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Role of Some Essential Plant Oils, Fungicides and Inducer Resistance Elicitors on The Management of Cucumber Downy Mildew

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ABSTRACT

The *in vitro* inhibitory effect of the essential plant oils (EPOs) of citronella, clove, neem and thyme, the fungicides Acrobat-mancozeb, Dithane M-45, Kocide 2000, Previcure-N and Ridomil-Gold-Plus and the inducer resistance elicitors (IREs), Bion (BTH), chitosan, humic acid and salicylic acid, on sporangial germination of the fungus-like *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov, the cause of cucumber downy mildew, was investigated *in vitro*. The role of the tested treatments in the management of cucumber downy mildew was, also, evaluated under greenhouse conditions. In addition, the effect of the combination of the sprayed, EPO (clove), the fungicide (Previcure-N) and IRE Bion (BTH) on the management of the disease under field conditions was investigated. The inhibitory effect of the tested EPOs, fungicides and IREs on sporangial germination of *P. cubensis* showed that they caused a significant reduction in the germinated sporangia compared with the control treatment. In this concern, the tested fungicides were the most efficient ones followed by the EPOs and then IREs. Management of the disease showed the same trend of *in vitro* assessment when they sprayed on artificially inoculated cucumber plants with the sporangial inoculum of the pathogen under greenhouse conditions. When cucumber plants were praying, under field conditions the combination of the sprayed, EPO (clove), the fungicide (Previcure-N) and IRE Bion (BTH) resulted in the superior treatment for managing the disease during 2021 and 2022 growing seasons at Giza governorate compared with plants sprayed with any of the EPO (clove), Previcure-fungicide and IRC (BTH) alone or in bi-combination, as well as control treatment.

Defense-related enzymes, i.e., chitinase, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase responsible were greatly increased in the leaves of all sprayed treatments by the tested materials compared with control treatment. In addition, plants sprayed with the tested IRC (BTH) followed by the EPO (clove) recorded the highest activity of all enzymes than plants sprayed with the tested Previcure-N fungicide.

INTRODUCTION

The cucumber (*Cucumis sativus* L.) is one of the most famous and popular cucurbit crops in Egypt for local consumption and exportation. It is liable to infection by bacterial, fungal and viral diseases in addition to nematode infection and physiological disorders. However, fungal diseases, especially downy mildew, are caused by the fungus-like *Pseudoperonospora cubensis*, (Berk. and Curt.) Rostov severely affected commercial cucumber production in the protective plantations and the open fields (Zitter *et al.*,1996). The causal fungus is air- transmitting pathogen, which its Oospores are unknown until now. However, the pathogen is distributed worldwide and threatens cucumber plantations.

The successful management of these pathogens is essential to reduce their hazardous effects on cucumber plantations. In addition, Egypt has a relative advantage of increasing the cultivated area and the pr0duced fruit yield, e.g., fertile soil, warm weather, sheep workers, pioneer farmers, enough am0unts of water etc. Angular chlorotic lesions - which appear on the foliage growth- are considered optimum symptoms of the disease. Bounding by leaf veins could cause appearing of lesions in an angular shape. During humid conditions, an inspection of the underside of the leaf reveals gray-brown to purplish-black fungal growth. This downy material is the sporulation of the pathogen. Magnification of the sporulation reveals the acutely and dichotomously branched sporangiophores bearing lemon-shaped sporangia. Eventually, leaves will turn necrotic and curlupwards. Generally, the downy mildew of cucurbits is sometimes called wildfire because of how rapidly it progresses, as if the crop were burned by fire. The pathogen must overwinter in an area that does not experience a hard frost, and where wild or cultivated cucurbits are present. The spores are dispersed via wind to neighboring plants and fields and often over long distances. Symptoms appear 4 to 12 days after infection. The pathogen thrives under cooland moist conditions but can do well under a wide range of conditions. Optimum conditions for sporulation are 15- 20°C with 6 to 12 hours of moisture present, often in the form of morning dew. Even when high daytime temperatures are not favorable for the pathogen >35°C, nighttime temperatures may be very suitable (Zitter *et al.*,1996). Chemical management of plant diseases is in most cases efficient in reducing the hazard of most plant diseases, but it extremely could cause environmental pollution and an increase in the accumulation of toxic substances in the chain of human food, especially in case of the fresh fruits. On theother hand, using other trials of disease management, viz. biological control, plant extracts, essential oils, inducer resistance elicitor, sanitary methods and agricultural practices, each alone, is not enough to give adequate results (Rhouma *et al.*, 2022).

This work was aimed to evaluate the efficiency of some EPOs, fungicides and IREs and, each alone (under greenhouse conditions) or in combination among clove, BTH and Previcure-N (under filed conditions) against cucumber downy mildew. Also, the activity of defense-related enzymes *i.e.*, chitinase, PAL, PO and PPO in the sprayed cucumberleaves with two EPOs, fungicides and IREs was estimated.

MATERIALS AND METHODS

1.Source of Essential Plant Oils*:*

The essential plant oil of citronella (*Cymbopogon nardus)*, clove (*Syzygium aromaticum=Eugenia caryophyllata*), neem (*Azadirachta indica*) and thyme (*Thymus capitatus)* were obtained from International Flavors and Plant oils Inc., Giza, Egypt. The essential oils were stored in dark bottles at 5ºC for further investigation.

2.Effect of some essential plant oils (EPOs), fungicides and inducer resistance elicitors (IREs), on sporangial germination of P.cubensis :

The effect of some EPOs, fungicides and IREs on the sporangial germination of the fungus-like *Pseudoperonospora cubincis* (Berk. and Curt.) Rostov *was investigated in vitro*.

2.1. Preparation of EPOs:

The essential plant oil of citronella, clove, neem and thyme was diluted to the concentrations of 1, 2, 3, 4 and 5% using distilled sterile water plus a few drops from Tween-20 (to make emulsion).

2.2. Preparation of the Tested Fungicides:

The concentrations of 100, 200, 300, 400 and 500 ppm. of the fungicides Acrobat-mancozeb (dimethmorph+mancozeb), Dithane M-45 (mancozeb), Kocide-2000 **(**copper hydroxide) Previcure-N (propamocarb hydroxychloride) and Ridomil-Gold Plus (metalxyl + copper hydroxide) were prepared to depend on their active ingredient.

2.3. Preparation of IRCs:

The inducer resistance elicitors (IREs) Bion (benzothiadiazole; BTH), chitosan (cellulose with the hydroxyl at position C2 substituted with an acetamido group) , humic acid (C187H186O89N9S1) and salicylic acid (monohydroxybenzoic acid) were prepared at 10, 20, 30, 40 and 50 mM depending on their molecular weight.

Naturally severely infected cucumber leaves by downy mildew were collected from a field located at Imbaba County,Giza governorate and incubated at 25±1°C under humid conditions to encourage formation of sporangia. The freshly formed sporangia were collected by sterilized brush from the infected leaves and put in each concentration of the tested EPOs, fungicides and IREs. One m1. of sporangial suspension was placed on each sterilized glass slide,borne on two glass rods in a sterilized Petri dish containing a piece of wetted cotton by sterilized distilled water to amend high relative humidity. The same was achieved for a spore suspension put in distilled sterilized water only as a control treatment. Five Petri-disease were used for each concentration of each EPOs, fungicide and IREs. Preparations were incubated in darkness at 25±1 °C for 24 hours. One drop of lactophenol cotton blue stain was added at the time of slide examination to kill and fix the germinated sporangia. The percentages of sporangial germination of the different treatments were counted as empty sporangium (cleared sporangium; which zoospores released out of the sporangium) in a total of 100 sporangia in each treatment. The germinated sporangia were counted and the average percentages of germination were estimated and recorded for each treatment.

3.Greenhouse Experiment:

Severely infected cucumber leaves by downy mildew collected from Imbaba County, Gizagovernorate were put in plastic bags provided by a piece of wetted cotton overnight in the laboratory (20-25ºC) to encourage the sporulation of the causal fungus. Using a sterilized camelbrush the sporangiophores and sporangia were put in a glass beaker (250 ml) containing sterilized distilled water. The prepared sporangial spore suspension was adjusted to $1X10^3$ sporangium/ml water with the aid of hemocytometer just before spraying on the cucumber plants.

The effect of the tested EPOs *i.e.*, citronella, clove, neem, and thyme, the fungicides *i.e.*, Acrobat-manzozeb, Diathane M-45, Kocide-2000, Previcure-N and Ridomil Gold Plus and the IREs *i.e.*, Bion(BTH), chitosan, humic acid and salicylic acxid) on the infection severity ofcucumber downy mildew caused by *P.cubensis* was achieved using artificial inoculation by sporangial suspension of the causal fungus under greenhouse conditions in order to choose the most efficient EPO, fungicide and IRE in order to estimate their efficacy on the management of the disease under field conditions, either alone or in different combinations.

Plastic pots (25 cm in diameter) containing disinfested clay soil by 5% formalin were seeded with cucumber seeds. Five seeds (cv. Amera) were sown in each pot, irrigated and left to grow then thinned into two plants in each pot, ten days after sowing. Five pots were prepared for each treatment. The grown plants (aged three weeks) were sprayed with the tested EPOs (5%), the fungicides at 150 g, 250 g, 250 g, 250 ml. and 200 g, respectively and IRCs (50 mM) four days before the artificial inoculation with sporangial suspension ($1x10^3$/ ml water) of the pathogen by sterilized plastic sprayer. Also, the plants were resprayed with tested EPOs, fungicides and IREs two times by the previous rates 10, 20 and 30 days after the inoculationby the tested pathogen. Plants sprayed with sporangial suspension only without other treatments were left as control treatments. The grown plants were irrigated when it was necessaryand fertilized with a local compounded fertilizer (one g for each pot), three weeks after sowing then 3 times weekly.

Disease severity was assessed one week after each spray of the tested treatments and the averages were recorded using a modified 0-9 scale (Call *et al.*,2012). Also, plant length (cm) in addition to foliage fresh weight (g) were estimated and recorded.

4-Field experiments:

A piece of land located at El-Badreasheen County, Giza governorate (clay soil) was prepared for sowing cucumber plants (cv. Amera), where cucumber downy mildew occurs yearly by severe infection. The land was prepared for planting cucumber plants by making ridgesof 100 cm width and then divided into plots (42 m^2). Cucumber transplants (15 days of age) were transplanted on the ridges 30 cm apart on mid of May 2021 and 2022. The transplants were left to grow under natural infection by the disease. All agricultural practices, *i.e.* irrigation, weeds and pest control as well as fertilization were applied according to the standard recommendations of Min. of Agric. and Land Recl.

The EPO (clove; 5 %), Previcure-N fungicide (250 ml/100 l water) and the IRC (BTH; 50 mM) were evaluated for their efficacy in the management of the disease under field conditions, under the natural infection by downy mildew. In this respect, the grown plants were left to the natural infection by the causal fungus like *P. cubensis* and then sprayed at the first appearance of downy mildew symptoms by the tested treatments *i.e.*, EPO (clove), the Previcure-N fungicide and the IRC (BTH), each alone or in different combination. Unsprayed plants with the tested treatments were left as control treatments. Three plots (each of 42 m^2) were used as replicates for each treatment. Downy mildew severity was rated in each season using the devised scale (0-9) by Call *et al.* (2012) and the averages were recorded. At the same time, fruit yield kg/ plot (42 m^2) was weighed in each harvest and the averages were calculated and recorded.

5.Disease Assessment:

The artificially and naturally infected plants were carefully examined to rate the severity of cucumber downy mildew depending on the devised scale (0-9) by Call *et al.* (2012) based on thepercentage of symptomatic infected leaf area (0 = 0%, 1 =1–5%, 2 = 6–10%, 3 = 11–20%, 4 = 21–30%, 5 = 31–50%, 6 = 51–65%, 7 = 66–80%, 8 = 81–99%, and 9 = 100%). The severity of the disease was calculated using the following formula:

$$\textbf{Disease severity \% = £ (nxv) X 100 / 9 N}$$

Where:

n = number of infected leaves in each category. v = numerical values of each category.N = total number of infected leaves.

Also, plant growth vigor was assessed as += Poor growth, ++= Good growth, +++=Very goodgrowth and +++++= Excellent growth.

6.Biochemical Activity of Defense-Related Enzymes:

The activity of chitinase (CH), phenylalanine ammonia-lyase (PAL) peroxidase (PO) and polyphenol oxidase (PPO), was measured in cucumber leaves free from the pathogen, inoculated with the pathogen, the EPOs (citronella and clove), IRCs (BTH and chitosan)-treated plants. Samples were taken one week after the second treatment of the plants by the different treatments for enzyme assays. Cucumber leaf samples (5 g) were homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) in an ice bath for enzyme assays. Crude leaf enzyme extract was prepared according to Ni *et al.* (2001). The homogenates were then centrifuged at 10,000 *g* for 10 min. Supernatants were used to analyze the defense-related enzymes CH, PAL, PO and PPO activities.

6.1. The activity of CH enzyme:

Chitinase activity was determined using the described method by Waterhouse *et al.* (1961)and represented as g N-acetylglucosamine µg N-acetylglucosamine × 10^3/min/g fresh weight (µgNAGA X 10^3/g FW).

6.2. The Activity of PAL Enzyme:

Phenylalanine ammonia-lyase (PAL) activity was measured according to the method of Burrell and Rees (1974). The reaction mixture contained 0.03M L-phenylalanine and 0.2 ml enzyme extract in a total of 2.5ml of sodium borate buffer (pH 8.8). This reaction mixture was kept in a water bath at 37°C for 1 h, and 0.5ml of 1 M (trichloroacetic acid)TCAwas added. The amount of trans-cinnamic acid formed from L-phenylalanine was measured spectrophotometrically at 290 nm. Enzyme activity was expressed as microgramsof trans-cinnamic acid $h^{-1}mg^{-1}$ protein.

6.3. The Activity of PPO Enzyme:

Polyphenol oxidase (PPO) activity was determined according to the method proposed by Mayer *et al.* (1965). The reaction mixture was containing 200 µl enzyme extract and 1.5ml of 0.01 M catechol. The activity was expressed as changes in absorbance at 495 nm·$min^{-1}mg^{-1}$protein.

6.4. The Activity of PO Enzyme:

To estimate peroxidase activity (PO) activity, 50 µl of enzyme extract was added to 2.85ml of 0.1 M phosphate buffer (pH 7.0) and mixed with 0.05 ml of 20 mM guaiacol reagent(Fu and Huang,2001). The reaction was started by the addition of 0.02 ml of 40 mM hydrogen peroxide to the mixture. The rate of increase in absorbance at 470 nm was measured over 1 min. One unit of enzyme activity was defined by the change in absorbance of 0.01 for 1 g fresh weight per minute.

7.Statistical Analysis*:*

Data were statistically analyzed using the standard procedures for complete randomized block and split designs as reported by Snedecor and Cochran (1989). The averages were compared at0.05 level using the least significant differenc (L.S.D.) according to Fisher (1948).

RESULTS

1.In vitro Effect Of Four EPOs On Sporangial Germination of *P.cubensis:*

Table (1) shows that the tested EPOs, *i.e.*, citronella, clove, neem and thyme resulted in significant inhibition to the germinated sporangia compared with the control treatment. At the concentration of 5% t, no sporangial germination was recorded. love oil was the superior treatment in this regard, with 31.8% sporangial germination, on average with 66.7% efficiency followed by thyme oil, being 36.3% sporangial germination, on average with 62.0% efficiency. Meanwhile, neem oil was the lowest effect on sporangial germination of the causal pathogen, being 41.6% sporangial germination, on average with 56.4%

efficiency. Citronella oil was of intermediate effect in this concern, being 38.6% sporangial germination, on average with 59.5% efficiency The inhibitory effect on the sporangial germination by the tested EPOs was gradually increased by increasing their concentration. Control treatment recorded 95.4% sporangialgermination.

Table 1. Effect of four essential plant oils (EPOs) on sporangial germination of *P.cubensis*, 24 hours after incubation at 25±1 °C.

EPOs	% Sporangial germination at concentration of (%)					Mean	% Efficiency
	1	2	3	4	5		
Citronella	72.8	61.6	43.4	11.0	0.0	38.6	59.5
Clove	69.4	54.4	28.8	6.6	0.0	31.8	66.7
Neem	79.0	62.0	48.8	18.0	0.0	41.6	56.4
Thyme	71.8	59.2	41.4	9.2	0.0	36.3	62.0
Control *	95.4	95.4	95.4	95.4	95.4	95.4	----
Mean	73.3	59.3	40.6	11.2	0.0		-----

* Control is not included in the mean.
L.S.D. at 5 % for: EPOs (E) = 2.2, Concentrations (C) = 2.9 and E x C = 3.2.

2.In vitro effect of four inducer resistance elicitors on sporangial germination of P. *cubensis:*

Table (2) reveals that the tested IREs *i.e.*, Bion (BTH), chitosan, humic acid and salicylic acid caused, also, significant inhibition to the germinated sporangia of the causal fungus compared with the control treatment. At the concentration of 5o mM no sporangial germination occurred. BTH was the most efficient one, being 41.5% sporangial germination, on average, with 56.9 % efficiency followed by chitosan, being 45.0 % sporangial germination, on average with 53.2% efficiency, then salicylic acid, being 46.0 % sporangial germination, on theaverage, with 52.2 % efficiency. Humic acid was the lowest effect in this concern, being 48.4% sporangial germination, on average with 49.7% efficiency The inhibitory effect of thetested IREs on the sporangial germination was gradually increased by increasing their concentration. Control treatment recorded 96.2% sporangial germination.

Table 2. Effect of four inducer resistance elicitors (IREs) on sporangial germination of *P. cubensis*, 48 hours after incubation at 25±1 °C.

IREs	% Sporangial germination at concentration (mM)					Mean	% Efficiency
	10	20	30	40	50		
Bion (BTH)	78.6	65.6	45.8	17.4	0.0	41.5	56.9
Chitosan	80.8	71.6	50.4	22.0	0.0	45.0	53.2
Humic acid	85.4	72.6	55.8	28.2	0.0	48.4	49.7
Salicylic acid	81.8	72.0	51.4	24.8	0.0	46.0	52.2
Control *	96.2	96.2	96.2	96.2	96.2	96.2	----
Mean	81.7	70.5	50.9	23.1	0.0		-----

* Control not included in the mean
L.S.D. at 0.05 for: Inducer resistance elicitors (I) = 2.1, Concentrations (C) = 2.7 andI x C = 3.5.3.

3.In vitro effect of five fungicides on sporangial germination of *P.cubensis*:

Data presented in Table (3) indicate that the tested fungicides caused a significant reduction in the germinated sporangia compared with the control treatment. No germination occurred at 500 ppm by Acrobat-mancozeb, Previcure-n and Ridomil Gold Plus. In addition, Previcure-N was the most efficient one followed by Ridomil Gold Plus then Acrobat-mancozeb, being 41.4, 43.7 and 42.8% sporangial germination, on average, of 56.3, 54.9 and 53.9% efficiency, respectively. Both Dithane M-45 and Kocide-2000 resulted in the lowest figures of efficiency, being 42.4 and 44.4%, respectively. The

inhibitory effect on the sporangial germination was gradually increased by increasing the concentration of the fungicide. Control treatment recorded 94.8% sporangial germination.

Table 3. Effect of five fungicides on sporangial germination of *P. cubinsis*,24 hours after incubationat 25±1°C.

Fungicides	% Sporangial germination at concentration of (ppm)					Mean	% Efficiency
	100	200	300	400	500		
Acrobat-Mancozeb	77.2	63.8	45.2	32.4	0.0	43.7	53.9
Dithane M-45	83.8	70.6	52.4	40.0	26.2	54.6	42.4
Kocide 2000	81.6	68.4	50.0	39.0	24.4	52.7	44.4
Previcure-N	74.0	60.6	43.4	28.8	0.0	41.4	56.3
Ridomil Gold Plus	76.8	61.8	45.6	30.0	0.0	42.8	54.9
Control *	94.8	94.8	94.8	94.8	94.8	94.8	-----
Mean	78.7	65.0	47.3	34.0	10.1	----	-----

* Control is not included in the mean.
L.S.D. at 0.05 for: Fungicides (F)= 2.3, Concentrations (C)=2.3 and F x C = 3.1.

4.Greenhouse Experiment:

Results shown in Table (4) show that a significant reduction in the severity of cucumber downy mildew with a significant increase in the plant length and foliage fresh weight occurred due to spraying the tested EPOs, fungicides and IREs compared with the control treatment. The tested fungicides were the most efficient in reducing the severity of the disease and increasing plant length(cm) and foliage fresh weight (g), being 6.1% (81.9% efficiency), 88.9 cm and 430.5 g followed by IREs, being, 12.4% (63.7 % efficiency), 81,0 cm and 420.8 g, then EPOs, being 13. 9% (58.4% efficiency), 78.1 cm and 415.2 g, on average, respectively. In addition,Previcure-N fungicide, the IRE BTH and The PEO clove were the most efficient materials in each of the fungicides, IREs and EPOs, being 4.4% (86.9% efficiency), 94.7 cm and442.4 g for Previcure-N, 11.1 % (67.1% efficiency), 84.0 cm and 426.1 g for BTH and 12.1 % (63.9% efficiency), 81.3 cm and 420.5 g for clove oil respectively. Meanwhile, the lowest effective materials of fungicides, IREs and EPOs were DithaneM-45, humic acid and neem. The respectivefigures were 77.6%,83.5 cm and 412.8 g for DithaneM-45; 60.6 %, 77.8 cm and 416.0 g for Humicacid and 54.3 %, 75.5 cm and 409.6 g for neem oil, respectively. The other materials recorded intermediate values.

Control treatment recorded 33.5% disease severity, 53.1 cm plant length and 249.8 g foliagefresh weight.

5.Field Experiments*:*

Table (5) reveals that a significant reduction in the natural infection by downy mildew was recorded due to spraying cucumber plants with the EPO (clove), Previcure-N fungicide, and IRE (BTH), each alone or in different combinations, compared with control treatment. In addition, the combination of clove oil +Previcure-N+ BTH was the superior and most efficient treatment in this regard, being 2.2% disease severity and producing the highest values of fruit yield, 104.9 Kg/plot (42m^2), followed by spraying the combination between Previcure- N+BTH (4.1% disease severity and 97.5 kg fruit yield/ plot 42 m^2) and Previcure-N+clove oil (4.5% disease severity and 95.8 kg fruit yield/ plot 42 m^2). On the other hand, spraying each of BTH, clove and Previcure-N alone recorded 12.3, 13.8 and 5.7 % disease severity and 78.4, 75.2, and 92.3 kg fruit yield/plot 42 m^2, respectively. Meanwhile, spraying of BTH+clove oil recorded intermediate values, being 8.1% disease severity and 82.7 kg fruit yield/plot 42 m^2.

Control treatment recorded the highest figure of disease severity (54.7%) and the lowestweight of the produced fruit yield (56.5 kg fruit yield/plot 42 m^2).

No significant effect was detected due to the effect of the growing seasons on theestimated disease severity and fruit yield.

Table 4. Effect of spraying cucumber plants (cv. Amera) with some EPOs, fungicides and IREs onthe severity of downy mildew, plant length and foliage fresh weight under greenhouse conditions.

Treatments	% Downy mildew severity 7 days after spraying (sprays)				Mean	% Efficiency	Plant length (cm)	Weight of the foliage growth (g)
	First	Second	Third	Fourth				
EPOs								
Citronella	7.9	10.8	16.7	23.2	14.7	56.1	76.7	412.0
Clove	6.6	9.1	13.1	19.4	12.1	63.9	81.3	420.5
Neem	8.0	11.2	17.9	23.9	15.3	54.3	75.5	409.6
Thyme	7.2	10.3	15.2	21.8	13.6	59.4	78.9	418.8
Mean	7.4	10.4	13.2	22.1	13.9	58.4	78.1	415.2
L.S.D. at 0.05 for: Treatments (T)= 2.7, Downy mildew severity (D) =1.3 and T x D = 3.0.						--------	LSD at 0.05=1.8	LSD at 0.05= 2.5
Fungicides:								
Acrobat	3.0	5.4	7.2	8.6	6.0	82.1	89.0	438.6
Mancozeb	5.0	7.0	8.9	9.4	7.6	77.6	83.5	412.8
Dithane M-45	5.0	7.0	8.5	9.2	7.4	77.9	84.4	418.5
Kocide 2000	2.5	3.4	5.5	6.2	4.4	86.9	94.7	442.4
Previcure-N	2.8	4.8	6.0	6.5	5.0	85.1	93.0	440.0
Ridomil Gol Plus								
Mean	3.7	5.5	7.2	8.0	6.1	81.9	88.9	430.5
L.S.D.at 0.05 for: Treatments (T)= 2.5, Downy mildew severity (D)=1.0 and T x D = 2.8.						----------	LSD at 0.05=2.6	LSD at 0.05= 2.8
IREs:								
Bion (BTH)	6.0	8.4	13.1	17.0	11.1	67.1	84.0	426.1
Chitosan	6.5	9.9	14.0	20.0	12.6	62.4	79.8	418.6
Humic acid	6.8	10.5	15.1	20.5	13.2	60.6	77.8	416.0
Salicylic acid	6.2	8.9	13.9	18.0	11.8	64.8	82.3	422.5
Control *	15.8	28.0	39.6	50.4	33.5	--------	53.1	249.8
Mean	6.4	9.4	14.5	18.9	12.4	63.7	81.0	420.8
L.S.D. at 0.05 for: Treatments (T)= 2.9, Downy mildew severity (D)=1.4 and T x D = 3.2.						-------	LSD at 0.05=1.5	LSD at 0.05= 2.9

* Control is not included in the mean.

Table 5. Effect of spraying the systemic Previcure-N fungicide, the EPO (clove) and the IRE (BTH) in different combinations on the severity of cucumber (cv. Amera) downy mildewand fruit yield, field experiment at Imbaba County, Giza governorate during 2021 and 2022 growing seasons.

Treatment	% Downy mildew severity during		Mean	Fruit yield / plot (42m²)during		Mean	Plant growth vigor
	2021	2022		2021	2022		
BTH	12.0	12.6	12.3	78.9	78.0	78.4	++
Clove (C)	13.5	14.1	13.8	75.8	74.5	75.2	++
Previcure-N (PN)	5.7	5.7	5.7	92.0	92.6	92.3	++++
BTH+C	8.2	8.0	8.1	82.4	83.0	82.7	+++
PN+BTH	4.1	4.0	4.1	97.5	98.0	97.8	++++
PN+C	4.4	4.5	4.5	95.8	95.0	94.6	++++
PN+BTH+C	2.1	2.3	2.2	105.4	104.3	104.9	++++
Control *	54.3	55.0	54.7	55.9	57.0	56.5	+
Mean	7.1	7.3	-----	89.7	89.3	-----	----

* Control is not included in the mean.

6.Biochemical activity of defense-related enzymes enzyymes:

Table (6) indicates that spraying the tested two materials of each of EPOs, (clove andneem), fungicides (DithaneM-45 and Previcure-N) and IREs (BTH and humic acid) on cucumberplants caused a considerable increase in the activity of defense-related enzymes *i.e.*, chitinase, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase in comparison with control treatment. Generally, BTH was the most efficient one in increasing the four enzymes *i.e.*, being 0.497, 0.585, 0.456 and 0.678, respectively in cucumber leaves compared with the other treatments and the control. Meanwhile, Dithane M-45 recorded the lowest activity of these enzymes, being0.468, 0.548, 0.429 and 0.623, respectively. The other materials resulted in intermediate activity for these enzymes. Un-sprayed cucumber leaves recorded the lowest activity for these enzymes, being 0.312, 0.504, 0.398 and 0.587, respectively.

Table 6. Effect of spraying cucumber plants (cv. Amera) with EPOs (clove and clove), Dithane M-45 and Previcure-N fungicides and IREs (humic acid and BTH) on the activity of fourdefense-related enzymes.

Treatments	Activity of *			
	Chitinase (CH)	Phenylalanine ammonia-lyase (PAL)	Peroxidase (PO)	Polyphenol oxidase (PPO)
Clove	0.487	0.567	0.451	0.645
Neem	0.476	0.522	0.446	0.639
DithaneM-45	0.468	0.458	0.429	0.623
Previcure-N	0.478	0.560	0.433	0.627
BTH	0.495	0.574	0.458	0.652
Humic acid	0.490	0.570	0.51	0.648
Control	0.312	0.504	0.398	0.587

* Expressed as absorption after 30 sec. at the appropriate wavelength.

DISCUSSION

During the last few decades, the world suffers from great pollution from many pollutants including agrochemicals,especially in developing countries.So, the strategy of management of plant pests, especially of vegetables and fruits depends on using alternative safe methods for pest management rather than pesticides. Therefore,scientists are trying to produce pesticides that are toxic to pests and do not have a harmful effect on the environment and living organisms, or at least produce pesticides that quickly decompose into non-toxic substances for living organisms.

In recent years, the emergence of downy mildew had a significant impact on the productionof cucurbits and disease control systems at multiple scales all over the world.In this concern, cucumber plants are liable to infection by many fungal diseases; however downy mildew caused by the fungus-like *Pseudoperonospora cubensis* is the most devastating one. It is an Oomycetes pathogen more closely related to water molds such as Phytophthora than to true fungi. The infection can destroy the grown plants in a few days if the Perivale environment condition is suitable for the fungus (Abel-Kader *et al.*,2012; Call *et al.*,2012; Khalil and Ashmawy, 2019; Falade,2021; Shoukry *et al.*,2021 and Rhouma *et al.*,2022).

There are multiple pathotypes of *P. cubensis;* watermelons, pumpkins,and squash are incompatible with several pathotypes,while cucumbers and cantaloupe are susceptible to them all. Each pathotype may have several strains,to which various cultivars of each type.

The tested EPOs citronella, clove, neem and thyme, the fungicides, *i.e.* Acrobat Mancozeb, Dithane M-45, Kocide 2000, Previcure-N and Ridomil Gold Plus and the IREs, *i.e.* Bion (BTH), chitosan, humic acid and salicylic acid resulted in significant inhibition of the germinated sporangia of the causal fungus compared with control treatment, The inhibitory effect was increased gradually by increasing the concentration of these materials compared to control treatment. In addition, the fungicides were the most efficient ones in this regard followed by EPOs then IREs.

Although our study found that spore germination was inhibited by the tested EPOs, fungicides and IREs, the levels of inhibition were different. This could be attributed to differences in the mode of action of each compound and spore sensitivity to these materials. Infection and spread of fungal pathogens occur mainly through spores. Therefore, inhibition of sporulation is desirable for targeting the pathogen to prevent or slow down intra-plant and interplant disease spread.

Greenhouse experiment revealed that there were significant reductions to the severity of cucumber downy mildew with a significant increase in the plant length and foliage fresh weight due to spraying the tested EPOs, fungicides and IRC compared with the control treatment. The tested fungicides were the most efficient in reducing the severity of the disease,plant length(cm) and foliage fresh weight (g), followed by IREs then EPOs. In addition, Previcure-N fungicide, the BTH and clove oil, were the most efficient materials in each of the tested fungicides, IREs and EPOs. Meanwhile, the lowest effective materials of fungicides,IREs and EPOs were DithaneM-45, humic acid and neem. The other materials recorded intermediate values.

Field experiments during 2021 and 2022 growing seasons revealed that spraying cucumber plants with Previcure-N fungicide, BTH as RIE and clove as EPO caused a significant reduction in the disease with a significant increment to the harvested fruit yield in comparison with the control treatment. significant reduction to the natural infection by downy mildew due to spraying cucumber plants with the EPO (clove), Previcure-N fungicide, and IRE (BTH), each alone or in different combinations, compared with the control treatment. In addition, the combination of clove oil +Previcure-N+ BTH was the superior and most efficient treatment in this regard, being 2.2% disease severity and producing the highest values of fruit yield, 104.9 Kg/plot ($42m^2$), followed by spraying the combination between Previcure-N+BTH and Previcure-N+Clove oil. Meanwhile, spraying of BTH+clove oil recorded intermediate values of disease severity and fruit yield.

Similar results were obtained by Rabea *et al.* (2003); Abdel-Kader *et al.* (2012); Mohamed *et al.* (2016); Utobo *et al. (*2016) and AL-Aswad and Al-Azzawi. (2021).

Because of destruction and aggressiveness of mildew disease, we could be forced to use chemical control and it may be difficult to control of disease without use of fungicides. Importance of fungicide's role is well known in reduction of the disease (Mc Grath, 2001) and many hypotheses could explain the role of IREs, where induced acquired resistance was induced by restricted infection is not due to a specific component of the pathogen, but rather to gradual appearance and persistence of a level of metabolic perturbation leading to stress on the host.

Due to the lack of significant resistance in cultivars and efficient fungicides, fungicides are often used to treat downy mildew in cucurbits. When climatic circumstances encourage disease growth, an active fungicide program is typically required for crop prevention and protection to minimize output losses. An effective, correctly scheduled fungicide application is the most crucial component of a successful downy mildew control program. As a result, when environmental circumstances promote disease growth, early and frequent fungicide treatments are essential to preserving the crop (Savory *et al*., 2011). So, the key to that is applying systemic fungicides targeted to the pathogen starting when there

is a risk of the pathogen being present. Systemic fungicides are needed for control on the underside of leaves.Each year there can be changes to the fungicides recommended as the pathogen develops resistance or new products are registered. Because these fungicides have targeted activity, additional fungicides must be added to the program when there is a need to manage other diseases such as downy mildew (Keinath *et al*., 2017 and Rhouma *et al.*,2022).

Because downy mildew is difficult to control if chemical sprays are not used promptly. As a result, an effective, appropriately scheduled fungicide treatment is the most crucial component of a successful downy mildew control strategy. Also, the key to doing so is using pathogen-specific systemic fungicides as soon as there is a danger of the pathogen being present. Broadly an integrated approach is important (Mc Grath, 2001; Lebeda and Cohen, 2011; Abada and Eid,2013; Holmes *et al*., 2015; Abada and Attia,2017; Patel et al., 2021; Salcedo *et al*., 2021 and Rhouma *et al.*,2022).

Liebenberg *et al*. (2005) evaluated *in vivo* the efficacy of selected plant extracts; neem (*Azadirachta indica)* derivatives (neem oil, neem cake powder and neem leaf powder) and the commercial fungicide Kocide DF, against bean rust. Results revealed a significantly high inhibitory effect on rust severity, incidence and uredospores germination obtained by neem oil. Zyton and Ahmed (2016) found that lemongrass, neem and thyme were efficient *invitro* and *in vivo* against *Uromyes appendiculatus the* causative agent of bean rust. Also, IREs were reported as an alternative and/ or safe trial for the management of many diseases, especially those of vegetable crops (Abada *et al.,* 2008; Abada and Abdel-Malek, 2011, Abada and Attia.,2017). Induction of acquired resistance is persistent and generally is pathogen nonspecific (Doubrava *et al.* (1988).

Spraying the tested two materials of each of EPOs, (clove and neem), fungicides (DithaneM-45 and Previcure-N) and IREs (BTH and humic acid) on cucumber plants caused a considerable increase in the activity of defense-related enzymes *i.e.*, chitinase (CH), phenylalanine ammonia-lyase PAL), peroxidase (PO) and polyphenol oxidase (PPO) in comparison with control treatment. Moreover, plants sprayed with ICEs recorded the highest activity of the three enzymes followed by those sprayed with the EPOS and then those sprayed with the fungicides. Generally, BTH was the most efficient one in increasing the four enzymes in cucumber leaves compared with the other treatments and the control. Meanwhile, Dithane M-45 recorded the lowest activity of these enzymes. The other materials resulted in intermediateactivity for these enzymes. It has been found that the reduction in disease severity was attributed to the increased levels of CH, PAL, PO and PPO enzymes.

Farkas and Kiraly (1967) and Morkunas and Gemerek (2007) reported that the peroxidaseenzyme oxidizes the phenolics to more fungal toxic compounds such as quinines, which inhibit both spore germination and fungal growth. Also, peroxidase was found to participate in thesynthesis of lignin. Moreover, Farkas and Kiraly (2008) and Melo *et al*. (2007) declared that the participation of an endogenous supply of phenolic compounds in plant disease resistanceis dependent upon the active phenol oxidase system.

Peroxidase activity was high in the tested treatments which are known to catalyze the reactive oxygen species and other lignans to strengthen the antioxidant systems by reinforcing the cell wall through cross-linking with hydroxyproline-rich glycoprotein-molecules. Wang and Fan (2014) reported that the tested treatment of tomato plants causes a hydrogen peroxide burst, which activates many immune-responsive genes, resulting in systemic resistance in the host to the virus. The treatments boosted the activity of catalase and peroxidase during postharvest storage in Newhall navel orange, resulting in extended shelf life(Zeng *et al.*, 2012).

Conclusions

This research provided information that cucumber downy mildew can be controlled withthe use of a combination of EPOs, fungicides and IRCs by adequate management field conditions rather than using each of them alone. Therefore, the successful development of such compounds as antifungals would not only provide a potent method for the management of vegetable foliar diseases but also could give promising success in multipurpose alternatives to conventional fungicides alone for the management of such plant diseases.

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