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Recent Approaches for Controlling Downy Mildew of Cucumber under Greenhouse Conditions

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Abstract

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The efficacy of biocontrol agents (effective microorganisms (EMs1), *Bacillus subtilus*, and *Bacillus pumilus*), zinc oxide nanoparticles, castor and clove oils, as well as of a recommended fungicide (famoxadone + cymoxanil) utilised during two growing seasons under greenhouse conditions against downy mildew of cucumber were evaluated. Furthermore, GC-MS analysis was carried out to identify the bioactive chemical components of plant origin oils (castor and clove). The effect of these treatments on some biochemical, growth, and yield characters of cucumber was also investigated. Famoxadone + cymoxanil was the most effective treatment in both test seasons, followed by clove oil, zinc oxide, EMs1, *Bacillus pumilus, Bacillus subtilus,* and castor oil. The results showed a significant effect of all the treatments on some biochemical (chlorophyll content, peroxidase, and polyphenoloxidase) as well as growth and yield characters (plant height, fruit number per plant, and fruit yield) of the cucumber plants relative to control.

Keywords: plant pathogen; cucurbits; plant oils; nanoparticles; bioagents

Downy mildew, caused by fungi like *Pseudoperonospora cubensis*, is one of the most destructive pathogen to cucumber. Symptoms firstly appear as pale green areas on the upper leaf surfaces. They change to yellow angular spots. A fine dark brown to grayish downy growth soon appears on the lower leaf surface. Infected leaves generally die but may remain erect while the edges of the leaf blades curl inward. Usually, the leaves near the centre of a hill or row are infected first. The infected area spreads outward, causing defoliation, stunted growth, and poor fruit development. The entire plant may eventually be killed (Bernherdt *et al.* 1988).

The fungus is easily carried by wind currents, rain splash, farm implements, or the hands and clothes of farm workers. It is favoured by cool to moderately warm temperatures, but tolerates hot days, although long periods of dry hot weather can stifle the spread of the disease (Hansen 2000). Unlike powdery mildew, it requires humidity to flourish. Therefore, downy mildew is most aggressive when heavy dews, fog, and frequent rains occur (Bernherdt *et al.* 1988).

The control of downy mildew disease has been almost exclusively based on the application of chemical fungicides. Several effective fungicides have been recommended for use against this disease, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, fungicide residues, and other health and environmental hazards. Moreover, the development of pathogenic fungi resistance towards synthetic fungicides is a great problem affecting significantly the future of chemical control by fungicides.

In this context, biocontrol approaches may help develop an eco-friendly control strategy for managing plant disease (Heydari & Misaghi 2003; Bharathi *et al.* 2004; Shahraki *et al.* 2008). Among biocontrol agents (BCA), antagonistic bacteria including *Pseudomonas* and *Bacillus* spp. have been shown to play very important roles in controlling several diseases

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(Collins & Jacobson 2003; Heydari & Misaghi 2003; Shahraki *et al.* 2008, 2009).

Moreover, it is important to explore novel antifungal agents, which may replace current control strategies. In recent years, nanoparticle (NPs) materials have received increasing attention due to their unique physical and chemical properties which differ significantly from their conventional counterparts (Stoimenov *et al.* 2002). Recent studies have demonstrated antimicrobial activities of various NP materials, including silver (Kim *et al.* 2008a,b; Kumar *et al.* 2008).

Aromatic plants have been used for centuries as spices and condiments to confer aroma and flavour to food and beverages. Additionally, due to their constituents, medicinal and aromatic plants can act as stabiliser agents, playing an important role in the shelf-life of foods and beverages (Salgueiro *et al.* 2010) but only in the last decade scientific research has focused its interest on their essential oils (EOs) and extracts as natural sources of antimicrobial and antioxidant compounds (Sagdic *et al.* 2003). EOs are volatile, natural, complex compounds characterised by a strong odour and are formed by aromatic plants as secondary metabolites (Bakkali *et al.* 2008).

This study aimed (1) to evaluate the efficacy of certain biocontrol agents (*Bacillus subtilis*, *Bacillus pumilus,* and effective microorganisms), zinc oxide nanoparticles, castor and clove oils, beside the recommended fungicide (famoxadone + cymoxanil), against *Pseudoperonospora cubensis*, the causative fungus of downy mildew in cucumber under greenhouse conditions in two growing seasons, (2) to identify the bioactive chemical components of plant origin oils (castor and clove) by the gas chromatography-mass spectrometry analysis, and (3) to investigate the effect of these treatments on some biochemical (chlorophyll content, peroxidase and polyphenoloxidase) and growth and yield characters (plant height, fruit number/plant, and fruit yield) of cucumber plants.

MATERIAL AND METHODS

Tested materials. Effective Microorganisms (EMs) formulation was obtained from the Egyptian Ministry of Agriculture and Land Reclamation, Giza, Egypt. This formulation contains 60 species of beneficial microorganisms grown in a special medium and produced in Egypt under supervision of the Japanese EMRO Scientific Organization. *B. subtilis* and *B. pumilus* as bioagents were obtained from the Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt. The tested fungicide used in this study was Equation Pro (famoxadone 16.6% + cymoxanil 22.1%). This fungicide was applied at its recommended field rate of 1 ml/l water. Zinc oxide nanoparticles were obtained from Egypt Nanotech Co. Ltd. (Giza, Egypt) with a purity of 99.99%. Clove and castor oils were obtained from El Captain Company for Extracting Natural Oils, Plants & Cosmetics, Cairo, Egypt.

Preparation of bioagent formulations. The talc-based formulations of each biocontrol agent were prepared with some modifications of the method developed by Commare *et al.* (2002). The selected biocontrol formulations were inoculated into the King B broth medium and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28 ± 2°C). One kg of talc powder was taken in a sterilised metal tray and pH was adjusted to neutral by adding $CaCO_3$ at the rate of 15 g/kg. 10 g of carboxymethyl cellulose (CMC) were added to each 1 kg of talc and mixed well and the mixture was autoclaved for 30 minutes. 400 ml of the 48-hour-grown suspension containing 9×10^8 CFU/ml water were mixed with the carrier cellulose mixture under aseptic conditions. After drying (approximately to 35% moisture content) for overnight, it was packed in polypropylene bag, sealed, and stored at room temperature (28 ± 2°C).

Control of cucumber downy mildew under greenhouse conditions. This study was carried out under greenhouse conditions at Sakha, Kafr Elshiekh Governorate at the protected agricultural locality during two growing seasons (2012–2013/2013–2014). Seedlings 21 day-old were transplanted at spacing of 50 cm using double row in each ridge. The plot was 3.5 m long and 1.5 m wide, each plot had 14 plants. The treatments were arranged in randomized complete block design with four replicates. All agricultural practices were carried as recommended by the Ministry of Agriculture and Land Reclamation. Plants were sprayed with *B. subtilis 1* and *B. pumilus* in a spray suspension with concentration levels of 1×10^8 CFU/ml. EMs formulation was applied at the concentration level of 5 ml/l water (Derbalah *et al.* 2013). Zinc oxide nanoparticles were carried out by preparing liquid solutions of this treatment at the concentration level of 0.5 g/l water (Derbalah *et al.* 2013). Castor and clove oils were applied at the concentration level of 5% using 0.01% of Tween 80 emulsifier. All treatments were applied three times in 10-day intervals between each spray. Disease se-

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verity was assessed one week after the last spray. Some growth and yield parameters (plant height, fruit number/plant, and fruit yield) of tomato plants were measured in the second season.

Disease assessment. Disease severity was determined according to the following equation (Descalzo *et al*. 1990):

$$R = \Sigma(a \times b)/N \times K \times 100$$

where: R – disease severity; a – number of leaves within infection grade; b – numerical value of each grade; N – total number of the examined leaves; K – the highest degree of infection in category; the area of lesions in each replicate (three plants with ten leaves each) was measured and the degree of infection was estimated by scale category

Reduction percentage in disease severity (DS) was determined according to the following equation:

$$\text{Reduction \%} = \{(\text{DS in control \%} - \text{DS in treatment \%})/\text{DS in control \%}\} \times 100$$

Biochemical changes associated with infection

Determination of chlorophyll. Two leaf discs (diameter 1 cm) were removed from the leaf sample and extracted with 5 ml *N*,*N*-dimethylformamide in the dark for 48 h at room temperature till the spectrophotometric measurements (Moran & Porath 1980). The density of colour was measured at 647 and 664 nm for chlorophyll *a* and *b*, respectively, using an UV-Vis Spectrophotometer 3700 (Shimadzu Corp., Kyoto, Japan). Chlorophyll *a* and *b* were calculated using the following equations:

$$\text{Ch. } a = 12.64(A_{664}) - 2.49(A_{647}) \quad (\mu g/ml)$$

$$\text{Ch. } b = 5.6(A_{664}) + 23.26 \quad (\mu g/ml)$$

Sample preparation and extraction for enzyme activities determination. The sample was ground with 0.1 M sodium phosphate buffer at pH 7.1 (2 ml buffer/g tissue) in a mortar. These triturated tissues were strained through four layers of cheese cloth and the filtrates were centrifuged at 3000 rpm at 6°C for 20 minutes. The supernatant fluid was used for enzyme assay of polyphenoloxidase and peroxidase using the UV-Vis Spectrophotometer 3700 (Shimadzu Corp.). The reference cuvette contained the same concentration of components as the sample cuvette, except that of the enzymes extract. The spectrophotometer cuvettes were thoroughly mixed and reading was recorded every 30 s for 2 minutes. The enzyme activity was expressed as the change in the absorbance per min per g (aa/min/g).

Polyphenoloxidase assay. For polyphenoloxidase determination, a colorimetric procedure was used (Matta & Dimond 1963). The reaction mixture contained 0.1 ml of enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH of 7.0, and 1.0 ml of 10^{-3} M catechol was brought to a final volume of 6.0 ml with distilled water. The activity of polyphenoloxidase was determined using spectrophotometric procedures at 495 nm wavelength.

Peroxidase assay. Peroxidase activity was determined according to the method described by Allam & Hollis (1972) through measuring the oxidation of pyrogallol in the presence of H_2O_2 at 425 nm. The reaction mixture contained 0.5 ml of 1% sodium phosphate buffer at pH 7.0, 0.3 ml of enzyme extract, 0.3 ml of 0.5 M pyrogallol, 0.1 ml of 1% H_2O_2 (v/v), brought to final volume of 3.0 ml with distilled water.

Chemical composition of caster and clove oils. Clove and caster oils were analyzed to identify the bioactive components in these oils that may be responsible for their antifungal activity against downy mildew. For the clove oil analysis, the sample was diluted with acetone and 1 µl of the sample was injected into a HP 6890N gas chromatograph equipped with a HP5975 mass detector and a fused silica capillary column HP5-MS (30 m × 0.32 mm, film thickness 0.25 µm) (all Agilent Technologies, Santa Clara, USA) was used. Helium was the carrier gas, and a split ratio of 1 : 100 was used. The oven temperature was maintained at 60°C for 8 min, then it was gradually raised at a rate of 30°C/min to 180°C and maintained at 180°C for 5 minutes. The temperature at the injection port was 250°C (Ayoola *et al.* 2008).

The analysis of castor oil was carried out using an Agilent HP 7890A GC gas chromatograph interfaced with a mass-selective detector (MSD, Agilent 7000 Triple Quad) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) (all Agilent Technologies). The carrier gas was helium with the linear velocity of 1 ml/minute. The oven temperature was set at 50°C for 2 min, then heating up to 100°C at a rate of 10°C/min with a hold-time of 3 min, then heating to 150°C at a 5°C/min rate with a 2 min hold-time, then to 200°C at a 10°C/min rate, and final increase at the rate of 20°C/min to 280°C. The injector and detector temperatures were 250°C and 300°C, respectively. The split injection mode with the split ratio 1 : 100 and 1 µl injected sample volume was used. The MS operating pa-

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Table 1. Effect of individual treatments on the severity of downy mildew of cucumber in two seasons

Treatments	Disease severity (%)	
	first season (2012–2013)	second season (2013–2014)
Bacillus subtilus	37.47 ± 2.63^{bc}	32.81 ± 0.54^{cd}
Bacillus pumilus	34.12 ± 2.1^{c}	35.22 ± 0.94^{bc}
ZnO	28.58 ± 1.19^{d}	25.31 ± 2.83^{e}
Effective microorganisms	39.15 ± 3.10^{c}	30.41 ± 2.02^{d}
Castor oil	43.33 ± 1.08^{b}	38.80 ± 1.36^{b}
Clove oil	19.51 ± 2.07^{e}	15.15 ± 1.17^{f}
Famoxadone + cymoxanil	7.90 ± 0.45^{f}	5.67 ± 0.56^{g}
Control	91.07 ± 1.4^{a}	88.31 ± 4.58^{a}

$^{a–g}$significance and non-significance between the means using Duncan's Multiple Range test

rameters were as follows: ionisation potential 70 eV, interface temperature 250°C, and acquisition mass range 50–800.

The relative percentage of the components was evaluated from the total peak area (TIC) by the apparatus software. The identification of components was based on the comparison of their mass spectra and retention times with those of the authentic compounds, on the computer matching with NIST and WILEY Library, as well as on the comparison of the fragmentation pattern of the mass spectral data with those reported in the literature

Statistical analysis. Data were statistically processed using the analysis of variance (ANOVA) test of SPSS software and the different means were compared by Duncan's Multiple Range test (Duncan 1955).

RESULTS

Efficacy of the tested materials against cucumber downy mildew under greenhouse conditions. The results showed that all the tested treatments significantly reduced the disease severity of downy

Table 2. Components of castor oil analysed by GC-MS

No.	Components	R.T.	Area (%)
1	Myristicin	14.63	13.17
2	β-Copaene	15.46	1.83
3	Arachidonic acid	15.6	0.59
4	Vitamin A aldehyde	15.7	0.48
5	10,13-Octadecadiynoic acid, methyl ester	16.088	3.20
6	Cubedol	16.33	1.92
7	Cervonic acid	16.38	1.17
8	β-Guaiene	16.49	0.92
9	3-Methylcatechol	16.98	3.50
10	Icosapentaenoic acid	17.18	4.46
11	10,12-Docosadiynedioic acid	17.36	0.67
12	Lutein	17.85	0.50
13	Arachic acid	18.27	3.18
14	Stearic acid	18.33	2.39
15	Erucic acid	18.75	16.59
16	*cis*-10-Nonadecenoic acid	19.7	16.94
17	Oleic acid, eicosyl ester	20.63	13.30
18	6-Octadecenoic acid	21.00	3.57

Table 3. Components of clove oil analyzed by GC-MS

No.	Components	R.T.	Area (%)
1	Eugenol	12.87	37.41
2	Phenol, 5-allyl-2-methoxy-	13.04	8.75
3	Caryophyllene	13.56	13.83
4	Longifolene	13.89	2.53
5	α-Humulene	14.07	0.40
6	*cis*-Thujopsene	14.24	0.71
7	Eugenol acetate	14.53	15.57
8	*trans-m*-Propenyl guaiacol	14.91	0.40
9	Longifolenaldehyde	15.11	0.57
10	Cholecalciferol	15.19	3.25
11	Santalol, *cis*, α-	15.24	1.61
12	Humulene-1,2-epoxide	15.49	0.62
13	(–)-Spathulenol	15.70	1.86
14	Cedrenol	15.90	1.41
15	Geranyl-α-terpinene	16.05	1.64
16	α-Bisabolol	17.45	0.66
17	Cyanidin cation	18.30	1.45
18	Hexadecanoic acid, ethyl ester	18.48	0.68
19	9-*cis*-Retinal	19.60	2.96
20	*cis*-13-Eicosenoic acid	21.37	3.67

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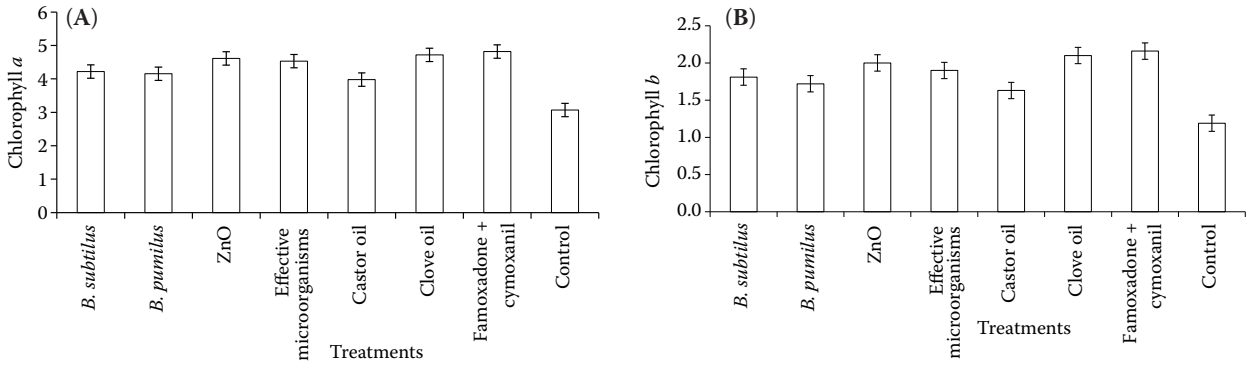

Figure 2. Effect of the tested treatments on (**A**) chlorophyll *a* and (**B**) chlorophyll *b* content in cucumber plants

mildew of cucumber relative to control treatment. Famoxadone + cymoxanil treatment was the most effective treatment against downy mildew followed by clove oil, zinc oxide nanoparticles, EMs1, *B. pumilus, B. subtilus*, and castor oil, respectively, in both tested seasons (Table 1).

Chemical composition of castor and clove oils. The identified compounds from castor and clove oils are listed in Tables 2 and 3. Eighteen compounds were identified for castor oil (Table 2) and twenty compounds were identified for clove oil (Table 3). The identified compounds belong to aldehydes, esters, alcohols, and fatty acids.

Effect of the tested treatments on some biochemical changes (chlorophyll content, peroxidase and polyphenoloxidase activity) in cucumber. The results showed a significant increase in all biochemical parameters (chlorophyll content, peroxidase and polyphenoloxidase activity) in cucumber plants under all tested treatments compared with control. The famoxadone + cymoxanil application resulted in the highest content of both chlorophyll *a* and *b* in the treated cucumber plants followed by clove oil, zinc oxide nanoparticles, EMs1, *B. pumilus, B. subtilus*, and castor oil, respectively (Figure 1). Also that famoxadone + cymoxanil caused the highest activity of both peroxidase and polyphenoloxidase enzymes in the treated cucumber plants followed by clove oil, zinc oxide, EMs1, *B. pumilus, B. subtilus*, and castor oil, respectively (Figure 2).

Effect of the tested treatments on some crop parameters. The results showed that plant height, fruit number/plant, and fruit yield were significantly increased under all the tested treatments compared with control. The highest crop parameters were recorded in cucumber plants treated with famoxadone + cymoxanil, followed by clove oil, zinc oxide, Ems1, *B. pumilus, B. subtilus*, and castor oil, respectively (Table 4).

DISCUSSION

Castor and clove oils significantly reduced the severity of downy mildew of cucumber (Table 1). The results are in agreement with previous studies covering a wide range of plant pathogens including bacteria and fungi (Bowers & Locke 2000, 2004; Momol *et*

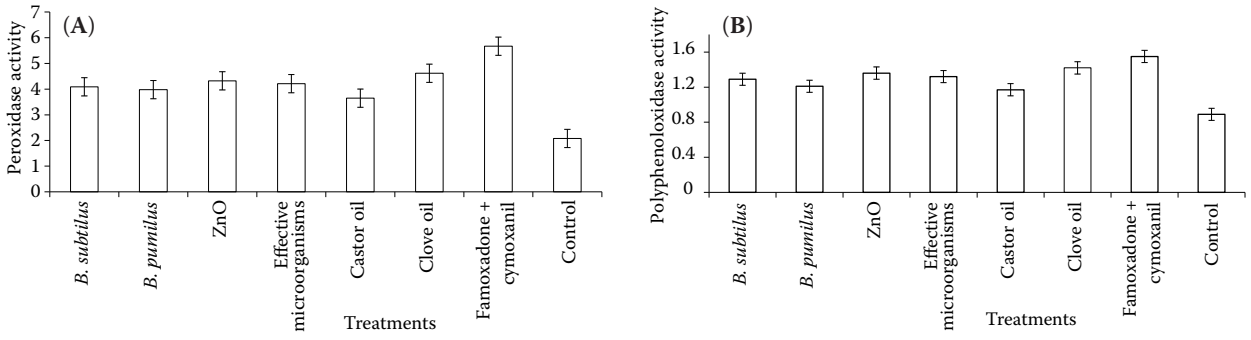

Figure 3. Effect of the tested treatments on (**A**) peroxidase and (**B**) polyphenoloxidase enzyme activity in treated cucumber plants

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Table 4. Effect of the tested treatments on some crop parameters (plant height, fruit number, and fruit yield) of cucumber plants in 2013–2014

Treatments	Plant height (m)	Fruit number/plant	Fruit yield (kg/plant)
Bacillus subtilus	2.63 ± 0.02^{cd}	25.33 ± 0.57^{c}	3.30 ± 1.0^{cd}
Bacillus pumilus	2.55 ± 0.05^{d}	23.00 ± 1.0^{d}	3.20 ± 1.0^{d}
ZnO nanoparticles	2.85 ± 0.05^{b}	26.00 ± 1.0^{c}	3.60 ± 1.0^{b}
Effective microorganisms	2.70 ± 0.10^{c}	25.33 ± 0.1.52^{c}	3.40 ± 1.0^{c}
Castor oil	2.65 ± 0.05^{cd}	21.67 ± 1.52^{d}	2.97 ± 0.15^{e}
Clove oil	2.98 ± 0.07^{a}	28.00 ± 1.0^{b}	3.70 ± 0.10^{b}
Famoxadone + cymoxanil	2.85 ± 0.05^{b}	30.00 ± 1.0^{a}	4.27 ± 0.05^{a}
Control	1.93 ± 0.02^{e}	19.00 ± 1.0^{e}	2.00 ± 0.10^{h}

$^{a–h}$indicate the significance and non-significance between the means using Duncan's Multiple Range test

al. 2000; Oka *et al.* 2000). The results suggested that the activity of castor and clove oils could be due to the drastic effect of fatty acids and their derivatives on the causal pathogen. Fatty acids, as well as their corresponding salts, have been reported to display an antimicrobial activity (Nieman 1995; Sprong *et al.* 2001; Skřivanová *et al.* 2005). Despite the lack of information, which would make the inhibitory mechanism fully understood, it has been assumed that the fatty acids penetrate the lipid membrane and dissociate in the more alkaline interior and cause metabolic disruption (Russel & Diaz-Gonzalez 1998). In addition, the high inhibition caused by the fatty acids could be due to their cytolytic activity by being a solvent of cellulose, a constituent of the cell membrane of fungi, while the fatty acids such as oleic acid have the potential of antifungal properties attributed to long chain unsaturation (Agoramoorthy *et al.* 2007). Thus, these findings could be significant for understanding the basis of clove and castor oils effect on the downy mildew pathogen. These plant essential oils, therefore, have the potential to be developed into an environmentally friendly and sound alternative to the use of fungicides in integrated management of plant diseases.

The results showed that zinc oxide nanoparticles significantly reduced the severity of downy mildew of cucumber relative to control, with no adverse effect on the treated cucumber plants. This may be due to the distorting and damaging effect on the sporangial membrane, resulting in a leakage of intracellular contents and eventually the death of sporangia or zoospores (Jin *et al.* 2009). This could be explained on the basis of the oxygen species released on the surface of ZnO, which cause fatal damage to microorganisms (Sunanda *et al.* 1998) and react with hydrogen ions to produce molecules of H_2O_2. The generated H_2O_2 can penetrate the cell membrane and kill the bacteria (Fang *et al.* 2006). The generation of H_2O_2 depends strongly on the surface area of ZnO, which results in more oxygen species on the surface and the higher antibacterial activity of the smaller nanoparticles (Ohira *et al.* 2008). This hypothesis for the physical mode of action of zinc oxide nanoparticles as nanocides via penetration of the cell membrane can overcome the resistance problem of fungi to fungicides, since the fungus is unlikely to become genetically selected or physiologically resistant to such a physical mechanism.

It has been found that the bioagents tested in this study (EMs1, *B. pumilus*, and *B. subtilus*) showed significant reduction in the severity of downy mildew in cucumber plants. Some isolates of *B. subtilis* are reported as growth-promoters or antagonists of several pathogens and biocontrol agents (BCAs) of numerous diseases (Brannen & Kenney 1997; Obagwu & Korsten 2003; Szczech & Shoda 2004; Romero *et al.* 2008). *B. subtilis* QST 713 strain contained in the commercial product Serenade™ is active against fungi and bacteria that cause scab, powdery mildew, sour rot, downy mildew, early leaf spot, early and late blight, bacterial spot, and walnut blight diseases (Anonymous 2005). Antagonism by *B. subtilis* QST 713 may be achieved in several ways, including nutrient competition, site exclusion, colonisation, and attachment of the bacteria to the fungal pathogen and induction of the natural systemic resistance of the plant.

Moreover, the efficacy of the tested bioagents against downy mildew may be due to nutrient competition, hyperparasitism, and/or antibiosis (Falk *et al.* 1995). Mycoparasitism is considered as a mecha-

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nism of plant pathogens control (Harman 2000), whereby a species or strain of microbe directly attacks and feeds on other fungi (Kendrick 1992). The production of antibiotics or enzymes that can inhibit the growth or reduce the competitive ability of other organisms is another control mechanism (Dunlop *et al.* 1989; Howell *et al.* 1993; Harman 2000). Control may also be achieved through competition for space and resources with highly competitive BCAs quickly colonising plant surfaces, creating an effective 'living barrier' to subsequent pathogen invasion (Cook 1988). Further mechanism is the mobilisation of nutrients in the soil, a process that makes compounds in the soil more available for plant uptake, resulting in increased general health and disease resistance (Harman 2000). BCAs may also induce changes in the plant that increase disease resistance similarly to the phenomena of induced and systemic acquired resistance (Handelsman & Stabb 1996; Harman 2000).

The biological treatment using effective microorganisms (EMs) formulation was also effective against the tested pathogen relative to control. This agrees with the findings of Elad and Kapat (1999), who established a successful antagonism of plant pathogens by saprophytic microorganisms operating by nutrient competition, hyperparasitism, antibiosis and/or induced host resistance.

The results of this study showed a significant increase of defense enzymes activity relative to control treatment. The role of defense related enzymes in disease resistance has been reported earlier (Zdor & Anderson 1992; Shewry & Lucas 1997; Chen *et al.* 2000). A biochemical analysis of rice crop raised from seeds treated with *P. fluorescens* exhibited early induction of peroxidase (POX) activity (Nandakumr *et al.* 2001). Sivakumar and Sharma (2003) showed that POX and polyphenoloxidase (PPO) activities were higher in plants raised from *P. fluorescens* treated seeds than in pathogen alone inoculated ones.

The results showed a significant increase in cucumber yield under different treatments relative to control. This may be due to the significant reduction in the severity of cucumber downy mildew under different treatments relative to control which subsequently resulted in increased crop yield (Cardwell *et al.* 1997). Foliar application of Zn and silica showed substantial influence both on yield and, particularly, crop quality (Khoshgoftarmanesh *et al.* 2010; Xiao-Fang *et al.* 2011). The cucumber yield differed in the first and in the second season which may be due to the variation in disease severity in cucumber within and among the seasons (Cardwell *et al.* 1997) subsequently affecting the crop yield.

In conclusion, this study showed the ability of the tested treatments to control downy mildew in cucumber. However, further studies are needed to evaluate the side effects of the used natural products to reflect their safety.

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