	-	-	+	(+)	+[M]	(+)	Graham et al. (1990)[I]	Neutral glucoside
6- malonyl daidzin	15.7	-	+>	+>	+ (+)	+[M]	+ (+)	Graham et al. (1990)[I], Word (1985)	glucoside acid
Malonyl genistin	17.3	-	+>	+>	+ (+)	+[M]	+ (+)	Graham et al. (1990)[c], Word (1985)	glucoside acid
Daidzein	18.9	-	-	+	(+)	+[M]	(+)	Graham et al. (1990)[c]	isoflavon
Glycetin 7-0-β-glucoside	19.6	+	-	-	+ / (+)	-	+ [B] (+)	Graham et al. (1990)[I], Gohnson et al. (1976)	glucoside
Genistein	21.6	-	-	-	(+)	+ +[M]	(+)	Graham et al. (1990)[I],	isoflavon
Kampferol	21.8	-	-	-	(+)	-	(+)	Graham et al. (1990)[I],	isoflavon
Kampferol glucoside	-	-	+>	+>	+[e] (-)	+ +>	+ (-)	Casio and MacClure (1984)[I]	glucoside
Formononetin	24.4	-	+	+	(+)	+	+ (+)	Graham et al. (1990)[I],	isoflavon
Formononetin glucoside	-	-	+>	+>	-	+>	+[B] (-)	Morris et al. (1991), Fett, (1984)[I]	glucoside
Glyceollin	25.8	-	-	+>	+	-	(+)	Graham et al. (1990)[I],	isoflavon
Biochanin A	27.7	-	+	-	+	-	(+)	Graham et al. (1990)[c]	isoflavon

( ) Refers to an identified compounds reported by the researchers who are indicated in the above table

a indicates the soybean organs studied by other researchers (refer to literature review)

b Retention time indicates in the above table are those detected using the HPLC procedure developed by Graham et al (1990)

NI non infected, I infected +or - between paranthesis refer to the identified compound in this study

> detected level of higher concentration of phenolic compound

e etiolated in lower level B leaves/bacteria interaction system M mature leaves

Table 35. Summarized HPLC analysis of the unknown compounds

Host/ organs /biocontrol agents / pathogens interaction system												
Hypocotyls												
Pathogen		biocontroll agents				pathogen + biocontrol agents						Control
M	F	B	S_1	S_2	T	P+B		P+S_1		P+S_2		P+T		
						M	F	M	F	M	F	M	F	
-	-	-	-	-	-	-		+l.p		-		+lp		-
-	+p	-	+lp	-	-	+lp		+M		+l.p			(+M)	-
+lp	+p	-	+lp	-	-	+p			-	+M			-	-
-	-	-	+lp	-	-		-	+lp			-		(+M)	-
-	+lp	-	+p	-	+lM		-		(+lM)		(+lM)		-	-
+lp	-	-	-	-	-	+lM			-	+lM			-	+lM
+p*	+lp	-	-	-	-	+p			-	+p			-	-
+p*	+p	+lM	-	-	-	+lp			-	+lp			(+lp)	+lp
+lp	-	-	-	-	-	+lp			-	+lp			(+lp)	-
+lp	-	-	-	-	-	+lp			-	+p			-	+lp
+p	-	-	-	-	-	+lp		+lp			-		(+lp)	-
+lp	-	+lp	-	-	-	+lp			-	+lp			-	+lp

M = *M. phaseolina* F = *F. solani* f.sp. *phaseoli* B = *B. subtilis* S_1 = *S. roseodiasticus* S_2 = *S. olivaceiscleroticus* P = pathogen

P* = highly prominent > $3x10^6$, $1x10^6$ < P = prominent < $3x10^6$ $1x10^5$ < p= less prominent < $1x10^6$ $4x10^4$ <+M=minor < $1x10^5$ lM=less minor < $4x10^4$

Cont.

Table 35. Summarized HPLC analysis of the unknown compounds

Host/ organs /biocontrol agents / pathogens interaction system										Unknown compounds	HPLC R_t(min)
Leaves											
biocontroll agents				pathogen + biocontrol agents					Control		
B	S_1	S_2	T	P+B		P+S_1		P+S_2		P+T				
				M	F	M	F	M	F	M	F			
-	-	-	-	-		-		-		-		+l.p	1	13.0-13.5
-	-	-	-	-		-		-		-			2	17.5-17.9
-	-	+l.p	+p	-		-		-		-		-	3	18.0-18.5
-	-	+M	+M	-		-		-		-		-	4	19.0-19.5
*	+M	+M	+lp	-		-		+lM		-		-	5	20.5-20.9
+p	-	-	-	-		-		-		-		-	6	21.0-21.5
-	-	-	-	-		-		-		+lM		-	7	22.5-22.9
-	-	+lM	+lp	+lp		(+lp)		(+lp)		-		-	8	23.0-23.9
+lp	-	-	-	-		-		-		-		-	9	24.5-24.9
-	-	-	-	-		-		-		-		-	10	25.0-25.5
-	-	-	-	-		-		-		-		-	11	26.5-26.9
+lp	-	-	-	-		+lM		(+lp)		+lp		-	12	27.0-27.5

M=*M. phaseolina* F=*F. solani* f.sp. *phaseoli* B=*B. subtilis* S_1=*S. roseodiasticus* S_2=*S. olivaceiscleroticus* P = pathogn

P* = highly prominent > $3x10^6$, $1x10^6$ < P = prominent < $3x10^6$ $1x10^5$ < p= less prominent <$1x10^6$ $4x10^4$ <+M=minor <$1x10^5$ lM=less minor < $4x10^4$

( )= Fusarium interaction system

4.6 Streoscaning Electron Microscopy

S.E.M. experiments described here were designed to provide a better understanding of the role of structural barriers, as a mechanism (s) of biocontrol resistance, attained in excised host organ; served as a representative model, during the very early stages of infection (penetration) by both pathogens of concern at the appropriate incubation temperature. This representative model was used, in particular, when the two pathogens were applied separately one day after the biocontrol agent had been applied at the inoculated sites. Two days later, these inoculated sites were subjected to the S.E.M. observations as outlined previously in the materials and methods.

It was hoped that the S.E.M. investigations can contribute to the unraveling of the mechanisms which are involved in the biocontrol defense as were previously demonstrated morphologically and biochemically in the preceding sections.

4.6.1 *Interaction Between Soybean-* M. phaseolina*/Biocontrol Agents Systems: Hypocotyls as Revealed by S.E.M.*

4.6.1.1 B. subtilis *system*

SEM observations showing the interaction between soybean hybocotyls-*M. phaseolina/ B. subtilis* system i.e., hypocotyls were firstly inoculated with the antagonsit, *B. subtilis* one day, then they were inoculated with the fungal pathogen *M. phaseolina* , at the same inoculated sites, three days after the first inoculation with the antagonist are as indicated in Figs. 43.1 and 43.2.

Fig. 43.1. *B. subtilis* cells in contact with hypha (Host) of *M. phaseolina.* Note the extracellular material of the biocontrol *B. subtilis* (arrow) at the close proximity of the bacterial cell with the epidermal cell wall. Note also attachment of the bacterial cells and integrity of the hyphal cell wall. The pitted appearance of the hypae (straight arrows). Pitting present on hyphae where bacterial cells are in close contact with the hyphae (arrow). Pitting appeared quite extensive at times.

Fig. 43.2. Similar to **Fig. 43.1,** however, note the extensive damage on the target hyphal pathogen, *M. phaseolina*, in the form of pitting appearance. Note also the presumably newly hyphae are very thin (compare the two arrows).

4.6.1.2 S. roseodiastaticus *(isolate No. 112) system*

SEM observations showing the interaction between soybean hybocotyls-*M. phaseolina*/ *S. roseodiastaticus* system i.e., hypocotyls were firstly inoculated with the antagonsit, *S. roseodiastaticus* one day, then they were inoculated with the fungal pathogen *M. phaseolina* , at the same inoculated sites, three days after the first inoculation with the antagonist are as indicated in Figs. 44.1, 44.2, 44.3 and 44.4.

Fig. 44.1. Overgrowth pattern form of the biocontrol, *S roseodiastaticus*. Note the extensive distortion of the target host (*M. phaseolina*). Coiling of growing host hyphae which was either still appeared normal or had become thinner or distorted (arrows).

Fig. 44.2. A magnification of the two different forms of the pattern of host hyphal appearance, i.e., compare the normal **(N)** and the abnormality in appearance which implies thin **(t)**, distorted **(d)** and concave **(c)** hyphae. Note the collapse of the epidermal cells.

Fig. 44.3. The biocontrol, *S. roseodiastaticus* conidiophore with conidia. Note the over growth of the biocontrol hyphae on the target hyphal pathogen *M. phaseolina* (arrow). Note the pitting appearance of the host hyphae (arrow), and also the collapse of the epidermal cells..

Fig. 44.4. Note the competition between the *M. phaseolina* **(M)** and *S.roseodiastaticus*in pentrating the stoma **(S).**

4.6.1.3 *T. harzianum system*

SEM observations showing the interaction between soybean hybocotyls-*M. phaseolina*// *T. harzianum* system i.e., hypocotyls were firstly inoculated with the fungal antagonsit, *T. harzianum* one day, then they were inoculated with the fungal pathogen ***M. phaseolina*** , at the same inoculated sites, three days after the first inoculation with the antagonist are as indicated in Figs. 45.1 and 45.2.

Fig. 45.1. Swelling of emerging hypha **(H)** from the *Macrophomina sclerotium* and the distortion **(D)** appearance of the penetrating and growing hyphae of the target host (*M. phaseolina*).

Fig. 45.2. The coiling pattern of growth of the biocontrol, *T. harzianum*, is distinct **(C)** along the growing hyphae of the target pathogen (host), *M. phaseolina*. Note that epidermal cells **(EC)** were collapsed. Note also the very thin lysed part (concave appearance) of the host growing hyphae (arrow).

4.6.2 *Interaction Between Soybean-* F. solani *f. sp.* phaseoli*/ Biocontrol Agents Systems: Hypocotyls as Revealed by S.E.M.*

4.6.2.1 B. subtilis *system*

SEM observations showing the interaction between soybean hybocotyls-*F. solani* f. sp. *phaseoli/ B. subtilis* system i.e., hypocotyls were firstly inoculated with the antagonsit, *B. subtilis* one day, then they were inoculated with the fungal pathogen *F. solani* f. sp. *phaseolil,* at the same inoculated sites, three days after the first inoculation with the antagonist are as indicated in Figs. 46.

Fig. 46. Collapse of the germinated spore **(GS)** of *F. solani* f.sp. *phaseoli* (host). Note the distortion appearance of both the Macroconidium and its germ tube (dotted lines). Bacterial cells **(B)** are very close to its site of contact and also attached to the germ tube. Epidermal cells **(EC)** appeared collapsed and electron dense and were slightly located further away from the site where the germinated spores are located, irrespective of the germinated spores and bacterial cells association.

4.6.2.2 S. roseodiastaticus *(isolate No. 112) system*

SEM observations showing the interaction between soybean hybocotyls-*F. solani* f. sp. *phaseoli/ S. roseodiastaticus* system i.e., hypocotyls were firstly inoculated with the antagonsit, *S. roseodiastaticus* one day, then they were inoculated with the fungal pathogen *F. solani* f. sp. *phaseolil,* at the same inoculated sites, three days after the first inoculation with the antagonist are as indicated in Figs. 47.1 and 47.2.

Fig. 47.1. Note distorted spores (arrow); swelling germ tube forming microconidia (head arrow), and also the swelling of the infection hyphae (dotted arrow) that were produced from the appressorium of the Fusarium (host). Note also the complete distortion of the penetrating structures. Collapse of the epidermal cells **(c)** within the inoculated site.

Fig. 47.2. Reaction of the epidermal cells of soybean excised hypocotyl towards its challenge by the biocontrol agent, *S.roseodiastaticus*:wo days after inoculation. Note the Hypersenstive Reaction **(HR)** of the epidermal cells.

4.6.2.3 T. harzianum *system*

SEM observations showing the interaction between soybean hybocotyls-*F. solani* f. sp. *phaseoli/T. harzianum* system i.e., hypocotyls were firstly inoculated with the fungal antagonsit, *T. harzianum* one day, then they were inoculated with the fungal pathogen *F. solani* f. sp. *phaseolil,* at the same inoculated sites, three days after the first inoculation with the antagonist are as indicated in Figs. 48.1 and 48.2.

Fig. 48.1. Collapsed spores (Macro and microconidia) of *F. solani* f.sp. *phaseoli* on soybean epidermal cells of excised hypocotyls. Very thin germ tube of fusarial microconidium (head arrow). Collapse of epidermal cells was observed one day, after inoculation with target pathogen in which its inoculated site was subjected to inoculation by the biocontrol, *T. harzianum,* one day before its challenge. Note host hyphae are either completely collapsed or looked thinner the parts (dotted arrows). TS = Trichoderma spore; FMa = Fusarium Macroconidium; FMi = Fusarium Microconidium, (scale bar = 10 μm).

Fig. 48.2. More advanced stage of the progress of *T. harzianum* (biocontrol) of the interaction site of Fusarium (host). Extensive collapsed area of epidermal cells induced by a distinct effect on the target pathogen (host) by the biocontrol *T. harzianum* coiling **(c)** pattern of growth. Note the coiled host hyphae appeared collapsed at same sites of interaction; other host hyphae were completely distorted **(d),** (lysed), and looked thinner and dark in its electron opacity.