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USE OF CULTURAL FILTRATES OF CERTAIN MICROBIAL ISOLATES FOR POWDERY MILDEW CONTROL IN SQUASH

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Abstract: Powdery mildew induces significant losses in yield and quality of squash. Therefore, culture filtrates of certain microbial isolates, (*Epicoccum nigrum, Epicoccum minitans, Epicoccum* sp., *Trichoderma harzianum, Trichoderma viride* and *Bacillus pumilus*) were used alone, and in combination with the fungicide penconazole to control powdery mildew in squash, under field conditions. Moreover, GC-MS analysis was carried out to identify the chemical components of the most effective culture filtrates against powdery mildew pathogen. The results showed that culture filtrates of different microbial isolates (except for *Trichoderma harzianum*) were more effective against powdery mildew in squash than the tested fungicide alone at the recommended levels, in both tested seasons. The results also showed that mixing different culture filtrates with penconazole improved efficiency against powdery mildew compared to using the fungicide alone, in both tested seasons. The efficacy of the culture filtrates of the tested microbial isolates against powdery mildew were due to the presence of a mixture of known antifungal compounds. The results suggest the possible use of the culture filtrates of the tested microbial isolates as alternative to fungicides, in powdery mildew control. Also, this study suggests the possible mixing of the culture filtrate of the tested biocontrol agents with fungicides to minimize the applied amount of fungicides.

Key words: powdery mildew, bio-control agents, fungicide, disease

INTRODUCTION

Cucurbits play a significant role in human nutrition. Cucurbit crops constitute a major portion of all vegetables and are grown in different regions in Egypt. Squash is a promising export crop which can be readily produced at a low cost during the winter season of Egypt.

Cucurbit powdery mildew, caused by to *Podosphaera* (sect. *Sphaerotheca*) xanthii (Castag.) Pollacci, is a serious disease on cucurbits grown worldwide. Powdery mildew occurs on leaves, stems and fruits. Major epidemics reduce crop yields by causing decreased fruit set, inadequate ripening, fruit cracking and deformation as well as reducing post-harvest storage time.

Control methods currently available under commercial conditions include the use of repeated applications of elemental sulphur (Kimati *et al*. 1980) and other fungicides. The constant use of fungicides, however, can result in environmental contamination and selection of resistant populations of *P. (sect. Sphaerotheca*) *xanthii* (Castag.) (McGrath 1996; McGrath *et al*. 1996). Beneficial microorganisms and insects are also negatively affected by some fungicides used against powdery mildew of cucurbits. Fungicides that contain high doses of sulphur are particularly harmful to beneficial microorganisms and insects (Calvert and Huffaker 1974).

These factors emphasize the need for new methods to control the diseases (Wilson *et al*. 1987) *i.e.* the usage of natural products or biocontrol agents (BCAs), culture filtrates of biocontrol agents, salts, plant extracts, and mineral oils alone or in combination (Horst *et al*. 1992; Falk *et al*. 1995; Belanger and Benyagoub 1997; Daayf *et al*. 1997; El-Kot and Hegazi 2008; Hegazi and El-Kot 2008; Pertot *et al*. 2007).

Also, the reduction of application rates is one of the dominant trends in the horticultural industry. This can be achieved in different ways, by the introduction of new chemicals that are applied at much lower rates, or by the purification of the chemicals, or by the combination of the formulation with an adjuvant or with plant extracts or culture filtrates of biocontrol agents. The adjuvants, plant extracts and culture filtrates of biocontrol agents can be incorporated into the formulation or added to the tank as a tank-mix product. The use of adjuvants, culture filtrates of biocontrol agents and plant extracts have gained more and more acceptance. This is mainly because the cost of the development of new active ingredients is still much higher than the cost of the development of new adjuvants. Bentonit is one of the adjuvants which has been used in combination with fungicides against plant pathogenic fungi. It has been used either alone, or in combination with the fungicides, Fundazol and Bayleton, against *Thielaviopsis basicola* (Berk. and Br.) (Bade 1995). Also El-Naggar (1996) used bentonit either alone, or in combina-

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tion with the fungicide Sumilex against *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* (Lib.) de Bary.

Recently, in some studies, volatile compounds produced by antagonistic fungi and bacteria have been shown to have potential antifungal activities (Alstrom 2001; Wheatley 2002; Fernando *et al*. 2005; Kai *et al*. 2006; Zou *et al*. 2007). Biological control of plant diseases by antifungal volatiles from fungal strains have also been carried out under the greenhouse conditions (Mercier and Manker 2005; Koitabashi 2005). Moreover, the use of culture filtrates of bio-agents mixed with fungicides to control some plant pathogens, have been reported (Fan *et al*. 2001; Yoshida *et al*. 2001).

Therefore, this study attempted to evaluate the efficiency of certain bacterial and fungal culture filtrates for controlling powdery mildew. The ability to minimize the recommended dose of the tested fungicide (penconzale) by mixing it with certain biocontrol agents filtrates was also investigated. Finally, identification of the biologically active components in the most effective culture filtrates against powdery mildew in squash was made.

MATERIALS AND METHODS

The tested microbial isolates

The microbial isolates which were used in this study were: *Epicoccum nigrum, E. minitans, Epicoccum* sp., *Trichoderma viride, Trichoderma harzianum* and *Bacillus pumilus*. Some of these microbial isolates *(E. nigrum, E. minitans* and *T. viride*) were kindly given to us from the Plant Pathology Dept. Agric. Research Institute, Giza, Egypt. While the other bio-agents (*Epicoccum* sp., *T. harzianum* and *B. pumilus*) were previously isolated and identified by El-Kot and Hegazi (2008). The fungal isolates which we used (*E. nigrum, E. minitans, Epicoccum* sp., *T. viride* and *T. harzianum*) were cultured on potato dextrose broth (PDB) for 15 days at 20–25°C. Then, the fungal and bacterial biomass was centrifuged at 10.000 rpm for 20 min, and the culture medium was discarded. Next, the supernatant was filtered by passing the culture broth through a sterile membrane filter (0.2 μm) (El-Boghdady 1993). *B. pumilus* was cultured on Kings-B medium in 250-ml Erlenmeyer flasks on a rotary shaker at 150 rpm at 28–30°C. After 24 h, bacterial cell suspension was pelleted by centrifugation at 7.000 rpm for 10 min. Then the supernatant was filtered using a glass filter to obtain cell-free culture filtrate (El-Boghdady 1993).

The tested fungicide

The tested fungicide used in this study was penconazole; trade name Topas EC 100%. This fungicide is used at a field rate of 5 ml/20l and produced by Syngenta Crop Protection Pty Limited. Penconazole has been highly recommended for control of powdery mildew in vegetable crops in Egypt.

Control of the powdery mildew disease on squash crop in the open field

We carried out our study at the experimental farm, run by the, Faculty of Agriculture, Kafr El-Sheikh University. The randomized complete block design with three replicates was used. Seeds of squash were sown in rows which were 1 m wide and 50 cm long. Plants were sprayed using 25 treatments (penconazole fungicide and/or culture filtrates of different microbial isolates) as shown in table 1, every ten days. Cultural practices, irrigation plus fertilization, and chemical control of pests were carried out as recommended in the program for the production improvement of cucurbits. A disease assessment was carried out, as mentioned below. The different treatments that were used to control powdery mildew were applied 6 h before artificial inoculation. Inoculation was carried out by shaking powdery mildew-infected leaves over the plants until the leaf surfaces were fully covered with powdery mildew spores. The mixing of the tested fungicide and culture filtrates of the tested bio-agents, was carried out according to the needed concentrations (75, 50 and 25% of fungicide or culture filtrate of each microbial isolate) in the total sprayed volume. The control treatment was carried out using water.

Table 1. List of different treatments used to control powdery mildew in squash crop

No.	Treatments
1	sprayed with water
2	sprayed using the recommended dose of fungicide penconazole
3	sprayed using 100% culture filtrates of *E. nigrum*
4	75% culture filtrates of *E. nigrum* + 25% of penconazole
5	50% culture filtrates of *E. nigrum* + 50% of penconazole
6	25% culture filtrates of *E. nigrum* + 75% of penconazole
7	100% culture filtrates of *E. minitans*
8	75% culture filtrates of *E. minitans* + 25% of penconazole
9	50% culture filtrates of *E. minitans* + 50% of penconazole
10	25% culture filtrates of *E. minitans* + 75% of penconazole
11	100% culture filtrates of *Epicoccum* sp.
12	75% culture filtrates of *Epicoccum* sp. + 25 % of penconazole
13	50% (vol.|vol.) sterilized water, culture filtrates of *Epicoccum* sp. + 50% of fungicide penconazole
14	25% culture filtrates of *Epicoccum* sp. + 75% of penconazole
15	100% culture filtrates of *T. harzianum*
16	75% culture filtrates of *T. harzianum* + 25% of penconazole
17	50% culture filtrates of *T. harzianum* + 50% of penconazole
18	25% culture filtrates of *T. harzianum* + 75% of penconazole
19	100% culture filtrates of *T. viride*
20	75% culture filtrates of *T. viride* + 25% of penconazole
20	50% culture filtrates of *T. viride* + 50%of penconazole
21	25% culture filtrates of *T. viride* + 75% of penconazole
22	100% culture filtrates of *B. pumilus*
23	75% culture filtrates of *B. pumilus* + 25% of penconazole
24	50% culture filtrates of *B. pumilus* + 50% of penconazole
25	25% culture filtrates of *B. pumilus* + 75% of penconazole

Disease severity

Disease severity was assessed after 10 days of the first inoculation (initial disease severity) and three times later with ten days intervals. In other words, the last assessement took place 40 days after the first application (the final disease severity). According to the method described by McGrath *et al*. (1996), the powdery mildew disease severity was estimated by counting visible sporulating mildew colonies on both adaxial and abaxial surfaces per leaf. Five old leaves per plant were examined for five plants in each plot (*i.e.* 25 leaves/treatment). Assessments were also made on fully expanded leaves from the middle and upper thirds of a plant. Data from all three age-classes of leaves were averaged together. The initial disease severity varied widely under field conditions, due to the presence of powdery mildew colonies on squash leaves before treatment. Thus, it was worth calculating the disease inhibition (DI %) to evaluate the real comparable efficacy of treatments as follows:

$$CDI\ \% = A - B/A \times 100$$

where:
CDI – corrected disease inhibition
A – the corrected final disease severity of treatment
B – the final disease severity of treatment

Therefore, the corrected final disease severity (A) was calculated as the product of corrected disease severity as shown in equation 2 according the method described by Kamel (2003)

$$\text{Corrected disease severity} = L/M \times N \qquad (2)$$

where:
L – the initial disease severity of a treatment
M – the initial disease severity of the check (control)
N – the final disease severity of the check (the control)

Statistical analysis

Data were analyzed statistically by the analysis of variance test and the different means were compared by Duncan's multiple range test.

Microscopic examination

The infected squash plant leaves that were treated with the most effective culture filtrate (100% culture filtrate *of E. minitans*) were selected to investigate the growth status of powdery mildew fungus. After 24 h of treatment, the leaves were immersed in dimethyl formamide until there was complete removal of chlorophyll pigment and the leaf tissue became clear. The fungal tissue was stained using Lactophenol. Then, the status of powdery mildew fungus on treated squash leaves relative to the control was investigated.

Chemical composition of bacterial and fungal culture filtrates

The most effective fungal *(E. minitans*) and bacterial (*B. pumilus*) culture filtrates were selected for analysis to identify the active components in these culture filtrates. GC/MS analysis was conducted on a HP 6,890 GC system coupled with a 5,973 network mass selective detector with a capillary column of HP-5MS (60 m x 0.25 mm, film thickness 0.25 m), according the method described by Mahboubi and Haghi (2008). The oven temperature program was started at 40°C, held for 1 min then turned up to 230°C at a rate of 3°C/min and held for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min, with a split ratio equal to 1/50. The detector and injector temperatures were 250 and 230°C, respectively. The oil compounds were identified by comparing their Retention Indices (RI), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library, and NIST (National Institute of Standards and Technology**)**. The samples were analyzed in the Agriculture Research Centre, Egypt.

RESULTS

Effect of penconazole and bacterial culture filtrate alone, and as a combination, on powdery mildew of squash under field conditions during the tested seasons

The results in tables 2 and 3 showed that fungicide and the bacterial culture filtrate significantly reduced the disease severity of powdery mildew in squash relative to the control treatment either alone, or as a combination, in both tested seasons. The effectiveness of the bacterial culture filtrate mixed with the tested fungicide was higher than the use of the culture filtrate or the tested fungicide used separately, in both tested seasons. When the bacterial culture filtrate and the tested fungicide were mixed, the mixture of 25:75%, respectively induced the highest effectiveness against powdery mildew compared to other mixing percentages, in both tested seasons. The effectiveness of the bacterial culture filtrate and its combination with the tested fungicide was slightly higher in the first season than in the second one.

Effect of penconazole and the tested fungal culture filtrates alone, and in combination, on powdery mildew of squash under field conditions during the tested seasons

The results in tables 2 and 3 showed, that the fungicide and the tested fungal culture filtrates either alone, or as a combination, significantly reduced the disease severity of powdery mildew in squash, relative to the control treatment, in both tested seasons. The results also showed, that use of *E. nigrum* culture filtrate alone, gave higher effectiveness against powdery mildew disease in squash crop than other treatments, in the first season. While the culture filtrate of *E. minitans* was the most effective treatment against powdery mildew disease in squash, in the second season. The effectiveness of culture filtrates of fungal isolates alone, were less effective than the use of fungicide alone, except for the culture filtrate of *T. harzianum*, in both tested seasons. The effectiveness of fungal culture filtrates alone, was improved when the filtrates were mixed with the tested fungicide in both tested seasons, except for the culture filtrates of *E. nigrum* and *E. minitans* isolates. Furthermore, among the tested fungal isolates, the combination of the tested fungal culture filtrates with the tested fungicide was more effective against powdery mildew than the use of fungicide alone, in both tested seasons, except for the culture filtrate of the *T. harzianum* isolate.

Table 2. Effect of penconazole fungicide, and culture filtrates of the antagonistic fungi alone, and in combination with penconazole, on powdery mildew of squash under field conditions during the first season

Treatments	Disease severity [%]		Disease inhibition (corrected) [%]
	after 10 days of inoculation	40 days after start of application	
100% of *Epicoccum* sp.	39.8	10.2	88.17 fg
75% of *Epicoccum* sp.+ 25% of penconazole	46.7	7.0	93.0 8c
50% of *Epicoccum* sp.+ 50% of penconazole	37.5	4.2	94.83 ab
25% of *Epicoccum* sp.+ 75% of penconazole	39.8	4.4	94.90 ab
100% culture filtrates of *E. nigrum* sp.	31.2	4.2	93.79 abc
75% of *E. nigrum* + 25% of penconazole	33.3	7.6	89.74 ef
50% of *E. nigrum* + 50% of penconazole	42.1	8.8	90.35 e
25% of *E. nigrum* + 75% of penconazole	59.8	17.7	86.33 h
100% of *E. minitans*	39.8	4.4	94.90 ab
75% of *E. minitans* + 25% of penconazole	44.4	7.0	92.72 cd
50% of *E. minitans* + 50% of penconazole	49.8	14.5	86.55 gh
25% of *E. minitans* + 75% of penconazole	51.7	20.4	81.78 i
100% of *T. viride*	31.2	9.4	86.10 h
75% *T. viride* + 25% of penconazole	39.8	8.8	89.8 ef
50% *T. viride* + 50% of penconazole	42.1	6.4	92.98 c
25% *T. viride* + 75% of penconazole	49.0	7.0	93.41 bc
100% of *T. harzianum*	31.2	25.4	62.42 m
75% *T. harzianum* + 25% of penconazole	25.4	16.6	69.83 l
50% *T. harzianum* + 50% of penconazole	27.0	13.4	77.01 k
25% *T. harzianum* + 75% of penconazole	33.3	14.5	79.89 j
100% culture filtrates of *B. pumilus*	44.4	13.4	86.07 h
75% *B. pumilus* + 25% of penconazole	42.1	12.6	86.18 h
50% *B. pumilus* + 50% of penconazole	49.0	9.4	91.15 de
25% *B. pumilus* + 75% of penconazole	46.7	4.7	95.35 a
100% R.D. of penconazole	51.7	16.6	86.94 gh
Mida (the control)	42.1	91.2	0.00 n

R.D. – recommended dose

a, b, c, d, e, f, g, h, I, j, k, l, and m indicate the significance and non-significance between means using Duncan multiple range test

Table 3. Effect of penconazole fungicide, and culture filtrates of the antagonistic fungi, alone, and in combination with penconazole, on powdery mildew of squash under field conditions during the second season

Treatments	Disease severity [%]		Disease inhibition (corrected) [%]
	after 10 days of inoculation	40 days after application	
100% of *Epicoccum* sp.	44.4	11.0	86.42 i
75% of *Epicoccum* sp. + 25% of penconazole	39.8	7.0	90.36 def
50% of *Epicoccum* sp. + 50% of penconazole	54.4	8.8	91.13 cde
25% of *Epicoccum* sp. + 75% of penconazole	51.7	7.6	91.94 bcd
100% culture filtrates of *E. nigrum* sp.	51.7	6.4	93.21 ab
75% of *E. nigrum* + 25% of penconazole	44.4	8.2	89.88 efg
50% of *E. nigrum* + 50% of penconazole	35.4	7.6	88.24 gh
25% of *E. nigrum* +75% of penconazole	42.1	12.6	83.60 j
100% of *E. minitans*	37.5	4.7	93.14 ab
75% of *E. minitans* + 25% of penconazole	46.7	6.4	92.48 bc
50% of *E. minitans* + 50% of penconazole	46.7	9.4	88.96 fg
25% of *E. minitans* + 75% of penconazole	49.0	10.2	88.59 fg
100% culture filtrates of *T. viride*	33.3	8.2	86.42 fi
75% of *T. viride* + 25% of penconazole	59.8	14.5	86.70 hi
50% of *T. viride* + 50% of penconazole	39.8	4.0	94.49 a
25% of *T. viride* + 75% of penconazole	37.5	3.7	94.60 a
100% of *T. harzianum*	13.4	8.8	63.99 m
75% of *T. harzianum* + 25% of penconazole	39.8	20.4	71.89 l
50% of *T. harzianum* + 50% of penconazole	18.8	7.0	79.58 k
25% of *T. harzianum* + 75% of penconazole	25.4	8.8	81.02 k
100% of *B. pumilus*	49.0	10.2	88.59 fg
75% of *B. pumilus* + 25% of penconazole	37.5	5.7	91.67 bcd
50% of *B. pumilus* + 50% of penconazole	33.3	5.0	91.78 bcd
25% of *B. pumilus* + 75% of penconazole	44.4	4.4	94.57 a
100% R.D. of penconazole	54.4	13.4	86.49 i
Media (control)	51.7	94.3	0.00 n

R.D. – recommended dose

a, b, c, d, e, f, g, h, I, j, k, l, and m indicate the significance and non-significance between means using Duncan multiple range test

Microscopic examination of the treated squash leaves

The squash leaves in the control treatment (sprayed with water) showed complete fungal growth of powdery mildew fungus, since the fungus hyphae developed full anastomosis and conidiophores in their right form. A series of conidia spores in natural form and size can be seen in figure 1A. However, the squash leaves treated with 100% culture filtrate of *E. minitans* (the most effective culture filtrate) induced morphological abnormalities such as vacuolation, swelling in hyphae and sporangium (Figs. 1B, C). Figure 1D shows hydrolysis in conidia spores. Then, the fungus completely decomposed as there are no conidiophores or conidia spores (the culture filtrate inhibited the formation of conidiophores) as seen in figure 1E.

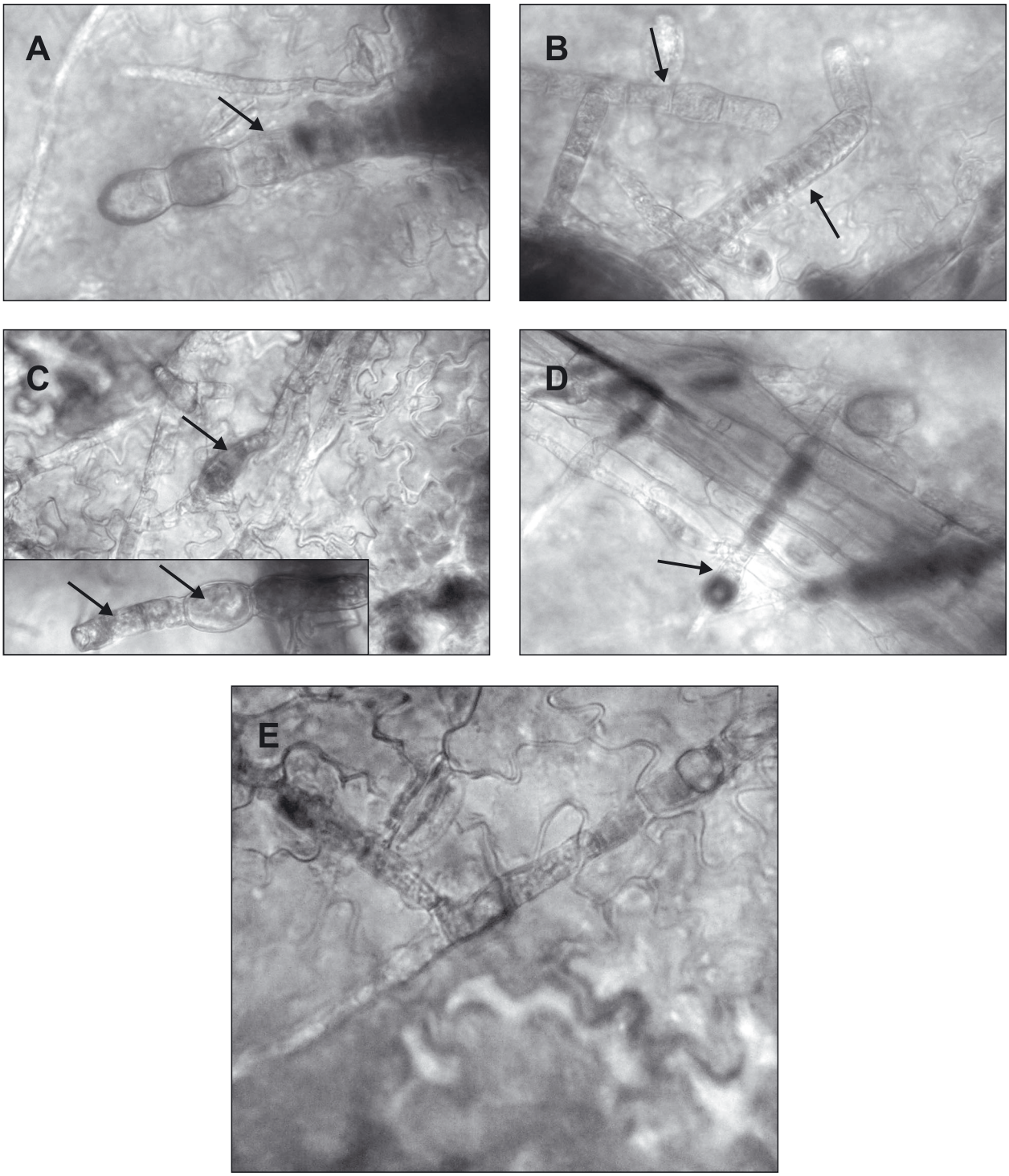

Fig. 1. The growth characters of powdery mildew fungi in the control (A) and after 24 h of treatment (B, C, D, and E) with a 100% culture filtrate of *E. minitans*

Composition of the most effective fungal and bacterial culture filtrates

The chemical constituent analysis of the most effective fungal (*E. minitans*) and bacterial (*B. pumilus*) culture filtrates, yielded the main volatile compounds for each culture filtrate as shown in tables 4 and 5. Nine and eleven compounds were identified from the fungal and bacterial culture filtrate, respectively, with different percentages as shown in tables 4 and 5. The identified compounds belonged to eldyhydes, esters, alcohols and fatty acids. Furthermore, overlapping patterns could be found in the identified compounds in the two culture filtrates. For example, the formation of 2-octenal was detected in both fungal and bacterial culture filtrates. The other detected compounds were found in each culture filtrate alone.

Table 4. The main constituents of *E. minitans* culture filtrate identified using GC-MS analysis

No.	Identified compounds	Retention time [min]	Area [%]*
1	2-Butyl ester of benzoic acid	5.59	31.37
2	Amino 6-methyl benzoic acid	6.22	13.01
3	6-Methyl 2 phenylindole	9.75	15.95
4	2-Pentyl ester of benzoic acid	10.52	2.95
5	Carvacrol methyl ether	13.79	5.44
6	Ethyl siloxane cyclic trimer	15.88	2.78
7	5-Methyl 2-phenyl indolizine	17.02	3.73
8	2-Butyl benothiazole	19.03	10.45
9	2-Octenal (E)	20.95	6.20

*percentage of each component in the analyzed extract depending on tis peak area

Table 5. The main constituents of *B. pumilus* culture filtrate identified using GC-MS analysis

No.	Identified compounds	Retention time [min]	Area [%]*
1	1H-indene, 1-methylene	5.55	3.1
2	n-Hexadecanoic acid	10	25.2
3	8-Methyl-1-decene	11.22	2.05
4	Oleic acid	11.99	2.87
5	2-heptenal	15.12	20.5
6	4-Hexen-1-yl ester of propanoic acid,	18.3	4.29
7	1H-Imidazole 1-ethanol	20.72	3.49
8	2-Octenal	21.87	6.75
9	Diethyl phthalate	22.1	2.7
10	1-Butanol, 3-methyl	22.5	2.07
11	3,4-Dimethyl-5-hexen-3-ol	22.85	5.6

DISCUSSION

The present study implies that culture filtrates of the tested fungal and bacterial isolates showed high effectiveness against powdery mildew pathogen in squash under field conditions. These findings in our study are in agreement with the findings of many researchers who reported that compounds produced by antagonistic fungi and bacteria have been shown to have potential antifungal activities against plant pathogens (Alstrom 2001; Wheatley 2002; Mercier and Manker 2005; Koitabashi 2005; Fernando *et al*. 2005; Kai *et al*. 2006; Zou *et al*. 2007). Also, many researchers found that *Bacillus* sp and their nonvolatile compounds made considerable contributions to the control of plant diseases (Algam *et al*. 2004; Hou *et al*. 2006)

This study implies that use of culture filtrates of the tested microbial isolates mixed with the tested fungicide worked better against the powdery mildew pathogen than fungicide used alone (Fan *et al*. 2001; Yoshida *et al*. 2001). However, this approach for the selected plant pathogen in this study, with the tested microbial isolates, is considered the first report in this regard. The results also revealed differential behavior of the culture filtrates of the tested microbial isolates with the tested fungicide, indicating a major contribution of the different agents depending on their nature, and possible antagonism rather than synergism in some cases.

It was observed, that analysis by GC-MS for the bacterial culture filtrate, resulted in the presence of different compounds, such as: n- hexadecanoic acid (palmitinic acid), 2-heptenal**,** 2-octenal and octadecanoic acid (oleic acid) with higher percentages (area percentages). The antifungal activity of the bacterial culture filtrate against the powdery mildew pathogen may be due to the presence of the previous fatty acids and their derivatives (Jay-Ran *et al*. 1998; Dale *et al*. 2004; Daniele *et al*. 2006; Prittee *et al*. 2007). Moreover, Pamela (2002), mentioned that the mode of action of bioagents such as *Bacillus* sp may be due to the production of lipopeptides such as hexadecanoic acid (palmitic acid), 9 octadecanoic acid (oleic acid) which have a direct effect on hyphae and sporangia of powdery mildew fungus.

Among these compounds identified by GC-MS, from the most effective fungal culture filtrate (*E. minitans*), Carvacrol methyl ester, 2-octenal; 5 methyl 2-phenylindole and 2 butyl benothiazole appeared to account for the largest percent. The antifungal activity of the fungal culture filtrate against the powdery mildew pathogen may be due to the presence of Carvacrol (Ultee *et al*. 2002; Goncalves *et al*. 2003; Neri *et al*. 2009) benzoic acid derivatives (Goncalves *et al*. 2003) benothiazole derivatives (Fernando *et al*. 2005), 2-octenal (Jay-Ran *et al*. 1998; Dale *et al*. 2004; Daniele *et al*. 2006) and 5 methyl 2-phenylindole (Abou-Elela *et al*. 2009).

Although, the antimicrobial activity of tested culture filtrates is attributed mainly to its major compounds, the synergistic or antagonistic effect of one compound present in a minor percentage in a mixture which recorded antifungal activity, has to be considered. Each of the culture filtrate components has its own contribution on the biological activity of the culture filtrate (Ragas *et al*. 2002).

The mode of action of the identified compounds in the tested culture filtrates against the powdery mildew pathogen, may be due to the inhibition of the melanin pigment formation. It was reported, that melanin pigment interrelated with the fungal pathogenicity and could endow fungi with certain special recovery functions, such as anti-radiation, anti-oxidation, and scavenging free radical (Bassam *et al*. 2002; Cao *et al*. 2006). Without such melanin, pathogenic fungi would lose pathogenicity. Bruce *et al*. (2003) found that volatiles from certain unidentified bacterial strains inhibited the growth and pigmentation of five sapstain fungi, which could affect the appearance of wood due to colonization by pigmented hyphae. In this study, we found that compounds identified from the tested culture filtrates, exhibited striking inhibition towards the pathogenic fungus. Therefore, the mode of action may be due to the ability to inhibit powdery mildew infection through the inhibition of melanin pigment formation. Moreover, the mode of action of the culture filtrates of the tested biocontrol agents (BCAs) may be carried out by inducing changes in the plant that increase its resistance to disease, which is similar to the phenomena of induced and systemic acquired resistance (Cook 1988; Howell *et al*. 1993).

The compounds identified by GC-MS analysis from the bacterial and fungal culture filtrate, contained alkenes, alcohol, esters, ketones, aldehydes and fatty acids. Therefore, the culture filtrates that contain a mixture of different biologically active compounds would be more effective for controlling pathogens and less likely to select for resistance than synthetic fungicides that are composed of a single compound (Wei *et al*. 2008). Finally, the culture filtrate of the tested bio-control agents can be regard as an effective and safe control of powdery mildew in squash. It is true that fatty acids and their derivatives identified in the culture filtrate of the tested bio-agents are used as treatment of inflammation, allergies, and anaphtlactic shock (Tasaka *et al*. 1988a; Tasaka *et al*. 1988b; Paterson 2006).

CONCLUSIONS

The results of this study suggest that culture filtrates of some biocontrol agents could be used as alternative of fungicides to control powdery mildew in squash. Furthermore, the results also implied the amount of the fungicides may be minimized. This may be done by mixing low doses of the fungacide with culture filtrates of some biocontrol agents. Such a mixture will help maintain good powdery mildew control and reduce environmental pollution. Microbial agents may be used while maintaining adequate monitoring and research to allow early detection and mitigation of detrimental biological and environmental impacts. There is increasing interest in the use of natural antimicrobial compounds as preservatives for foods. Examples of such natural antimicrobial, promising compounds are carvacrol, which is present in the essential oil fractions of fungal culture filtrate (9.48%), and benothiazole (7.49%) that is known as a synthetic fungicide.

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POLISH SUMMARY

WYKORZYSTANIE FILTRATÓW POCHODZĄCYCH Z HODOWLI WYBRANYCH IZOLATÓW MIKROORGANIZMÓW DO ZWALCZANIA MĄCZNIAKA KABACZKA

Mączniak powoduje poważne straty zarówno w wielkości, jak i jakości plonu kabaczka. W celu zwalczania mączniaka kabaczka wykorzystano filtraty pochodzące z hodowli wybranych izolatów mikroorganizmów: *Eppicocum nigrum*, *Eppicocum minitans*, *Eppicocum* sp., *Trichoderma harzianum*, *Trichoderma viride* i *Baccilus pumilus*. Filtraty zastosowano indywidualnie oraz w kombinacji z fungicydem penkonazol w warunkach polowych. Ponadto, przeprowadzono analizę GC-MS w celu identyfikacji komponentów najskuteczniejszych filtratów przeciwko mączniakowi. Wyniki badań wykazały, że filtraty różnych izolatatów mikroorganizmów (z wyjątkiem *Trichoderma harzianum*) wykazywały wyższą skuteczność w porównaniu do skuteczności samego fungicydu zastosowanego w zalecanych dawkach, w obu sezonach badań. Wykazano również, że łączne stosowanie filtratów pochodzących z hodowli różnych mikroorganizmów z fungicydem penkonazol przeciwko mączniakowi prawdziwemu, dawało lepsze wyniki w porównaniu z fungicydem zastosowanym samodzielnie. Skuteczność testowanych filtratów mikroorganizmów była warunkowana obecnością mieszanki znanych grzybobójczych składników. Wyniki badań wskazują na możliwość alternatywnego stosowania filtratów pochodzących z hodowli wybranych mikroorganizmów w zwalczaniu mączniaka kabaczka. Łączne stosowanie czynników biologicznego zwalczania z fungicydami pozwoli na zmniejszenie ilości stosowanego preparatu.