

EFFECT OF DIFFERENT TYPES OF MEDIA ON ANTAGONISTIC ACTION BETWEEN SOME SOIL-BORNE FUNGI

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ABSTRACT

The comparison between the effects of the used different media (PDA, Czapek-Dox agar, malt extract agar and soil extract agar) in growing both parasitic fungi (*Rhizoctonia solani*, *Fusarium oxysporum* F. sp. cucumerinum and *Sclerotinia sclerotiorum* and the antagonistic fungi (*Trichoderma harzianum*, *Gliocladium roseum* and *G. virens*) showed that the available media for growing the antagonistic fungus *T. harzianum* in the presence of the pathogen *R. solani* were respectively malt extract agar followed by P.D.A., while the opposite results for growing the pathogenic fungus *R. solani* in the presence of *T. harzianum* was soil extract agar followed by Czapek-Dox agar.

Also, we found that the available media for growing the antagonistic fungus *T. harzianum* in the presence of the pathogen *F. oxysporum* were Czapek-Dox agar followed by soil extract agar, P.D.A. and malt extract agar, respectively. While the best media for growing the same antagonistic fungus *T. harzianum* in the presence of *S. sclerotiorum* were soil extract agar, Czapek-Dox agar, P.D.A. and malt extract agar media, respectively. In case of the antagonist *G. virens* with the *R. solani*, we found that the available media for the best growth was the P.D.A., while the other three media type gave negative growth for *G. virens* and positive growth for *R. solani*. Also, the whole media were available for growing the antagonist *G. virens* in the presence of the pathogen *F. oxysporum* cucumerinum but the P.D.A. was the best between all media for growing the two fungi, while the best media for growing the same antagonistic fungus in the presence of the pathogen *S. sclerotiorum* were malt extract agar and soil extract agar followed by P.D.A. and Czapek-Dox agar media. In the presence of *Gliocladium roseum* with the three pathogenic fungi (*R. solani*, *F. oxysporum* F. sp. cucumerinum and *S. sclerotiorum*), all media gave less growth (decreased) for the antagonistic fungus *G. roseum* and positive growth (increased) all pathogenic fungi.

Key words: Antagonism, media, soil-born fungi, biological, *trichoderma*.

INTRODUCTION

The number of scientific articles on biological control that have appeared in recent years reflects a strong wave on interest in the development of economical pest control methods which promise to reduce the intensity of chemical pesticide usage. Most of these works explore the

general principles and practices of this subject and a few concentrated on microorganisms as a biological agents. Among the microorganisms, some bacteria in particular exert controlling influences on other organisms in nature.

It is obvious that in harnessing organisms as a biological advantage in that fieldly their propagules can be raised in huge numbers in artificial media to produce suspensions which can be applied in green house and in the field. Therefore, the aim of our research is to compare the effect of different media on growing both parasitic fungi (*Rhizoctonia solani*, *Fusarium oxysporum* *F. sp. cucumerinum* and *Sclerotinia sclerotiorum*) and three antagonism fungi (*Trichoderma harzianum*, *Gliocladium virens* and *G. roseum*).

The effects of tap water, soil extract and potato dextrose agar on growth and interactions of *S. sclerotiorum*, *Botrytis cinerea* and *R. solani* and their antagonists *Coniothyrium minitans* and *G. roseum* were studied by Whipps and Magon (1987), they found that the different media had significant effects on growth rates of all fungi, but PDA, did not always give maximum growth, also antibiotic production by the antagonistic fungi depended on the type of the used medium.

Also, Whipps (1987) regarded that the same media had significant effects on the growth rates of *R. solani*, *F. oxysporum* and *T. harzianum* and on the production of volatile or non-volatile antibiotic compounds and on the hyphal interactions, while Wu *et al.* (1986) stated that *T. harzianum* was most strongly on PDA plates, and sterilized soils among different isolates of *Trichoderma spp.* Di-Ercole (1984) found that the *T. harzianum* gave complete covering on the pathogen *R. solani* on PDA, while Phillips-Ajl (1989) found that the mycelial growth of *S. sclerotiorum* was reduced by *G. vermes*, *T. harzianum*, *Coniothyrium mintans* and was slightly inhibited by *G. roseum* on PDA. Benhamou and Chet (1993), Xu *et al.* (1993), Singh (1991), Xu and Li (1993) and Hader *et al.* (1979), reported that the isolates of *T. harzianum* inhibited the hyphal growth of *R. solani*, *F. oxysporum*, *F. sp. cucumerinum* and *S. sclerotiorum*. While, Di-pietro *et al.* (1993), Tu (1980), Hyeong and Kim (1987) and Sreenivasprasad and Manibbushanrao (1990) found that *G. virens* is a destructive mycoparasite of *R. solani*, *S. sclerotiorum* and *F. oxysporum* *F. sp. cucumerinum*.

Burges *et al.* (1997) regarded that incubation of *Botrytis cinerea* on PDA amended with culture filtrates of *G. roseum* gave no significant growth reduction.

MATERIALS AND METHODS

Evaluation of the antagonistic potential of *Trichoderma harzianum*, *Gliocladium roseum* and *G. virens* to *Rhizoctonia solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* were studied. All the isolates of pathogenic fungi except *F. oxysporum* *F. sp. cucumerinum* were isolated from damping-off diseased seedlings of cucumber, cowpea and bean. Identification of fungal isolates for species level was done according to Gilman (1957) and Barnett and Hunter (1972). Pure cultures of *F. oxysporum*

F. sp cucumerinum and *T. harzianum* were obtained from Institute of Plant Pathology Research (A.R.C.), Cairo, while culture of *G. virens* and *G. roseum* were obtained from Institute of Plant Protection, Researchers, Budapest, Hungary. Dauol culture system of growing all fungi on agar medium were evaluated on potato dextrose agar media (PDA), malt extract agar (MEA), soil extract agar (SEA) and Czapek-Dox agar media (CzDA). Soil extract agar was supplemented with 1 g NaNO₃ and 10 g glucose per liter of medium. A 9 cm petri dish each containing 15 ml of the different media was inoculated with P.D.A. mycelial discs (5 mm diameter) of both fungi antagonistic and pathogenic fungi. The fungus discs were placed opposite to other and relatively closed to periphery of the plates (Johnson *et al.*, 1953).

Control treatments were carried out by placing mycelial discs of pathogenic fungi alone. Four replicates of plates were carried out for each treatment. All the inoculated plates were incubated at 25°C for 6 days.

RESULTS AND DISCUSSION

The results of comparing the effect of the used media in growing both parasitic and antagonistic fungi showed that the best medium for having best growth of the antagonist *T. harzianum* with the pathogen *R. solani* was the malt extract agar giving 54 mm diameter average of the growing zones of *T. harzianum*, while the *R. solani* zone growth average was 36 mm on the same medium with the same antagonist. The second class of growth level of both *T. harzianum* and *R. solani* happened on P.D.A. medium, the average colony diameter of *T. harzianum* was 48 mm and 42.0 mm for the *R. solani*. The third growth level of the two tested fungi from growing on Czapek-Dox agar medium gave 36.0 mm diameter average for *T. harzianum* grown colonies, while it was 54.0 mm for colonies diameter average of *R. solani*. The least effective medium was the soil extract agar giving 33.0 mm colony diameter for *T. harzianum* and 57.0 mm colony diameter average for *R. solani*.

From these results we find that the available media for growing the antagonistic fungus *T. harzianum* in the presence of the pathogen *R. solani* were malt extract agar followed by P.D.A. medium, while the opposite results for growing the pathogenic fungus *R. solani* in the presence of the antagonist *T. harzianum* was the soil extract agar followed by Czapek-Dox agar medium, results are shown in Table (1).

We found in the same table that these result agree with Whipps and Magon (1987) who reported that the PDA is not always the best medium for giving the maximum growth for *T. harzianum*, while Wu *et al.* (1986) stated that growing the *T. harzianum* on the PDA gives strong growth in the presence of *R. solani*. The maximum growth of the antagonist *G. virens* with the pathogen *R. solani* was the PDA medium giving 45.0 mm colony diameter average for both *G. virens* and *R. solani*. While, the other three media types the malt extract agar, the Czapek-Dox agar and the soil extract agar gave less growth for *G. verens* and positive growth for *R. solani* giving (36.0 and 54.0 mm), (33.0 and 57.0mm) and (30.0 and 60.0 mm) diameter

averages, respectively. These results are in agreement with Whipps (1987) who reported that significant growth of *G. virens* was obtained from growing on PDA better than tap water or soil extract.

The third treatment of growing the antagonist *G. roseum* and the pathogen *R. solani* on the four media types show the all media gave less growth for the antagonist *G. roseum*, while it increased the growth of the pathogen *R. solani* to be superior, the obtained growth diameter of colonies averages of both *G. roseum* as antagonist and *R. solani* as pathogens were (27.0 and 63.0 mm) on the malt extract agar, (24.0 and 66.0 mm) on PDA, (12.0 and 78.0 mm) on soil extract agar and (9.0 and 81 mm) on Czapek-Dox agar, respectively, all these results are mentioned in Table (1) and Fig. (1). Such result are given by Phillips (1989) who stated that growing *G. roseum* on PDA slightly inhibited the growth in presence of *R. solani*.

Table 1. Growth of antagonistic fungi and *Rhizoctonia solani* on different fungi.

	PDA	MFA	SEA	Czapek
<i>R. solani</i>	42	36	57	54
<i>T. harzianum</i>	48	54	33	36
<i>R. solani</i>	45	54	60	57
<i>Gliocladium virens</i>	45	36	30	33
<i>R. solani</i>	66	63	78	81
<i>Gliocladium roseum</i>	24	27	12	9
L.S.D. 5%	2.93	2.69	2.53	1.58
	3.41	2.04	1.49	2.01

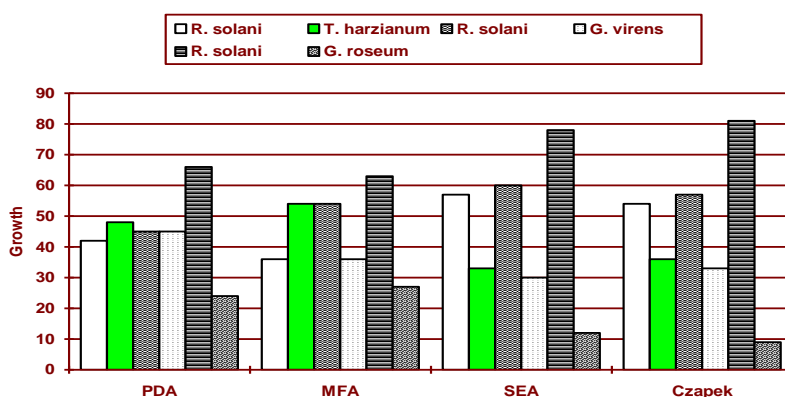


Fig. 1. Growth of antagonistic fungi and *Rhizoctonia solani* on different fungi.

The available media for having the best growth of the antagonist *T. harzianum* with the pathogen *F. oxysporum* sp. *cucumerinum* was the Czapek-Dox agar medium giving 66.0 mm diameter average of the growing zones of *T. harzianum*, while the *F. oxysporum* zones growth average was 24

mm on the same medium. The second growth level of both *T. harzianum* and *F. oxysporum* happened on soil extract agar medium, the average colony diameter of *T. harzianum* was 63.0 mm and 27.0 mm for the *F. oxysporum* *F. sp. cucumerinum*. The third growth level was obtained from the two tested

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Growth and antagonistic effect of some soil borne on different types of media incubated at 28c+ 2c for 6 days.

A=R. Solani (control)

B=R. Solani+ T.harzianum

C+R.solani+ G.virens

D=R.solani +G.roseum

Growth and antagonistic effect of some soil borne on different types of media incubated at 28c+ 2c for 6 days.

A= *F.oxysporum f. Sp.Cucumerinum* (control)

B= *F.oxysporum f. Sp.Cucumerinum +T.harzianum*

C= *F.oxysporum f. Sp.Cucumerinum +G.virens*

D= *F.oxysporum f. Sp.Cucumerinum +G.roseum*

Growth and antagonistic effect of some soil borne on different types of media incubated at 28c+ 2c for 6 days.

A= *S.sclerotiorum* (control)

B= *S.sclerotiorum* + *T. harzianum*

C= *S.sclerotiorum* + *G. virens*

D= *S. sclerotiorum* + *G. roseum*

fungi from growing on P.D.A. giving 62.0 mm diameter average of *T. harzianum* grown colonies, while the colony diameter average of *F. oxysporum* *F. sp. cucumerinum* was 18.0 mm. The least effective medium on the growth of the two tested fungi was the malt extract agar giving 57 mm colony diameter average in case of *T. harzianum* and 33.0 mm colony diameter average for *F. oxysporum*.

From these results we find that the listing of the most available media for growing the antagonistic fungus *T. harzianum* in the presence of the pathogen *F. oxysporum* *F. sp. cucumerinum* were Czapek-Dox agar followed by soil extract agar, P.D.A. and Malt extract agar, respectively (See Table 2). It is obvious also in the same table that the best growth of the antagonist *G. virens* with the pathogen *F. oxysporum* *sp. Cucamerinum* was the P.D.A. giving 66.0 mm and 24.0 mm diameter average, respectively. While, the other three media types, the Czapek-Dox agar, soil extract agar and malt extract agar gave 63.0 mm diameter for growth *G. virens* and 27.0 mm for *F. oxysporum* *sp. cucamerinum* on Czapek-Dox agar, 57.00 mm for *G. virens* and 33.0 for *F. oxysporum* *F. sp. cucamerinum* on malt extract agar, respectively. The third treatment of growing the antagonist *G. roseum* and the pathogen *F. oxysporum* *F. sp. cucamerinum* on the four media types show that all media gave less growth for the antagonist *G. roseum*, while it helped the growth of the pathogen *F. oxysporum* *cucamerinum*, the obtained growth diameters of colonies averages of both *G. roseum* and *F. oxysporum* *F. sp. cucamerinum* were 66.0 and 24.0 on malt extract agar (54.0 and 36.0 mm.) on Czapek-Dox agar, (51.0 and 39.0 mm) on P.D.A. and (48.0 and 42.0) on soil extract agar, respectively, all these results are mentioned in Table (2) and Fig. (2). These results agree with Whipps (1987) who reported that *T. harzianum* and *G. virens* gave good growth on PDA and on soil extractm, while *G. roseum* gave less growth on the same media in the presence of *F. oxysporum*.

Table 2. Growth of antagonistic fungi and *Fusarium oxysporum* on different fungi.

	PDA	MFA	SEA	Czapek
<i>F. oxysporum</i>	18	24	33	27
<i>T. harzianum</i>	62	66	57	63
<i>F. oxysporum</i>	24	27	39	33
<i>Gliocladium virens</i>	66	63	51	57
<i>F. oxysporum</i>	51	54	66	48
<i>Gliocladium roseum</i>	39	36	24	42

L.S.D.	5%	4.15	3.33	3.33	1.49
		2.53	1.58	2.67	2.41

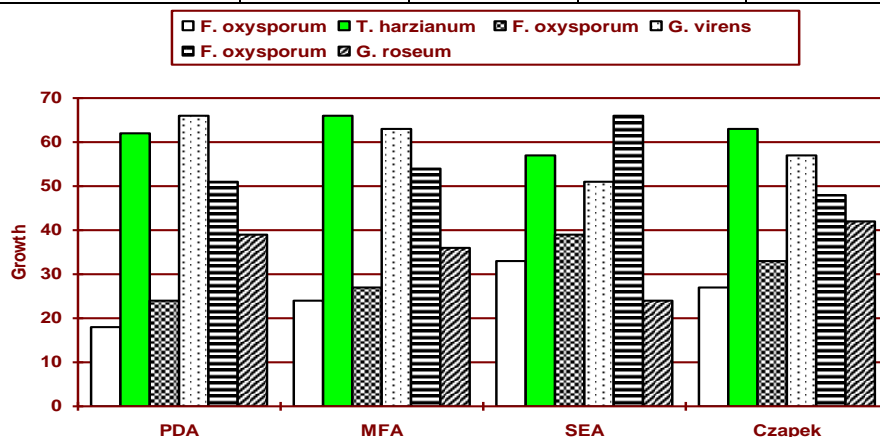


Fig. 2. Growth of antagonistic fungi and *Fusarium oxysporum* on different fungi.

The best media for having the best growth of the antagonistic *T. harzianum* with the pathogen *S. sclerotiorum* was soil extract agar medium which gave 75.0 mm diameter average for the growing zones of *T. harzianum*, while the *S. sclerotiorum* zones growth average was 15.0 mm. The second class on growth of both *T. harzianum* and *S. sclerotiorum* happened on Czapek-Dox agar, the average colony diameter of *T. harzianum* was 63.0 mm and 27.0 mm for the *S. sclerotiorum*. The third class growth level was obtained from these two tested fungi grown on P.D.A. giving (57.0 and 33.0mm) colony diameters averages, respectively.

The least effective media on the growth of both *T. harzianum* and *S. sclerotiorum* was the malt extract agar giving 51.0 mm and 39.0 mm colony diameter averages, respectively. From these results we find that available media for growing the antagonistic fungus *T. harzianum* in the presence of the pathogen *S. sclerotiorum* were soil extract agar, Czapek-Dox agar, P.D.A. and malt extract agar media, respectively. We find also that the best growth of the antagonist *G. virens* with the pathogen *S. sclerotiorum* was the soil extract agar and malt extract agar giving 57.0 and 33.0 mm colony diameter averages for *G. virens* and *S. sclerotiorum*. While, the other two media types the P.D.A. and Czapek-Dox agar gave 54.0 and 36.0 mm and 51.0 and 39.0 mm diameter averages for *G. virens* and *S. sclerotiorum*, respectively. The third treatment of growing the antagonist *G. roseum* and the pathogen *S. sclerotiorum* on four media types show that all gave negative growth results for the antagonist *G. roseum*, while it helped the growth of the pathogen *S. sclerotiorum* to be superior, the obtained growth diameter averages of both *G. virens* as antagonist and *S. sclerotiorum* as pathogens were (27.0 and 63.0 mm) on Czapek-Dox agar, (27.0 and 63.0

mm) on malt extract agar (30.0 and 60.0 mm) on P.D.A. and (24.0 and 66.0mm) on soil extract agar, respectively (Table 3, Fig. 4).

Table 3. Growth of antagonistic fungi and *Sclerotinia sclarum* on different fungi.

	PDA	MFA	SEA	Czapek
<i>Sclerotinia sclarum</i>	33	27	39	15
<i>T. harzianum</i>	57	63	51	75
<i>Sclerotinia sclarum</i>	36	39	33	33
<i>Gliocladium virens</i>	54	51	57	57
<i>Sclerotinia sclarum</i>	60	63	63	66
<i>Gliocladium roseum</i>	30	27	27	24
L.S.D. 5%	2.84	1.49	1.97	3.41
	2.58	1.82	2.69	3.83

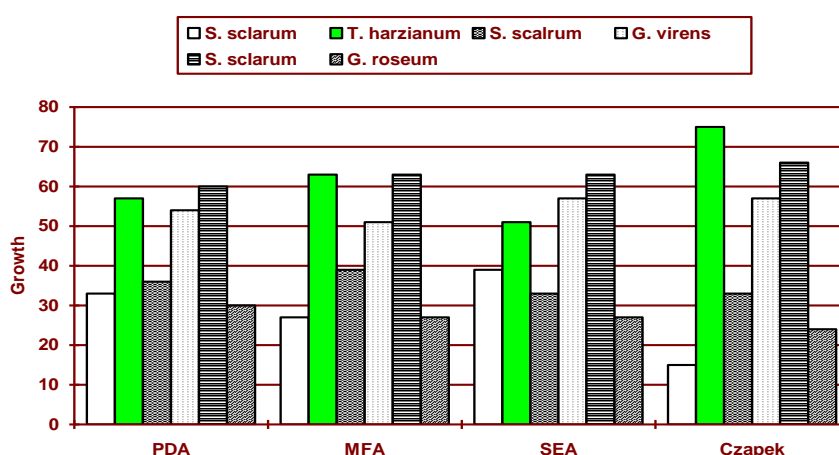


Fig. 3. Growth of antagonistic fungi and *Sclerotinia sclarum* on different fungi.

Such results are in agreement with Phillis-Ajl (1989) who found that the mycelial growth of *S. sclerotiorum* was reduced by *T. harzianum*, *G. virens* and less inhibited by *G. roseum*.

Finally, we state that the majority of results obtained from this work agree with those given by Whipps (1987) to volatile and non-volatile antibiotic compound by *T. harzianum* and *G. virens* and hyphal interaction in presence of *R. solani*, *F. oxysporum* F. sp. cucumerinum had significant effect, also Whipps and Magan (1989) who mentioned that the antibiotic production depended on the media types.

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تأثير أنماط مختلفة من البيئات المغذية على حالة التضاد بين بعض فطريات التربة.

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للمقارنة بين دراسة استخدام بعض البيئات المختلفة مثل بيئة Czapek-Dox agar, Potato dextrose agar, soil extract agar, Malt extract agar المضادة مثل تريكودرما هارازيانم *Trichoderma harzianum* وجليوكلاديم فيرنس *Gliocladium verens* وجليوكلاديم روزيم *G. roseum* ضد الفطريات الكامنة في التربة مثل ريزوكتنيا سولاني *Rhizoctonia solani* وفيوزاريم أوكس سبورم كيوكاميرنيم *Fusarium oxysporum* F. sp. *Sclerotinia sclerotiorum* Cucamerinum وذلك تحت ظروف المعمل حيث أوضحت النتائج المتحصل عليها أن أفضل البيئات المستخدمة والتي تسمح بنمو الفطر المضاد *T. harzianum* وذلك في وجود الفطر الممرض *R. solani* هي بيئة مستخلص المولت تبعها بيئة P.D.A. ولكن على العكس كانت بيئة Czapek-Dox هي التي ينمو عليها الفطر الممرض وكذلك بيئة مستخلص التربة بدرجة أفضل من النمو الميسلومي للفطر المضاد *T. harzianum* وفي تواجد الفطر الممرض *Fusarium oxysporum* F. sp. *Cucamerinum* ثم Czapek-Dox agar ثم Soil extract agar ثم P.D.A. ثم Malt extract agar على التوالي 0 ولكن كانت أفضل البيئات لنمو نفس الفطر المضاد في تواجد الفطر الممرض *S. sclerotiorum* كانت بيئة Soil extract agar ثم Czapek-Dox agar ثم Malt extract agar 0 في حالة إلتقاء الفطر المضاد *G. viren* مع الفطر الممرض *R. solani* كانت أفضل البيئات والتي تسمح بنمو الفطر المضاد بدرجة أفضل هي بيئة P.D.A. ولكن الثلاث بيئات الأخرى المستخدمة كانت ذات تأثير أقل أي أن الفطر الممرض *R. solani* ينمو بدرجة أفضل من الفطر المضاد *G. virens* ولكن عند تواجد الفطر المضاد *G. virens* مع الفطر الممرض *Fusarium oxysporum* F. sp. *Cucamerinum* وجد أن كل البيئات المستخدمة تسمح بنمو الفطر المضاد بدرجة أفضل وكان أفضل هذه البيئات هي بيئة P.D.A. بينما كانت أفضل البيئات عند تواجد نفس الفطر المضاد مع الفطر الممرض *S. sclerotiorum* هي بيئة Soil extract agar, Malt extract agar تبعها بيئة P.D.A. و Czapek 0

أما في حالة تواجد الفطر المضاد *G. roseum* مع الثلاث فطريات الممرضة السابقة الذكر فقد وجد أن جميع البيئات تكون أفضل لنمو الفطر الممرض عن الفطر المضاد 0