



EVALUATION OF CERTAIN BIOCIDES AND CHEMICALS INDUCING RESISTANCE IN MANAGEMENT OF CUCUMBER DOWNY MILDEW UNDER PROTECTED CULTIVATION

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Received: 04/12/2019; Accepted: 05/01/2019

ABSTRACT: Downy mildew of cucumber is caused by the fungus-like pathogen *Pseudoperonospora cubensis* (Berk and Curtis), which causes loss in the yield in Egypt especially under protected cultivation where low temperature and high humidity are prevalent. The efficiency of two Bio-control agents *i.e.* T34 (*Trichoderma asperellum*, 10^9 spore/cm³) and Bio-Cure-B (*Pseudomonas fluorescens*, 10^8 spore/cm³) as well as three resistance inducing chemicals (RICs), *i.e.* potassium dihydrogenphosphate (K₂HPO₄), salicylic acid (C₇H₆O₃), and potassium nitrate (KNO₃) were evaluated for controlling cucumber downy mildew under greenhouse and plastic house conditions. Greenhouse experiment data revealed that, spraying cucumber plants with either biocontrol agent or tested RICs two times *i.e.* 5 days before and/or nine days after artificially inoculated by the *P. cubensis* significantly reduced percentage of disease severity, meanwhile plant length and foliage fresh weight were increased in comparison with control treatments. The treatments increased the activity of peroxidase, β-1,3-glucanase, beside increase in total carbohydrates and total phenolic contents. Under plastic house, all tested biocides and/or RICs either alone or double combinations caused significant reduction in disease severity associated with significant increment in some growth parameter of cucumber plants (plant length and fruit yield) when compared with control treatments in both seasons. However, application of tested RICs mixture with tested biocides was more effective in this regard than the only application of them individually. In this respect, tested biocides were also more efficient than resistance inducing chemicals (RICs).

Key words: Cucumber, downy mildew, induced resistance, Biocides, *Pseudomonas fluorescens*, *Pseudoperonospora cubensis* and *Trichoderma asperellum*.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops grown under protected cultivations in Egypt. Downy mildew of cucumber, caused by *Pseudoperonospora cubensis* (Berk and Curtis), is one of the most prevalent and distributed foliar diseases of protected cultivation, that reduce the production considerable from early spring until autumn seasons (Abd El-Kereem, 1998; Ahmed *et al.*, 2000). Downy mildew greatly affects both yield and quality and has been a serious problem world-wide on various cucurbitaceae crops (Thomas, 1996). Fungus spores can survive in a

wide range of environmental conditions which makes them also adaptable against various control methods used in crop protection (Chowdhury *et al.*, 2015). Azoxystrobin is a systemic fungicide which inhibits cell respiration by binding to the protein subunits in the mitochondrial cytochrome bc1 complex (Fisher and Meunier, 2008). Induced resistance can be split broadly into systemic acquired resistance (SAR) and induced systemic resistance (ISR). ISR is phenotypically similar to pathogen- SAR in that it confers an enhanced defensive capacity against diseases caused by fungi, bacteria, viruses, and nematodes (Ran *et al.*, 2005). Systemic acquired resistance (SAR)

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is effective against a wide range of pathogens and requires the synthesis of phenolic signaling compound, salicylic acid. It is of note that ISR is independent of the SA production and pathogenesis-related proteins (PR) induction but requires the operation of plant growth hormones jasmonic acid (JA) and ethylene signaling pathways. Although both SAR and ISR are effective against different types of pathogens, it was found that both SAR and ISR requires NPR1 gene in systemic plant defenses, suggesting the interplay of those systemic resistance (Feys and Parker, 2000). Spraying cucumber plant with phenol (0.5%), salicylic acid (0.5%), anhydrous sulfanilic acid (0.5%) and potassium chloride (0.3%); then inoculated plants with the downy mildew pathogen at the 4-leaf stage decreased disease rates than the controls (Xing *et al.*, 1997). Solution of KH_2PO_4 with concentration 50, 100 and 150 mM sprayed as foliar treatment against downy mildew of cucumber, reduced the disease incidence by 75.8, 74.2 and 67.7%, respectively compared to the untreated control (Abd El-Karem, 1998). Successful biological control of foliar diseases such as blights, blast, powdery and downy mildews has been achieved by a number of researchers under greenhouse and in field trials using fungal and bacterial antagonists (Mosa, 2002; Abd El-Moity *et al.*, 2003; George 2003; Xing *et al.*, 2003; Hussein *et al.*, 2007).

The objective of the present work was to study the role of different biological control agents and/or resistance inducing chemicals (RICs) (instead of fungicides) as safety alternatives control methods against cucurbit downy mildew in relation to their ability to induce systemic resistance mechanism in cucumber plants.

MATERIALS AND METHODS

Source of Cucumber Seeds

Seeds of Tifa hybrid of cucumber cultivar used in these study were kindly provided by Vegetable Crop Research Institute, Hort. Res. Inst., ARC, El-Dokki, Egypt.

Source of Biocides and Resistance Inducing Chemicals (RICs)

Potassium dihydrogens phosphate (K_2HPO_4), salicylic acid ($\text{C}_7\text{H}_6\text{O}_3$), and potassium nitrate

(KNO_3) were obtained from Company of the Republic of Chemicals., Cairo, Egypt, and were tested as resistance inducing chemicals (RICs). The biocides biocontrol agents T34 (*Trichoderma asperellum*, 10^9 spore/cm³) was obtained from Shoura Chemicals Company and Bio-Cure-B (*Pseudomonas fluorescens*, 10^8 spore/cm³) was obtained from T. Stanes and Company limited, India.

Source of Pathogenic Fungus

During spring season 2017, naturally infected leaves of cucumber with typical angular lesion of downy mildew were collected from Kaha Research Farm Station of Kalubia Governorate and used directly for preparing spore suspension that adjusted to 5×10^4 sporangia/ml using a Haemocytometer slide. Spore suspension was used to carry out artificial inoculation onto two weeks aged cucumber seedlings.

Inoculation Technique

Inoculation with sporangia of *P. cubensis* was performed onto three weeks aged cucumber plants, where the plants with fully expanded true leaves. Sporangial suspension was sprayed onto the upper and lower surfaces of the leaves. Each plant received 10-20 ml suspension. Plants were bagged using plastic bags to ensure even the infection process for 48 hr.

Greenhouse experiments

Two biocides, Bio-control T34 (*Trichoderma asperellum*, 10^9 spore/cm³) and Bio-Cure-B (*Pseudomonas fluorescens*, 10^8 spore/cm³) as well as three resistance inducing chemicals (RICs), *i.e.* potassium dihydrogens phosphate (K_2HPO_4), salicylic acid ($\text{C}_7\text{H}_6\text{O}_3$), and potassium nitrate (KNO_3), were evaluated for their effects against the infection of cucumber downy mildew caused by *P. cubensis*, under greenhouse conditions in order to select the most efficient biocides and RICs. During mid of March 2017, pots (25-cm in diam.), were filled with sand clay soil (1:1, *W:W*) disinfested by 5% formalin. Each pot was sown with 5 seeds. Pots were irrigated and seeds were left to germinate for two weeks to form young seedlings. Cucumber plants (3 weeks-old) were thinned into two plants/pot after the previously mention period. The grown plants (3-week-old) were artificially inoculated as mentioned before, five

days after spraying with either recommended doses of tested biocides or 75 mM of tested RICs. Second spray of either biocides or RICs was applied nine days after artificial inoculation. Five replicates were used for each treatment. Five pots, unsprayed with biocides or RICs, were served as infected check treatments. Fungicide Azoxystrobin was used as additional infected check treatments at the rate of 50 ml / 100 Liter H₂O. The grown plants were irrigated when necessary and fertilized twice, *i.e.* three and five weeks after sowing, by the crystalon compounded (1g/pot). Disease severity was assessed two weeks after the second spray of either fungicides or RICs. Also, plant length (cm) and foliage fresh weight (g/plant), were determined 8 weeks after sowing.

Biochemical Analysis

Analysis of enzymatic activity

Enzyme extraction from plant tissues

Inoculated leaves were harvested at 24, 48, 72 hr., post-inoculation and stored at -80°C for subsequent analysis. Samples (one gram fresh weight) were ground in liquid nitrogen and used for extraction of individual enzymes by homogenization in sodium acetate buffer 50mM pH5.2 (3ML/GM) AT 4°C. The homogenate was centrifuged at 10,000 g for 15 min at 4°C and the supernatant was used for enzyme assay according to **Nandeeshkumar *et al.* (2008)**.

Peroxidase activity assay

Peroxidase activity was assayed spectrophotometrically according to the method described by **Hammerschmidt *et al.* (1982)**. The reaction mixture for the peroxidase assay included 25 µl of crude enzyme solution, 1ml of potassium phosphate buffer (10 mM pH 6.9), 1ml of 25% guaiacol and 1ml of 100 mM H₂O₂. One unit of the peroxidase activity has been defined as the change in absorbance at 470 nm per minute per gram fresh weight of leaf tissues.

β-1, 3-glucanase activity assay

β-1, 3-glucanase (E.C. 3.2.1.39) activity was colourmetrically assayed using laminarin-dinitrosalicylic acid method described by **Dann and Deverall (2000)** at wavelength 610 nm. The enzyme activity was expressed as mg glucose released min⁻¹ mg⁻¹ of sample.

Determination of phenolic content

Total phenols were determined using the Folin-Ciocalteu reagent as mentioned by **Snell and Snell (1953)**. The absorbance was measured at 520 nm and the phenolic content was expressed as mg/g fresh weight.

Determination of carbohydrates

Carbohydrates were extracted as described by **Snell and Snell (1953)** and color optical density of the reacted mixture was measured on absorbance 540 nm using the picric acid technique described by **Thomas and Dutcher (1924)** and results were expressed as mg/g fresh weight.

Plastic house experiments

Effect of biocides and their mixtures with RICs on natural downy mildew infection (under protected cultivation)

Two experiments were carried out under unheated plastic houses at Kaha Research Station, Kalubia Governorate, Egypt during the two successive spring seasons of 2017 and 2018 to study the effect of two biocides at the rate of 85 g/100 Liter H₂O and three RICs at concentration 75 mM either individually or in mixtures, against natural infection by *P. cubensis* (Berk and Curtis) the causal pathogen of cucumber downy mildew. The plastic house was divided into five ridges. Each ridge was 1x59 m. Cucumber transplants (Tifa, hybrid F1) with three true leaves (20 days old) were sown in the plastic house with 25 cm distance between plants. Culture practices such as drip irrigation, fertilizer and insect control were carried out as recommended by Ministry of Agriculture, Egypt. The plants from each assigned treatment were sprayed with tested biocides at the rate of 85g/100 Liter H₂O as a single treatment and/or combined with tested RICs at concentration 75 mM, *i.e.* C₇H₆O₃, K₂HPO₄ and KNO₃ as well as Bio-control T34+K₂HPO₄, Bio-control T34+K₂HPO₄, (Bio-control T34+KNO₃, Bio-Cure-B+C₇H₆O₃, Bio-Cure-B+K₂HPO₄ and Bio-Cure-B+KNO₃ at 10 days intervals. Four plots, each consisted of one row X 10m long (40 plants/row), were used as replicates for each treatment. A set of plants, unsprayed with biocides or RICs, served as infected check treatments. The fungicide Azoxystrobin treatment was used as

additional infected check treatments at the rate of 50 ml / 100 Liter H₂O. Disease severity (DS) was measured at 60 and 75 days after sowing. Also, the fruit yield (kg/plot) in each treatment was recorded at each harvesting time and the average was calculated. Randomized complete design with four replicates for each treatment was used. Each replicate consisted of 40 plants.

Disease Assessment

The plants were rated for disease severity (DS), the former as percentage of infected leaves on the plant and the next as the severity percentage of disease damage. DS was measured according to **Reuveni (1983)** using color index and infected area. The color index was calculated as follows:

0= no symptoms 1= greenish 2= yellowish 3= yellow 4= brown

The infected area index was measured as follows:

1= symptoms on 25% or less

2= >25-50 %

3= >50-100% of leaf area.

Multiplication of the color and infected area indices of each leaf yields a value of disease severity. Reduction percentage of disease severity was determined according to the following equation:

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

Statistical Analysis

Data were statistical analyzed using analysis of variance (ANOVA) among treatments. Means were compared by least significant differences (LSD) at $p \leq 0.05$ as described by **Song and Keane (2006)**.

RESULTS

Greenhouse Experiments

Results presented in Table 1 show that all tested biocides and resistance inducing chemicals (RICs) caused significant reduction in

the severity of downy mildew with significant increase in plant length and foliage fresh weight of cucumber plants in comparing with check treatments. However, tested biocides were more efficient in these regards than RICs. Bio-control T34 was the most effective biocide tested followed by Bio-Cure-B, as they significantly reduced the disease severity to 70.3 and 67.5%, respectively. These treatments increased plant length to 72.7 and 70.6 cm and foliage fresh weight to 148.3 and 142.2 g/plant, respectively. Meanwhile, potassium nitrate was the most effective RIC followed by potassium dihydrogen phosphate, as they reduced disease severity to 64.3 and 60.6% and increased the plant length to 70.3 and 68.7 cm and the foliage fresh weight to 142.3 and 135.9 g/plant, respectively.

Biochemical Analysis

Effect of spraying with two biocides and three RICs as biotic inducers on peroxidase activity

Results indicated that in all treatments tested, the peroxidase activity was increased in comparison with control (Table 2). The mean recorded levels of peroxidase were significantly higher in leaves treated with Bio-control T34 and Bio-Cure-B followed by potassium dihydrogen phosphate, potassium nitrate and salicylic acid. The estimated peroxidase activity reached 2.57, 2.40, 2.35, 2.25 and 2.14 IU/ml, respectively compared with that in untreated leaves (1.1 IU/ml). Plants treated with Azoxystrobin showed higher activity of peroxidase in comparison with the other tested treatments.

Effect of spraying with two biocides and three RICs on β -1, 3 glucanase activity

Results presented in Table 2 show that all tested biocides and resistance inducing chemicals (RICs) caused significant increases in β -1,3-glucanase activity compared with control. Maximum enzyme activity was obtained from plants treated with Azoxystrobin, being 9.3 mg/g fresh weight. Moreover, among two biocides tested it was noticed that treatment with Bio-control T 34 caused high activity of β -1,3-glucanase if compared with treatment with Bio-Cure-B. The corresponding values glucose representing enzymes activity was 6.92 and 4.60 mg/g fresh weight.

Table 1. Effect of spraying with two biocides and three RICs on the disease severity of cucumber downy mildew and plant growth parameter, under greenhouse conditions

Treatment		Disease severity (%)	Reduction (%)	Average plant length (cm)	Average foliage fresh weight (g/plant)
Tested biocide	Biocontrol T34	15.6	70.3	72.7	148.3
	Bio-Cure-B	17.1	67.5	70.6	142.2
	K ₂ HPO ₄	20.7	60.6	68.7	135.9
Tested RIC	C ₇ H ₆ O ₃	23.1	56.1	76.5	135.0
	KNO ₃	18.4	64.3	70.3	142.3
Azoxystrobin50 ml /100 H ₂ O*		2.8	94.7	84.3	167.6
Control**		52.6	---	60.2	101.2
LSD at 0.05%		1.4	----	1.7	2.7

*Cucumber plants inoculated with the tested fungus, and sprayed with Azoxystrobin50 ml /100 H₂O and served as control.

**Cucumber plants were inoculated with the tested fungus only and served as control.

Table 2. Effect of spraying with two biocides and three RICs on enzymatic activity

Treatment		Enzymatic activity	
		Peroxidase 470 nm/mg protein	β- 1,3-glucanase mM/60min/mg protein
Tested biocide	Biocontrol T34	2.24	6.92
	Bio-Cure-B	2.21	4.60
	K ₂ HPO ₄	2.12	4.60
Tested RIC	C ₇ H ₆ O ₃	1.92	4.10
	KNO ₃	1.95	3.89
Azoxystrobin50 ml /100 H ₂ O		2.96	9.30
Control		1.10	3.23
LSD at 0.05%		0.433	1.77

Analysis of phenolic and carbohydrate contents

Illustrated results in Table 3 show that total phenols were increased as a result of application of the bioagents in cucumber plant tissues in all the investigated treatments compared with the control and even of Azoxystrobin treatment. The same trend was also detected for most of the applied bioagents in increasing the total carbohydrates. Plants treated with KNO₃ denote great increase in both total phenols and total carbohydrate contents, followed by Bio-control T34.

Plastic House Experiments

Effect of biocides and their mixtures with RICs on natural downy mildew infection (under protected cultivation)

On the efficient basis all tested biocides and resistance inducing chemicals (RICs) were chosen to evaluate their efficiency in managing the natural disease infection under plastic-house conditions. Presented results (Table 4) indicate that, all tested biocides and/or RICs caused significant reduction in the natural infection of downy mildew of cucumber plants compared

Table 3. Effect of spraying two biocides and three RICs on total phenols and total carbohydrates of cucumber leaves

Treatment		Total phenols (mg/g)	Total carbohydrates (mg/g)
Tested biocide	Biocontrol T34	3.14	9.11
	Bio-Cure-B	2.88	8.42
	K ₂ HPO ₄	2.33	8.22
Tested RIC	C ₇ H ₆ O ₃	1.95	7.41
	KNO ₃	3.77	9.87
Azoxystrobin50 ml /100 H2O		1.57	6.41
Control		1.34	5.34

Table 4. Effect of spraying with two biocides and three RICs either individually or in double combination on the disease severity of cucumber downy mildew under protected cultivations during growing seasons 2017and 2018

Treatment	First Season (2017)		Second season (2018)	
	Disease severity (%)	Reduction (%)*	Disease severity (%)	Reduction (%)*
Biocontrol T34	14.5	72.4	14.2	72.8
Bio-Cure-B	17.3	67.0	17.2	67.0
C ₇ H ₆ O ₃	23.1	56.0	23.0	65.5
K ₂ HPO ₄	20.6	60.7	20.9	60.0
KNO ₃	18.3	65.1	18.0	65.5
Biocontrol T34+ C ₇ H ₆ O ₃	9.2	82.5	9.3	82.2
Biocontrol T34+K ₂ HPO ₄	9.8	81.3	9.7	81.4
Biocontrol T34+KNO ₃	8.3	84.2	8.4	83.9
Bio-Cure-B+K C ₇ H ₆ O ₃	12.0	77.1	12.2	76.6
Bio-Cure-B+K ₂ HPO ₄	11.4	78.3	11.0	78.9
Bio-Cure-B+KNO ₃	10.0	81.0	10.2	80.5
Azoxystrobin	3.08	94.1	3.0	94.3
Control 1**	52.5	---	52.2	----
LSD at 0.05%	1.987		1.876	

* Comparing with the untreated control.

** Cucumber plants sprayed only with water and served as control.

with the control, and enhanced the average fruit yield in both growing seasons (Table 5). In this respect, tested biocides were also more efficient than resistance inducing chemicals (RICs). Moreover, application of tested RICs mixture with tested biocides was more effective in this regard than the application each of them individually. Application of Azoxystrobin 50 ml/100 H₂O recorded the highest value, in reducing the disease severity in 2017 and 2018 growing seasons (3.08 and 3.0%, respectively).

On the other hand, results in Table 4 show that, applying any tested biocide alone recorded the lowest averaged efficiency in reducing disease severity (67.5 and 72.4%). At the same time, application of tested biocide recorded the highest value in rising the fruit yield (88.4 and 92.0 kg/plot) and increased the plant length (246.8 and 249.6 cm) for Bio-Cure-B and Bio-control T34 in season 2017, respectively (Table

5). Also, the same trend was recorded in 2018 growing seasons.

DISCUSSION

During the few decades, the world is suffering great pollution by many pollutants including pesticides and fungicides. Therefore, the current strategies of pest management, especially on vegetables and fruits, depend on using biocides and resistance inducing chemicals (RICs) rather than pesticides, fungicides and/or applying these chemicals at the first periods of plant growth prior to fruit maturity. In this respect, cucumber plants are liable to infection by downy mildew under open fields as well as protected cultivation and the peak of infection reaches its maximum at fruit harvesting. Hence, this research aimed to use two biocides, *i.e.* Bio-control T34 and Bio-Cure-B as well as three antioxidants, *i.e.* salicylic

Table 5. Effect of spraying with two biocides and three RICs either individually or in double combination on the average of cucumber yield under protected cultivations, during growing seasons 2017 and 2018

Treatment	Yield parameters			
	Season 2017		Season 2018	
	Average plant length (cm)	Average yield (kg/plot) #	Average plant length (cm)	Average yield (kg/plot) #
Biocontrol T34	249.6	92.0	255.6	99.6
Bio-Cure-B	246.8	88.4	249.8	92.8
C ₇ H ₆ O ₃	233.8	75.2	242.8	76.8
K ₂ HPO ₄	234.8	77.2	241.8	82.0
KNO ₃	245.7	83.2	251.7	83.6
Biocontrol T34+ C ₇ H ₆ O ₃	287.0	104.0	294.0	105.6
Biocontrol T34+K ₂ HPO ₄	289.4	110.0	295.4	112.4
Biocontrol T34+KNO ₃	296.5	123.2	291.5	131.6
Bio-Cure-B+K C ₇ H ₆ O ₃	292.3	97.2	300.4	100.0
Bio-Cure-B+K ₂ HPO ₄	291.9	100.4	299.0	104.0
Bio-Cure-B+KNO ₃	290.5	116.8	278.5	122.0
Azoxystrobin	297.5	69.2	277.5	70.4
Control 1**	193.2	45.2	195.2	46.0
LSD at 0.05%, Treatment (T) = 2.3		2.8	2.4	2.9
Season (S) = n.s. , T x S = 3.1				

Each figure represents the mean of 4 replicates (40 plants).

acid, potassium dihydrogen phosphate and potassium nitrate as safe substances to evaluate either individually or mixture for their efficiency in managing the artificial inoculation with the causal agent of the disease under greenhouse conditions. Moreover, all tested biocides and resistance inducing chemicals (RICs) were applied under protected cultivation in two growing seasons (2017 – 2018) to manage the natural infection of the disease.

The obtained results of pot experiment showed that the tested biocides, Bio-control T34 and Bio-Cure-B as well as three antioxidants, *i.e.* salicylic acid, potassium dihydrogen phosphate and potassium nitrate, caused significant reduction in the disease severity associated with significant increase in plant length and foliage fresh, compared with check treatments. On the other hand, Azoxystrobin was more efficient than tested biocides and RICs in this regard. It is well known that RICs were reported as alternative and/or safe compounds for management of many diseases, especially those of vegetable crops (Muhanna, 2006; Abada *et al.*, 2008). The plant defense mechanisms against pathogen attack include the accumulation of antimicrobial secondary metabolites known as phytoalexins (Agrios, 2005); activation and/or synthesis of defense peptides and proteins that can have direct or indirect action during pathogenesis. (Castro and Fontes, 2005). In various plant species, resistance can be induced with elicitors such as SA, MeJA and CHI against a wide range of pathogens (Sharathchandra *et al.*, 2004; Amin *et al.*, 2007).

Results indicated that, all investigated treatments, increased peroxidase activity as compared with control (Table 2). The study by Ramos *et al.* (2008) suggested that two different responses to pathogen challenge are possible which are dependent upon the specific PGPR strain. Alkahtani *et al.* (2011) reported that treating cucumber plants with different tested abiotic inducer showed significant reduction in powdery mildew disease severity as well as there was increasing in enzymatic activities of peroxidase, polyphenoloxidase, chitinase and β -1,3-glucanase. Another study by Hamiduzzaman *et al.* (2005) indicated that some biochemical changes at the cellular level upon infection with

Perrospora viticola (the causal organism of downy mildew in grapevine) were observed. Callose deposition and lignification at the cellular level could contribute to prevent the infection of *P. viticola* in β -aminobutyric acid (BABA)-treated plants. In pearl millet, peroxidase activity has already been described to be associated with reduction in the rate of pathogen multiplication and spread (Shivakumar *et al.*, 2003). In the present study it was found significant increases in the activity of β -1, 3-glucanase in treated plant either with biocides or RIS compared to control. The obtained findings in the current study are in agreement with Ji and Kuc (1996) who reported an antifungal activity of cucumber β -1, 3- glucanase and chitinase.

In general, biocides and resistance inducing chemicals (RICs) proved significant effects to downy mildew control and reduces powdery mildew sporulation as well as the production of chasmothecia may eventually kill the entire mildew colony (Kiss *et al.*, 2004).

REFERENCES

- Abada, K.A., M.R. Hilall and S.H. Mostafa (2008). Induced resistance against powdery mildew in cucumber. *J. Biol. Chem. Environ. Sci.*, 3 (3): 45-56.
- Abd El-Kereem, F. (1998). Induction of resistance to some diseases of cucumber plants grown under greenhouse conditions. Ph.D. Thesis, Fac. Agric., Ain Shams Univ., Egypt.
- Abd El-Moity, T., M. Abd El-Moneim, M. Atia, A. Aly, M. Tohamy and A. Abou-Hadid (2003). Biological control of some cucumber diseases under organic agriculture. *Acta Hort.*, 608: 227-236.
- Agrios, G.N. (2005). *Plant Pathology*. 5th Ed. Academic Press, San Diego, USA.
- Ahmed, S., U. Narain, R.K. Prajati and C. Lal (2000). Management of downy mildew of cucumber. *Anna. Pl. Prot. Sci.*, 8: 254-255.
- Alkahtani, M., S.A. Omer, M.A. El-Naggar, E.M. Abd-El-Kareem and M.A. Mahmoud, (2011). Pathogenesis-related proteins and phytoalexin induction against cucumber powdery mildew. *Int. J. Plant Pathol.*, 1-9.

- Amin, A.A., E.S.M. Rashad and H.M.H. El-Abagy (2007). Physiological effect of indole-3-butyric acid and salicylic acid on growth, yield and chemical constituents of onion plants. *J. Appl. Sci. Res.*, 3 (11): 1554-1563.
- Castro, M.S. and W. Fontes (2005). Plant defense and antimicrobial peptides. *Protein and Peptide Letters*, 12: 11-16.
- Chowdhury, S.P., A. Hartmann, X. Gau and R. Borriss. (2015). Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42-A review. *Frontiers in Microbiol.*, 6 (780): 1-11.
- Dann, E.K. and B.J. Deverall (2000). Activation of systemic disease resistance in pea by an avirulent bacterium or a benzothiadiazole, but not by a fungal leaf spot pathogen. *Plant Pathol.*, 49 (3): 324-332.
- Feys, B.J. and J.E. Parker (2000). In replay of signaling pathways in plant disease resistance. *Trends Genet.*, 16: 449-455.
- Fisher, N. and B. Meunier (2008). Molecular basis of resistance to cytochrome bcl inhibitors. *FEMS Yeast Res.*, 8: 183-192.
- George, K. (2003). Downy mildew control in cucurbits. www.atra.Ncat.org, 1-5.
- Hamiduzzaman, M.M., G. Jakab, L. Barnavon, J.M. Neuhaus and B. Mauch-Mani (2005). β -aminobutyric acid (BABA)-induced resistance against downy mildew in grapevine acts through the potentiation of callose formation and JA signaling". *Mol. Plant-Microbe Int.*, 18: 819-829.
- Hammerschmidt, R., E. Nuckles and J. Kuc (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Plant Pathol.*, 20: 73-82.
- Hussein, M.A.M., M.H.A. Hassan, A.D.A. Allam and K.A.M. Abo-Elyousr (2007). Management of Stemphylium blight of onion by using biological agents and resistance inducers. *Egypt. J. Phytopathol.*, 35 (1): 49-60.
- Ji, C. and J. Kuc (1996). Antifungal activity of cucumber β 1-3-glucanase and chitinas. *Physiol. Mol. Plant Pathol.*, 49: 257-265.
- Kiss, L., J.C. Russell, O. Szentivanyi, X. Xu and P. Jeffries (2004). Biology and biocontrol potential of *Ampelomyces mycoparasites*, natural antagonist of powdery mildew fungi. *Biocontrol Sci. Technol.*, 14: 635-651.
- Mosa, A.A. (2002). Induced resistance in rice against blast disease using abiotic and biotic agents. *Ann. Agric. Sci., Ain Shams Univ., Cairo*, 47 (3): 993-1008.
- Muhanna, N.A.S. (2006). Pathological studies on root-rot and vine decline of cantaloupe in Egypt. Ph.D.Thesis, Fac. Agric., Cairo Univ., 218.
- Nandeeshkumar, P., J. Sudisha, K.K. Ramachandra, H.S. Prakash, S.R. Niranjana and H.S. Shekar (2008). Chitosan induced resistance to downy mildew in sunflower caused by *Plasmopara halstedii*. *Physiol. Mol. Plant Pathol.*, 72 (4-6): 188-194.
- Ramos, S.B., M.J. Barriuso, M.T. Pereyra de la Iglesia, J. Domenech and Gutierrez F.J. Manero (2008). Systemic disease protection elicited by plant growth promoting rhizobacteria strains: Relationship between metabolic responses, systemic disease protection, and biotic elicitors. *Phytopathol.*, 98: 451-457.
- Ran, L.X., L.C. Vanloon and P.A.H.M., Barker (2005). No role for bacterially produced salicylic acid in rhizobacterial induction of systemic resistance I *Arabidopsis*. *Phytopathology*, 95: 1349-1355.
- Reuveni, R. (1983). Resistance of Cucumis melo to *Pseudoperonospora cubensis*. *Ann. Appl. Biol.*, 102: 533-537.
- Sharathchandra, R.G., S. Niranjana Raj, N.P. Shetty, K.N. Amruthesh and H. Shekar Shetty (2004). A chitosan formulation Elexa induces downy mildew disease resistance and growth promotion in pearl millet. *Crop Prot.*, 23:881-888.
- Shivakumar, P.D., H.M. Geeth and H.S. Shetty (2003). Peroxidase activity and isozyme analysis of pearl millet seedlings and their implications in downy mildew disease resistance. *Plant Sci.*, 164: 85-93.

- Snell, F.D. and C.T. Snell (1953). *Colorimetric Methods of Analysis*. Toronto. New York, London: D. Van., Nostrand Company IVC., 111: 606-612.
- Song, W. and A.J. Keane (2006). Parameter screening using impact Factor and Surrogate-Based ANOVA Techniques, AIAA-2006-7088, 11th AIAA/ISSMO Multidisciplinary Anal. and Optimization Conf., Renaissance Portsmouth, Virginia, 6-8 Sep 2006.
- Thomas, C.E. (1996). Downy mildew. In: Zitter, T.A., D.L. Hopkins, C.E. Thomas (eds.): *Compendium of Cucurbit Diseases*, APS Press, St. Paul, MN, 25-27.
- Thomas, W. and R.A. Dutcher (1924). The determination of carbohydrate in plants by picric acid reduction method. The estimation of reducing sugar and sucrose. *J. Ame. Chem. Sci.*, 46 (6): 162-166.
- Xing, L., Z. Ding, Y. Wenxiang, D. Li and L. Daqun (2003). A study on the effect of *Bacillus* on downy mildew of cucumber. *Plant Prot.*, 29 (4): 25-27.
- Xing, F.Y., L.R. Xi, M.L. Guo and H.X. Wen (1997). Induced resistance to downy mildew in cucumber by chemicals. *Acta Phytopylacica Sinica.*, 24 (2): 159-163.

تقييم فعالية بعض المركبات الحيوية والمواد الكيماوية المستحثة للمقاومة في مكافحة البياض الزغبي على الخيار تحت ظروف الزراعة المحمية

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مرض البياض الزغبي في الخيار الذى يسببه الفطر *سيديوبيرنوسبورا كيوبنسس* والذي يسبب خسائر في المحصول في مصر خاصة تحت ظروف الزراعة المحمية حيث تتوافر درجات الحرارة المنخفضة والرطوبة العالية، تم اختبار فعالية رش الخيار باثنان من الكائنات المضادة في صورة مركبات تجارية ترايكودرما اسبريليم (بيوكنترول- تي ٣٤) وسيكومونس فلوريسنس (بايو- كيور- بي) وثلاثة من الكيماويات المستحثة للمقاومة وهى فوسفات البوتاسيوم ثنائية القاعدية وحامض السالسليك و نترات البوتاسيوم لدراسة تأثيرها علي مكافحة الإصابة بمرض البياض الزغبي تحت ظروف كل من صوبة الاصص و صوب الإنتاج، أوضحت نتائج صوبة الاصص عن أن رش نباتات الخيار باستخدام إما الكائنات المضادة أو الكيماويات المستحثة للمقاومة مرتين، إحداهما قبل ٥ أيام والثانية بعد تسعة أيام من إجراء العدوى الصناعية بالمسبب المرضى *سيديوبيرنوسبورا كيوبنسس*، أدى إلى انخفاض معنوي للمرض مع حدوث زيادة معنوية لطول النبات والوزن الطازج للمجموع الخضري مقارنة بمعاملة الكنترول، وكذلك أدت إلى زيادة في نشاط بيروكسيداز، بيثا ١,٣ - جلوكاناز، إلى جانب زيادة في الكربوهيدرات الكلية والفينول الكلي، وقد أجريت تجارب البيوت البلاستيكية (صوب الإنتاج) خلال موسمي ٢٠١٧ و ٢٠١٨ و تحت ظروف العدوى الطبيعية بالمسبب المرضى *سيديوبيرنوسبورا كيوبنسس* أوضحت النتائج المتحصل عليها أن معاملة نباتات الخيار بالكائنات المضادة و الكيماويات المستحثة للمقاومة إما بشكل فردي أو في مخلوط أحدث نقصاً معنوياً للمرض مع إحداهما زيادة معنوية في المحصول الناتج وزيادة في طول النبات في كلا الموسمين مقارنة بمعاملة الكونتورول، كانت معاملة نباتات الخيار بخليط من الكائنات المضادة مع الكيماويات المستحثة للمقاومة أكثر فعالية من رش كل منها منفرداً، في هذه الدراسة، كانت المبيدات الحيوية التي تم اختبارها أكثر فعالية في مقاومة المرض عن الكيماويات المستحثة للمقاومة.

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