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ISOLATION OF INDIGENOUS BIOAGENTS AND ESTIMATION OF THEIR EFFICIENCY FOR CONTROL of some SOIL- AND AIR-BORNE FUNGI OF CUCURBITS.

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ABSTRACT

In order to save the Egyptian ecosystem from pollution with pesticides. Five bioagents were isolated from the Egyptian soil. These bioagents were identified as *Trichoderma hamatum*, *Gliocladium virens*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces* sp. The isolates proved to be antagonistic to *Rhizoctonia solani*, *Pythium* sp. and *Fusarium* sp. in *vitro*. Under field conditions, good control of soil borne diseases of cucurbits was achieved as a result of these bioagents.The application of bioagents as seed coat resulted in higher efficiency than applying them as soil drench. The bioagents affected positively the root system and the foliage.

A new method to asses the bioagent antagonistic potency in *vitro*, was developed. The bioagents showed satisfactory control of downy mildew disease on cucumber and powdery mildew on squash, with significant increasing in the yield. In all cases the fungicides showed better results.

INTRODUCTION

The cucurbits are a world wide important vegetable crops, They are subject to be attacked by many pathogens either in the open field or under covered agricultura conditions resulting in great economic losses. These pathogens may be soil borne i.e. *Fusarium* spp., *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium* spp., which attack the root system resulting in root rot diseases or airborne i.e. *Pseudoperonospora cubensis* and *Sphaerotheca fulginea* the causal organisms of downy mildew and powdery mildew respectively (Maximlian, *et al.* 1976)

However, good control of these diseases can be achieved by chemical control products, The hazardous of the pesticides on the environment and consumers (Epstein *et al.*, 1967; Fawcett and Spencer, 1970; Dubey and Mall, 1972; Beye, 1978 and Javoraska, 1978) limit the use of these pesticides and emphasize the need for new saver methods to control plant diseases (Wilson *et al.*, 1987). The biocontrol of plant diseases as an alternative strategy has received an increasing attention at the last tow decades (Papavizas, 1985; Baker, 1987 Lockwood, 1988 and El- Kafrawy ,2002).

This investigation is an attempt to isolate some indigenous bioagents from the Egyptian field to save the Egyptian ecosystem, and to study the ability of these biological agents to suppress the soil- and air-borne pathogens on cucurbits in addition to clarify the influence of these bioagents on the vegetative growth of the plants and it's crop. It aims also to develop a standard method which enables the investigators to calculate the antagonistic efficiency of the bioagents.

MATERIALS AND METHODS

I- Isolation of different bio-agents from the soil :

Healthy plants of cucumber grown in the Gemmiza experimental farm were chosen and plucked out with roots and rhizosphere soil, The roots and rhizosphere soil were crushed thoroughly and ten grams of each soil sample were suspended in 90 ml sterilized distilled water in 200 ml. flasks and shacked for 20 min. using high speed shaker. Standard dilution plating technique (Wollum, 1982) was followed to isolate the different bio-agents, where. serial dilutions up to 10^{8} were done by repeated addition of 1 ml. soil suspension to 9 ml. sterilized distilled water. The resulted suspensions were used for the isolation of fungi, bacteria and actinomyces.

a- Isolation of fungi :

0.250 ml. of each dilution was spread on the surface of peptone dextrose agar in Petri dishes using sterile glass rod . Five Petri dishes were used for each dilution. The dishes were incubated at 25°C for 3 days. The separated grown colonies were subcultivated on PDA medium.

b- Isolation of bacteria and actinomyces:

0.250 ml. of each dilution was spread on the surface of soil extract agar in Petri dishes in case of bacteria and Jensen's agar medium in case of actinomyces using sterile glass rod . Fife Petri dishes were used for each dilution. The dishes were incubated at 28°C for 2 days. The separated grown colonies were subcultivated on slant nutrient agar medium in case of bacteria and Jensen's agar medium in case of actinomyces

Ii- Prescreening of the isolated organisms for antagonistic potency in*vitro*:

In order to screen the antagonistic potency of the isolated fungi, these isolates were cultured on PDA medium in Petri dishes for five days. while the bacterial isolates were cultured in nutrient-yeast broth medium in flasks, incubated with shaking at 150 rpm for 48 to 72 h. at 28° C. then the bacterial; suspensions were centrifuged , washed and resuspended in 20 m N buffer, pH 7.0.

Petri dishes containing 1/5 PDA were inoculated in the center with *Rhizoctonia solani, Fusarium* spp or *Pythium* spp. disks, 4 mm in diameter, taken from the edge of 5 days old culture. The same Petri dishes were inoculated with 4 disks taken from the edge of 5 days old culture of 4 different isolated fungi near the edge of the dishes, or with 0.1 ml. of the different actinomyces or bacterial suspension . Each of the candidate bio-agent (the isolated fungi, bacteria and actinomyces) was inoculated in five plates. The plates were incubated at 25 ° C. for 5 days and any noticed inhibition zone or hyper parasitism was recorded.

The organisms, which showed valuable inhibition zone or hyper parasitism, were tested again to assest their antagonistic potency using the following new developed technique. On the 1/2 PDA medium plates, a disk of the pathogen, *Rhizoctonia solani, Fusarium* sp. or *Pythium* sp. was mounted at distance of 3 cm. from the plate edge, opposite to it , on the same radius and at distance of 1.5 cm. from the opposite plate edge, one

disk of the candidate fungus , or a droplet 0.1 ml. of the candidate of bacteria or actinomyces suspension was mounted . Five replicates were used for each and the plates were incubated at 25 ^{O} C. until the pathogen growth reach the rear edge of the plate.

The antagonistic potency of the candidates were estimated as the percentage of the growth inhibition area of the pathogen, calculated according to the following new developed equation.

$AP = [(6.08 – 1.5 D_1) * (D – D_1)] / 3.14 * D^2$

since :

AP = antagonistic potency

D = diameter of the pathogen growth on the rear site of the fungus disk.

D1 = diameter of the pathogen growth on the nearby site of the fungus disk which faces the bioagent candidate.

$(6.08 – 1.5D_1)$ = equation of the common chord resulted from the interference between the growth of the pathogen and the area of antagonistic effectThis equation was generated using Main Trend sub program, Excel computer program, Office Microsoft.

p.s. This equation was examined and proved to be adequate when the pathogen and bioagent candidate were placed at the aforementioned distance only. Other wise it must be modified before use.

The candidate bioagents, which proved their antagonistic potency were identified in plant pathology research institute according to Rifai, 1969; Bissett, 1991 and Domsch 1980 in case of fungi, while the bacterial and actinomyces isolates were identified according to Bergey's Manual of Determinative Microbiology,1954 (Holt ,1984).

III- Assessment of biological control potency of the different bioagent candidates under field condition:

For assessment of biological control potency of the different bioagent candidates under field conditions, two field experiments were performed through two successive years (2000 and 2001) in El-Gemmiza Experimental Station, El-Gharbia governorate; on two cucurbits crops, cucumber (vr.Beta Alfa) and squash (vr. Alexandrani).

a- Preparation of the bioagent inocula :

Fungal bioagent candidates were grown on PDA medium for 7 days at 28°C. Fungal spores were harvested by adding 10 ml of sterilized distilled water to each plate and scraped gently. The suspensions were filtered through sterilized filter paper (Wattman No. 1). The number of fungal spores in the aqueous suspension was adjusted to approximately 25 x 10^8 spore/1 ml by heamocytometer.

Bacterial isolates were grown on nutrient glucose broth (NGB), while *Streptomyces* sp. was grown on starch nitrate broth (SNB) described by Waksman, (1962). The cultures were incubated for 3 days at 30°C. in a shaking incubator, then the cultures were centrifuged at 5000 rpm for 10 min. Precipitate mass from each culture was resuspended into 30 ml sterilized distilled water. the bioagent suspensions were incorporated into talc powder (50 ml suspension/100 gm powder) and 2 ml of 0.1% carboxyl methyl

cellulose and mixed thoroughly, then were air dried in laminar flow hood for over night. The dried formulations were stored in sealed polyethylene bags.

b- Assessment of the efficiency of bioagent candidates in controlling the soil borne diseases:

A formulation slurry was prepared by adding 4 g. of the bioagent formulation in 25 ml of sterilized distilled water and mixed thoroughly. Seeds of cucumber and squash were mixed with the bioagents slurry at the rate of 40 g/kg. The treated seeds were dried in laminar flow hood over night. The treated seeds were sown in the field, in plots (3 m x 5 m.) in three replicates for each treatment. At the same time untreated seeds were sown in equal number of plots , after sowing, the formulation of the bioagents were suspended in water separately in a ratio of 400 g./10 L of water and then were applied to soil surface as soil drench. Seeds treated with Monceren at the rate 3 g./kg. were sown in three plots to serve as check. Pre-, Post-emergence damping off and healthy survived plants were calculated according to Shatla *et.al.* ,1983.

c- Assessment of the efficiency of bioagent candidates in controlling the airborne diseases:

For the assessment of the efficiency of the bioagent candidates for controlling the airborne diseases, the bioagent candidates were tested for their effectiveness against powdery mildew of squash and downy mildew of cucumber by using the bioagents formulation at the rate of 400 g./10 L of water as foliar spraying three times after the infection in 15 days intervals. Ridomil plus was used at the rate of 15 g./10L water to serve as check for downy mildew disease and micronized sulphur was used at the rate of 25 g./ 10L. water in the case of powdery mildew.

The disease severity was estimated as follow:

Degrees of infection with downy- or powdery-mildew diseases were estimated according to the flowing scales:

Numerical value (Infection category)	Infection
0	No infection spots on the leaf
1	Very small infection spots occupy less than 1/10 of the leaf area.
2	Infection spots cover > 1/4 of the leaf area.
3	Infection spots cover > 1/4 and < 1/2 of the leaf area.
4	Infection spots cover > 1/2 of the leaf area, or the leaf is destroyed .

Disease severity was calculated using the equation developed by Towsend and Heuberger, (1943).

$$P = \frac{\Sigma(n \times v)}{4N} \times 100$$

where :

P = disease degree

n = number of leaves within infection category.

v = numerical value of each category.

N = total number of leaves.

The averages were compared at 0.05 and 0.01 levels using the least significant difference (LSD) after transforming percentages into arc sine.

RESULTS AND DISCUSSION

I- Prescreening of the isolated organisms for antagonistic potency in *vitro*:

From a huge number of the isolates, isolated and prescreened for their antagonistic potency, only four isolates proved to be highly antagonistic to *Rhizoctonia solani*, *Fusarium* sp. and *Pythium* sp. These isolates were, identified and were found to be, two fungal isolates (*Trichoderma hamatum* and *Gliocladium virens*), two bacterial isolates (*Bacillus subtilis* and *Pseudomonas fluorescens*) and an isolate of *Streptomyces* sp. These isolates were subjected to a further antagonistic test.

II- Estimation of antagonistic potency in *vitro*:

The antagonistic effect of biocontrol agents against cucumber and squash damping-off pathogens was determined (Table 1). Data revealed that *Trichoderma hamatum* and *Gliocladium virens* were the most antagonistic agents with no significant difference. They resulted in 24.8 and 21.4 % reduction in the growth of *Rhizoctonia solani*, and 27.2 and 22.5% In the growth of *Pythium* sp. No significant difference was found among *Trichoderma hamatum*, *Gliocladium virens* and *Bacillus subtilis* in case of *Fusarium* sp.

Pseudomonas fluorescens was the least effective one. Streptomyces sp. Showed moderate effect on *Rhizoctonia solani* and *Fusarium* sp. but lower effect on *Pythium* sp.

Table 1: Antagonistic potency (AP) of the different bioagents candidates against *Rhizoctonia solani*, *Fusarium* sp. and *Pythium* sp. in *vitro*.

Pathogen	*R. solani*		*Fusarium* sp.		*Pythium* sp.	
Bioagents	D_1* in cm.	AP * %	D_1* in cm.	AP * %	D_1* in cm.	AP * %
T. hamatum	1.30	24.8	1.25	26	1.30	27.2
G. virens	1.45	21.4	1.53	20.3	1.40	22.5
B. subtilis	1.60	18.2	1.35	23.6	2.50	4.0
Ps. Fluorescens	2.30	6.0	2.2	7.0	2.10	8.0
Streptomyces sp.	1.75	16.2	1.85	14.3	2.40	5.0
L.S.D. at 0.05	0.27		0.32		0.35	

D_1 = Mean of nearby growth diameter D_1 (cm)
AP = Antagonistic potency

II- Assessment of the isolated bioagents candidates for their potency in controlling soil borne diseases of cucurbits under field conditions:

The biological potency of the 5 bioagent candidates in controlling soil born diseases of cucumber and squash was estimated compared with Monceren fungicide, Date are demonstrated in Table (2).

It is obvious that, either in the case of cucumber or squash all the treatments resulted in significant reduction in pre- and post emergence damping off. the fungicide Monceren was the most effective treatment, it

resulted in the highest percentage of healthy survived plants. In most cases, *T. hamatum, G. virens* and *B. subtilis* proved to be the most effective tested bioagents. Results showed that seed coating with biocontrol agents or fungicide was more effective than soil drench.

Table 2: Biological potency of the bioagent candidates in controlling soil borne diseases of cucumber and squash represented as the percentage of pre-, post emergence damping off and survived plants at the year 2000 .

Treatment	Cucumber						Squash					
	Seed coating			Soil drench			Seed coating			Soil drench		
	%Pre.	%post-	%surv.	%Pre.	%post-	%Surv.	%Pre.	%post-	%Surv.	%Pre.	%post-	%Surv.
T. hamatum	29.46	9.82	60.72	40.17	8.03	51.80	28.57	7.14	64.29	40.87	10.71	49.12
G. virens	31.25	8.03	60.72	41.07	8.03	50.90	33.92	7.14	58.94	30.39	11.07	58.54
B. subtilis	25.89	11.60	62.51	42.85	9.82	47.33	37.50	10.71	51.79	48.21	8.92	42.17
P. fluorescens	35.71	9.82	54.47	49.10	8.03	42.87	49.15	9.82	41.08	57.14	5.35	37.51
Streptomyces sp.	32.14	9.82	58.04	33.03	9.82	57.15	46.42	8.92	44.66	54.46	5.35	40.19
Monceren	19.64	5.35	75.01	32.14	8.92	58.94	13.39	9.82	76.79	25.00	2.67	72.33
Control	57.14	10.71	32.15	55.60	7.34	37.06	58.92	7.14	33.94	58.92	7.14	33.94
L.S.D. 5%	0.158	0.26	0.356	0.131	0.233	0.244	0.83	0.74	0.859	0.635	0.327	0.69

This date were confirmed when the same experiment was repeated at the next year (Table 3). Similar data were obtained, all the treatments resulted in significant retained reduction in the number of the damped off plants. The fungicides its high efficacy and resulted in the highest number of survived plants, however limited difference among the different treatments was noticed. Seed coating proved to be superior to soil drench treatment.

Table 3: Biological potency of the bioagent candidates in controlling soil borne diseases of cucumber and squash represented as the percentage of pre-, post emergence damping off and survived plants at the year 2001 .

Treatment	Cucumber						Squash					
	Seed coating			Soil drench			Seed coating			Soil drench		
	% Pre.	%post-	%surv.	% Pre.	%post-	%Surv.	% Pre.	%post-	%Surv.	% Pre.	%post-	%Surv.
T. hamatum	25.89	10.71	63.40	36.60	8.03	55.57	26.78	7.14	66.08	35.71	13.39	50.9
G. virens	27.87	7.14	64.99	38.39	7.14	54.47	31.25	6.25	62.5	35.71	10.71	53.58
B. subtilis	25.00	8.03	66.97	39.28	9.82	50.90	33.92	10.71	55.57	43.75	9.82	46.43
P. fluorescens	32.14	9.82	58.04	46.42	8.03	45.55	44.64	8.92	46.24	53.47	6.25	40.28
Streptomyces sp.	28.57	8.03	63.40	30.35	8.92	60.73	43.75	8.03	48.22	50.89	6.25	42.86
Monceren	22.32	6.25	71.43	31.25	5.35	63.40	15.17	11.60	73.23	21.42	17.85	60.73
Control	53.57	11.60	34.83	53.57	11.60	34.83	57.14	5.35	37.51	57.14	5.35	37.51
L.S.D. 5%	0.368	0.183	0.216	0.173	0.253	0.298	1.032	0.476	1.069	0.641	0.63	0.949

III- Side effect of the isolated bioagents on root system and foliage.

The effects of seed and soil treatments with the bioagent candidates on root and shoot lengths (cm) of cucumber and squash plants was measured after 30 days of sowing (Table 4).

Table (4):Effect of seed and soil treatments with the bio agent candidates on root and shoot length (cm) of cucumber and squash plants after 30 days of sowing under field conditions at two successive years.

Bioagents	2000				2001			
	Cucumber		Squash		Cucumber		Squash	
	Root length (c m)	Shoot length (cm)	Root length (c m)	Shoot length (cm)	Root length (c m)	Shoot length (cm)	Root length (c m)	Shoot length (cm)
T. hamatum	3.75	10.50	9.25	24.00	4.50	11.25	10.25	25.00
G. virens	4.00	10.00	7.25	27.75	4.00	10.50	8.25	28.75
B. subtilis	3.50	8.50	7.75	29.50	3.50	9.50	8.25	28.75
Ps. Fluorescens	3.75	9.75	5.75	26.00	4.25	10.75	7.00	27.00
Str. Sp.	3.00	7.50	5.75	24.75	3.75	8.25	6.50	25.75
Monceren	3.75	10.00	7.25	24.25	4.75	10.75	8.00	25.25
Control	2.50	7.00	4.5	22.25	3.25	8.0	5.25	23.00
L. S. D.5%	1.4	2.7	3.8	4.8	1.75	3.8	1.6	4.6

Data in Table (4) showed that, no significant differences was found in cucumber root length except at the year 2001 when the cucumber seeds were treated with *Gliocladium virens*.

In case of squash, clear difference can be noticed. *T. hamatum* was the most effective treatment. When squash seed were coated with *Trichoderma hamatum* , the root length reached 9.25 compared with 4.5 in the control treatment at the year 2000 and 10.25 compared 5.25cm. in the control treatment at the year 2001.

Concerning the side effect on the foliage of cucumber, little significant difference was noticed at the year 2000, however no significant difference was found at 2001. In case of squash, similar data was found. No significant difference was found except in the case of *Gliocladium virens*, since significant increase in shot length was recorded. At the year 2001, *Gliocladium virens* and *Bacillus subtilis* showed significant increase in squash root length. No treatment showed bad effect on either root or the foliage of both crops through the two successive year.

IV- Estimation of the isolated bioagent candidates for their biocontrol potency against airborne diseases of cucurbits :

a- Downy mildew on cucumber

Results in Table (5) imply that all treatments were effective in reducing disease severity through two successive years except *Streptomyces* sp which at the first year failed to show satisfactory control of the disease. Ridomil plus fungicide was the most effective treatment. It resulted in 54.76 and 66.61% at the year 2000 and 2001 respectively, compared with 45.23 and 59.62 % resulted by *T. hamatum* at the two successive years. This disease reduction was reflected on the yield. All the treatments resulted in significant increase in the yield during the two years. This increase in the yield ranged from 4 ton/ feddan, produced by the untreated plots , and 7.66 ton/feddan produced in plots treated with *T. hamatum* at the year 2000 and 7.00 ton/feddan in plots treated with Ridomil plus.

Table (5): Assessment of biological potency of the bioagents candidates in controlling downy mildew disease on cucumber and the yield compared with fungicides.

Bioagents	2000			2001		
	% disease severity	Biocontrol efficiency %	Yield/Feddan (ton)	% disease severity	Biocontrol efficiency %	Yield/Feddan (ton)
T. hamatum	11.50	45.23	7.66	10.28	59.62	6.82
G. virens	12.50	40.47	6.50	11.50	54.83	6.66
B. subtilis	13.16	37.33	6.30	12.30	51.68	6.56
Ps. Fluorescens	16.00	23.8	6.25	15.26	40.06	6.00
Streptomyces sp.	21.50	0.00	6.00	19.36	23.95	5.75
Ridomil plus	9.50	54.76	6.75	8.50	66.61	7.00
Control	21.00	-	4.00	25.46	-	4.00
L.S.D. at 0.05	2.44	-	0.35	4.51	-	0.365

b- Powdery mildew incidence and yield of squash:

The biological potency of the candidate bioagents was assessed under field conditions in controlling powdery mildew disease on squash and the yield compared with fungicides. The obtained data are presented in Table (6).The data in Table (6) indicate that, at the year 2000 all treatments except *P. fluorescens* and *Streptomyces* sp. resulted in significant reduction in the disease severity. Micronized sulphur gave the most effective treatment; followed by T. *hamatum* then *G. virens* and *G. virens.* This reduction in disease severity was combined with increase in the yield. The yield increased from 7.250 ton/feddan resulted from the untreated plots (control treatment) to 10.68, 10.5, 10.32 and 10.26 in plots treated with Micronized-sulphur, *T. hamatum, G. virens,* and *B. subtilis* respectively. At the year 2001 similar data were obtained, except that, *P. fluorescens* in that year resulted in significant reduction in disease severity. All the treatments resulted in significant increase in the yield over the none treated plots. Plots treated with Micronized-sulphur yielded 11.0 ton/ feddan compared with 7.5 ton from untreated plots. The yield of the other treatment ranged from 10.83 to 9.83 ton / feddan.

Table (6): Assessment of biological potency of the bioagents candidates in controlling powdery mildew disease on squash und the yield compared with fungicides.

Treatment	2000			2001		
	Disease severity%	Treatment efficiency %	Yield/Feddan (ton)	Disease severity%	Treatment efficiency %	Yield/feddan (ton)
T. hamatum	12.50	47.91	10.50	11.46	48.33	10.83
G. virens	14.00	41.66	10.32	13.43	39.44	10.45
B. subtilis	14.50	39.58	10.26	15.33	30.88	10.36
P. fluorescens	18.30	23.75	9.80	17.40	21.55	10.00
Streptomyces sp.	19.00	20.08	9.72	18.36	17.22	9.83
Micronized- sulphur	11.00	54.16	10.68	10.43	52.97	11.00
Control	24.00	-	7.250	22.18	-	7.500
L.S.D. at 0.05	5.80	-	0.307	4.10		0.659

DISCUSSION

Saving the environment has become an obligate demand, therefore minimizing the pesticides application is an international incumbency. For this target substitution of the pesticides with other environment safely procedures is a pressing goal.

Biological control of plant diseases can be a useful element to minimize the use of fungicides. Sinc biological control depend upon the multiplication and introduction of an organism in the ecosystem..A great attention must be done to get positive results and to hinder any disruption of the ecosystem. Finding bioagents originated from the Egyptian environment is very important to save the Egyptian ecosystem and to insure the efficiency and the continual of the control process. In this work, from a huge number of isolates, isolated from the Egyptian soil, 5 bioagents were obtained .These bioagents were identified as *Trichoderma hamatum* , *Gliocladium virens*, *Bacillus subtilis, Pseudomonas fluorescens* and *Streptomyces* sp. These isolates Proved to be antagonistic to *Rhizoctonia solani*, *Pythium* sp. and *Fusarium* sp. in *vitro*. Under field conditions, good control of soil borne diseases of cucurbits was achieved as a result of these bioagents. Earlier, Zhang, *et.al.*; (1996) controlled successfully Fusarium colonization of cotton roots and Fusarium wilt by seed treatments with *Gliocladium virens* and *Bacillus subtilis.* At the same time Lewis *et.al.*. (1996) controlled damping-off diseases caused by *Rhizoctonia solani* and *Pythium ultimum* with alginate pills of *Gliocladium virens*, *Trichoderma hamatum*.El-Mersawy (2000) Controlled downy mildew in maize caused by *Peronosclespora sorghi* with *Bacillus subtilus* .

Since there was no standard method to asses the bioagent antagonistic potency in *vitro*, specially in the case of non-spore former fungi;. it was important to develop such method. The developed method based on the calculation of the relative inhibition area, resulted from the interference between the growth area of the pathogen and the antagonistic area of the bioagents, referred to the whole pathogen growth area. This method is adequate to non-spore former as well as spore former fungi., and can be a good tool to calculate the antagonistic efficacy of such bioagents.

Applying the bioagent as seed coat resulted in higher reduction of pre- and post-emergence damping off compared with applying them as soil drench. That may due to that when the bioagents were used as seed coats, the bioagent concentrated around the seeds forming protective parriers around them. On the long run, with the repeated application of the bioagent, the bioagent will establish in the soil, and in such case the two application methods may gave the same results.

However, the fungicides proved to be more effective in controlling the soil- and air-borne diseases of cucurbits, but risk assessment may accuse the use of the bioagents. Further studies have to be done on the compatibility between the bioagents and the fungicides which may lead to found out a combination between a low dose of fungicide compatible with the bioagent and can result in increase the efficiency of both of them.

REFERENCES

Baker, R. (1987). Molecular biology in biological control of soil borne pathogens. Pages. 115-124 in C.J. Arntzen and C. Ryan, eds., Molecular strategies for crop protection. Alan. R. Liss, NY.

Beye, F. (1978). Insecticides from the vegetable kingdom pL. Res. Dev., 7: 13-31.

Bissett, J. (1991). A revision of the genus *Trichoderma*. Infragenetic classification. Can. J. Bot., 69: 2357-2372.

Domsch, K.H.; W. Gams and T.H. Anderson (1980). Compendium of soil fungi. Academic Press, London, 859 pp.

Dubey, P.S. and L.P. Mali (1972). Herbicidal pollutive, pollen damage by herbicide vapours. Sc. Cult., 39: 556-558.

El- Kafrawy,A. A. (2002). Biological control of bean damping off caused by *Rhizoctonia solani* .Egypt. J. Agric. Res. ,80 (1) :57-70.

El-Mersawy,E. M. (2000) . Biological control of maize downy mildew disease caused by *Peronosclespora sorghi* in Egypt.J. Agric .Sci. Mansoura Univ., 25(11): 6787-6794.

Epstein, S.S.; J. Andreae; H. Jaffec; S. Joshu; H. Folk and N. Mantel (1967). Carcinogenicity of the herbicide meleic hydrazide. Nature, 215: 1388-1390.

Fawcett, C.H. and D.M. Spencer (1970). Plant chemotherapy with natural products. A. Rev. Phytopath., 8: 403-418.

Holt,G. G. (1984).Bergey,s Manual of Determinative Microbiology , 1954 Eight edition .The Williels and Wilkins Company / Baltimore.

Javoraska, T. (1978). Effect of combined herbicideds on the occurrence of morphoses in the spikes of spring barley . Agrochemia,!8 :37-42.

Lockwood, J.L. (1988). Evolution of concepts associated with soil borne plant pathogens. Ann. Rev. Phytopathol. 26: 93-121.

Lewis, J.A.; R.D. Lumsden, and J.C.Locke (1996). Biocontrol of damping-off diseases caused by Rhizoctonia solani and Pythium ultimum with alginate prills of Gliocladium virens, Trichoderma hamatum and various food bases. SO - Biocontrol-sci-technol.Abingdon, Oxfordshire : Carfax Publishing Co., 6:163-173.

Maximlian, K.; E. Muhle; E. Renmuth and H. Bochow (1976). Phytopathologie and phlanzenschutz. Akademic. Verlag.Berlin,915 pp.

Papavizas, G.C. (1985). *Trichoderma* and *Gliocladium*; Biology, Ecology and Potential for biocontrol. Ann. Rev. Phytopathology, 23: 23-54.

Rifai, M.A. (1969). A revision of the genus *Trichoderma* Mycol. Papers. CMI Association of Applied. Biologists: Kew Surrey, UK, 116: 1-56.

Shatla, M. N.; Z. El-Shennawy; A. M. Besiony; S. El-Khateeb and E.Z. Khalifa (1983)Interaction between *Rhizoctonia solani* and*Rhizobium Japonicum* on soybean. Menofiya J. Agric. Res., 6-48-59

Towsend, G. K. and T. W. Heuberger, (1943). Methods for estimating losses caused by diseasess experiments. Plant Dis. Rept.27:340-343.

Waksman, S.A. (1962). The actinomycetes. III-Antibiotics of Actinomycetes. The Williams and Wilkins Comp. USA.

Wilson, Ch; J.D. Fanklin and B.E. Otto (1987). Fruit volatiles inhibitory to *Monilinia frueticola* and *Botrytis cinerea*. Plant Dis., 71(4): 316-319.

\Wollum A. G. (1982). Cultural methods for soil microorganisms. Pages 751-802 in : Methods of soil analysis, Part 2, Chemical and Microbiological Processes. American Society of Agronomy and Soil Science. Madison, WI.

Zhang, J.; C.R Howell,and J.L. Starr(1996).Suppression of Fusarium colonization of cotton roots and Fusarium wilt by seed treatments with *Gliocladium virens* and *Bacillus subtilis*. Biocontrol-sci-technol. Abingdon, Oxfordshire : Carfax Publishing Co,. 6 : 175-187.

عزل عوامل المكافحة الحيوية و تقدير كفأتها لمكافحة الفطريات الأرضيـــة و الهوائيـــة التى تصيب القرعيات.

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للحفاظ على البيئة المصرية من التلوث بالمبيدات تم عزل ٥ عـــزلات مـــن الكائنـــات الدقيقـــة لإستخدامها فى المكافحة الحيوية . تمتعريف هذه العزلات وكانت عزلتان من الفطـــر هـــا الترايكودرمـــا هاماتوم ؛ الجليوكالديوم فيرنز وعزلتين من البكتريا هما باسيلوس سائلوس ؛ سيدوموناس فلوريسانس وعزله ستربتومايسن واحدة . أثبتت هذه العزلات قدرتها على تضاد فطريات الريزوكتونيـــا ســـولانى ، البثيـــوم ، الفيوزاريوم فى المعمل عند إستخدام هذه العزلات فى الحقل أدت إلى مكافحة أمراض عفن الجذور ومـــوت البادرات فى الخيار والكوسة وكان لها تأثير جيد على المجموع الجذرى والخضرى ، كما يلاحظ أن أستخدام هذه الكائنات لتغطيه البذور أعطى كفاءة أعلى فى مكافحة المرض عنه عن إستخدامه رشا على التربة . تـــم إستنباط طريقة جديدة لحساب كفاءة وقدرة التضاد للكائنات فى المعمل تعتمد على حســـاب مســـاحة منطقـــة التثبيط منسوبة إلى المساحة الكلية لنمو الفطر الممرض . تم الحصول على نتائج جيدة عندما أستخدمت هـــذه الكائنات رشا على نباتات الكوسة والخيار لمكافحة مرض البياض الدقيقى والزغبى على الـــترتيب ونلاحـــظ زيادة معنويه فى المحصول ، وفى كل الحالات أعطت المبيدات كفاءة أعلى من الكائنات المضادة .