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Evaluation of the Efficiency of some Environmentally Safe Means for Controlling Rust Disease of Anise (*Pimpinella anisum* L.), as Important Medicinal Plant in Egypt

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Efficacies of Ethephon, Et (200 & 300 ppm/L), Indol Acetic acid (10 & 20 ppm/L), Kinetine, Ki (10 & 20 ppm/L), Humic acid, HA (1.5 & 2.5 ml/L), Nicotinic acid, NA (1 & 2 mg/L), Salicylic acid, SA (200 & 400 mg/L), Plant Guard, PG (2 & 4 ml/L), Eminent, Em (0.5 & 1.0 ml/L), Plantvax, Pl (0.5 & 1.0 ml/L) and Tilt, Ti (0.5 & 1.0 ml/L) for controlling rust disease of anise were evaluated in field experiments (2004/2005 & 2005/2006 seasons) as spraying treatment.

The rusted leaf area, number of rust pustules/leaf and rust pustule size were significantly minimized than the control with all concentrations of the treatments tested in both seasons, except IAA, SA and Em at the low concentrations. Moreover, significant reductions between the low and the high concentrations in the rusted leaf area were found, while these results were achieved in most cases with the other criteria. The highest reductions in the rusted leaf area and No. of rust pustules/leaf , however, were recorded with HA , IAA, Pl and SA at the high concentration , while similar result in the size of rust pustule was recorded with PG , followed by NA, Pl and Ti at the same concentration. On the other hand, yield fruits /plant was significantly maximized than the control with all treatments tested in both seasons. The high concentration of all treatments, except HA and NA, gave significant increases in plant yield than the low one. Pl (1ml/L), however, was significantly superior than the other in increasing yield fruits/plant, followed by SA (400mg/l) and Pl (0.5ml/ l).

Activities of chitinase and peroxidase enzymes in anise leaves were increased as a result of spraying with the treatments under study. Increases, relative to control, reached 1.924% (HA) to 93.089% (IAA) in case of chitinase, while they were 11.896% (HA) to 80.733 (Ki) with peroxidase. On the other hand, only SA (13.035%) and Pl (10.316%) increased total phenol contents in plant leaves, while free phenol contents were increased using all treatments, except Et, Em, Ti and PG. Increases, however, ranged between 46.693% (NA) and 281.288 (KI) were found. Also, conjugated phenol contents were only increased with SA (3.864%). Three or more of the detected six phenolic compound fractions were found in anise leaves with each treatment. Occurrence (mg/g fresh leaves) of each fraction greatly varied according to the treatment kind. Control treatment was free from phenol, 4-chlorophenol and 4-chloro-3-methyphenol fractions, while they were found with 10 (all treatments), 6 and 2 treatments, respectively.

Key words: Anise rust, chitinase, induced resistance, peroxidase, phenols content and rust criteria.

Anise (*Pimpinella anisum* L.) is one of the most important umbelliferous medicinal plants in Egypt. The rust disease (*Puccinia pimpinellae* (F. Strauss) Mart.) is the most prevalence and destructive disease allover the country during the last three years. Since the disease was recorded in Egypt (Ghoneem, 2003) there are annually considerable losses in yields of fruits and its essential oil. Moreover, 26% of seed lots were found to be infected by the rust fungus. Therefore, evolving concepts of effective and environmentally safe measures to control the disease is very important.

Several strategies for controlling rust diseases on several crops have been introduced but serious losses occur largely to plants because the effectiveness of these approaches is variable and often short-lived. Current thinking about plant protection and the environment suggests alternatives to pesticides (Tuzun & Kloepper, 1995). A number of compounds that do not have direct antimicrobial activity increase resistance or at least decrease symptoms, in some host-pathogen interactions (Kessmann *et al.*, 1994 and Hammerschmidt & Smith, 1997). These compounds cause induced resistance, the phenomenon that resistance of plants to pathogens can be enhanced. The induction of resistance is associated with metabolic and structural changes inside plants (Benhamou & Belanger, 1998). On the other hand, Kauss *et al.* (1992) identified salicylic acid and its derivatives as potential activators for induction resistance in plants. Moreover, certain growth regulators such as ethephon (Bulman and Smith 1993; Sallam, 1997 and El-Nagar, 1998) and IAA (Van Andel and Fuchs, 1972 and Sallam, 1997) were recorded in this respect.

The objective of the present investigation was to evaluate the efficacy of various compounds against rust disease in comparison with three common rust fungicides. Also, efficacy of the compounds tested on the activity of chitinase and peroxidase enzymes as well as phenolic compound contents in anise leaves were determined.

Materials and Methods

During stage of growth in the field, six potential chemical activators of systemic acquired resistance were applied at two concentrations in anise crop to control rust disease caused by *Puccinia pimpinellae*. Three growth regulators namely, IAA, Ethephon and Kinetin as well as Salicylic acid, Humic acid and Nicotinic acid as systemic resistance inducers in addition to the fungicides Eminent ((±)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl) propyl 1,1,2,2-tetrafluoroethyl ether) (IUPAC), Tilt (1-(2-(2,4-dichlorophenyl)4-propyl-1,3-dioxolan-2-ylmethyl)-1H-1,2,4-triazole)and Plantvax (5,6-dihydro-2-methyl-N-phenyl-1,4-oxathin-3-carboxamide 4,4-dioxide, the dioxide of vitavax) as well as the biocide Plant Guard were used in this respect.

Field trials were carried out in two successive seasons, on November12nd, 2004/2005 and 2005/2006 at Mashtol County, Sharkiya governorate. Plot area was 3 x 1.8 m with three ridges per plot; each ridge has ten hills which contain 3plants per each. Treating plants by foliage spraying started on Feb.10th, the second spraying was 15 days later. Two concentrations in liter were used from each compound as follow: IAA and Kinetin 10, 20 ppm, Ethephon 200, 300 ppm, Humic acid 1.5, 2.5ml, Nicotinic acid 1, 2mg, Salicylic acid 200, 400 mg, Eminent, Plantvax and Tilt 0.5, 1.0 ml and Plant Guard 2, 4 ml. The control plants were sprayed with water.

Rust disease assessment:

Rust disease severity was recorded after complete appearance of rust symptoms by natural infection as:

1. Percentages of infected leaf areas according to James (1971):

The rusted leaves were classified to six categories, *i.e.* 1, 5, 20, 50, 70 and 80%. Disease severity was calculated as disease index percentage as follows:

$$DI = \frac{\text{Sum of } (n \times v)}{6\,N} \times 100$$

Whereas: DI= Percentage of rusted leaves [disease index].
n = Number of leaves in each category.
v = Numerical value of each category.
N = Total number of leaves in samples.

2. Number of pustules per leaf surface:

Ten leaves were randomly chosen per each plant. Number of pustules on the lower surfaces were counted and mean was registered.

3. Pustule size:

Leaves samples of all treatments were taken 20 days after the rust symptoms started to measure the pustule size. The sampled leaves were boiled in lactophenol ethanol (1:2 v/v) solution for three minutes for fixation (Shipton and Brown, 1962). The length (L) and width (W) of 60 randomly chosen pustules were measured for three leaves of each treatment. The pustule size (µm) was calculated according to the suggested formula by Broers and Wallenburg (1989):

$$\text{Pustule size} = \tfrac{1}{4}\,\pi\, LW$$

Whereas: $\pi = 22/7$, L= Length and W= Width.

4. Fruit yield:

Fruit yields per 60 plants of each treatment were collected, 20 plants per each replicate. The average yield per each plant (g) was then calculated.

Extraction of plant leaves and assay of enzyme activity:

Samples of leaves from each treatment and control plants were collected after complete appearance of rust symptoms in order to assay the activity of enzymes and determine the total and free phenolic compounds.

A. Peroxidase enzyme activity:

One gram of leaf tissues was rapidly ground with 2 ml of 0.1 M sodium phosphate buffer at pH 7.1 in a mortar. The ground tissues were strained through four layers of cheesecloth. Then, the homogenate was centrifuged at 3000 rpm for 20 minutes at 6°C. The supernatant fluid was used to determine the peroxidase enzyme activity according to the method adopted by Allam and Hollis (1972). The enzyme activity was estimated by measuring the oxidation of pyrogallol to purpurgallin in the presence of H_2O_2 at 425 nm. using Beckman DB Spectrophotometer. The peroxidase activity was expressed as the change in the absorbance per minute (DA/min.).

B. Chitinase enzyme activity:

Samples of fresh leaves (5 g for each) were homogenized with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM B-mercaptoethanol at the rate of 1:3 (w:v). The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was used to determine the chitinase activity (Tuzun *et al.*, 1989) according to the colorimetric method of Monreal and Reese (1969), using 1% colloidal chitin substrate prepared from chitin powder according to the method described by Ried and Ogryd-Ziak (1981).

The chitinase enzyme activity was expressed as mM N-acetyl-D-glucosamine (NAG) equivalent, released from colloidal chitin per gram fresh weight tissue per 60 minutes at 540 nm by using Spectrophotometer (Reissig *et al.*, 1955).

Determination of total and free phenolic compounds:

Total and free phenols were colorimetrically determined in the treated and untreated (control) leaves of anise plants using phosphotungistic-phosphomolybdic acid reagent (Folin and Ciocalteu phenol reagent) at 520 nm and against a reagent blank as described by Snell and Snell (1953).

Total and free phenols were determined as milligrams equivalent of chlorogenic acid per gram fresh weight of leaf material, using a constructed standard curve. Conjugated phenols were calculated as difference between free and total phenols.

Statistical analysis:

The data were statistically analyzed according to the standard procedure in a completely random design or split plot as mentioned by Snedecor and Cochran (1982).

Results

I. Effect of various compounds on rust disease control:

A. Percentages of the infected leaf area (disease severity):

All concentrations of the treatments tested (Table, 1) significantly reduced rust disease severity than the control in both trial seasons, 2004/2005 and 2005/2006. Reductions ranged between (24.71-77.0%) and (60.14-91.62%) were recorded in the 1st season with the 1st and 2nd concentrations tested, respectively. While, they were (25.55-79.22%) and (69.71-98.27%) in the 2nd season. Significant reductions in disease severity, however, were always occurred by increasing concentrations in both trial seasons. The highest significant reductions were recorded with Humic acid (2.5ml/L), Plantvax (1ml/L), IAA (20ppm/L), Salicylic acid (400 mg/L) and Kinetine (20 ppm/L) in the 1st and the 2nd season.

B. Number of rust pustules on leaf surface:

The number of pustules per lower leaf surface (Table, 2) was significantly reduced than the control in both seasons tested as a result of spraying with each one of the compounds under evaluation. Reduction than the control, however, reached (34.87-72.20%) and (61.32-83.99%) with the 1st and 2nd concentrations, respectively, in 2004/2005 season. Whereas, they were (49.42-95.07%) and (88.84-99.67%) in 2005/2006 season. Reductions than the control in number of

Table 1. Effect of spraying anise plants with various compounds on rust disease severity, 2004/2005 and 2005/2006 seasons

Treatment	Concentration / l water	2004/2005 season		2005/2006 season	
		Disease severity (%)	Reduction* (%)	Disease severity (%)	Reduction* (%)
Ethephon	200 ppm	36.44	31.41	27.96	34.64
	300 ppm	20.36	61.68	10.74	74.89
IAA	10 ppm	14.68	72.37	11.48	73.17
	20 ppm	5.67	89.33	00.74	98.27
Kinetine	10 ppm	13.15	75.25	10.19	76.18
	20 ppm	7.33	86.20	3.15	92.64
Humic acid	1.5 ml	13.20	75.16	11.11	74.03
	2.5 ml	4.45	91.62	1.67	96.10
Nicotinic acid	1.0 mg	18.25	65.65	11.30	73.59
	2.0 mg	9.15	82.78	5.74	86.58
Salicylic acid	200 mg	12.22	77.00	8.89	79.22
	400 mg	6.11	88.50	2.99	93.01
Plant Guard	2.0 ml	32.84	38.19	24.46	42.82
	4.0 ml	21.18	60.14	12.96	69.71
Eminent	0.5 ml	40.00	24.71	31.85	25.55
	1.0 ml	8.75	83.53	3.89	90.91
Plantvax	0.5 ml	16.12	69.66	13.33	68.84
	1.0 ml	5.13	90.34	2.78	93.50
Tilt	0.5 ml	19.52	63.26	13.70	67.98
	1.0 ml	11.24	78.84	6.11	85.72
Control	0.0	53.13	-	42.78	-
L.S.D. at 5% for:		1st season		2nd season	
Treatment (T) =		1.03		1.81	
Concentration (C)=		0.67		0.79	
T x C =		2.13		2.51	

* Reduction relative to the control.

Table 2. Effect of spraying anise plants with various compounds on number of pustules/leaf, 2004/2005 and 2005/2006 seasons

Treatment	Concentration / l water	2004/2005 season		2005/2006 season	
		No. of pustules/leaf	Reduction* (%)	No. of pustules/leaf	Reduction* (%)
Ethephon	200 ppm	30.80	38.74	12.15	55.98
	300 ppm	19.45	61.32	1.26	95.43
IAA	10 ppm	15.11	69.95	3.69	86.63
	20 ppm	8.05	83.99	0.09	99.67
Kinetine	10 ppm	18.33	63.54	2.68	90.29
	20 ppm	11.00	78.12	0.79	97.14
Humic acid	1.5 ml	13.98	72.20	3.04	88.99
	2.5 ml	9.23	81.64	0.18	99.35
Nicotinic acid	1.0 mg	18.87	62.47	1.36	95.07
	2.0 mg	10.50	79.12	0.96	96.52
Salicylic acid	200 mg	16.13	67.92	2.20	92.03
	400 mg	9.18	81.74	0.31	98.88
Plant Guard	2.0 ml	24.13	52.01	13.08	52.61
	4.0 ml	17.07	66.05	3.08	88.84
Eminent	0.5 ml	32.75	34.87	13.96	49.42
	1.0 ml	10.08	79.95	0.45	98.37
Plantvax	0.5 ml	17.93	64.34	2.80	89.86
	1.0 ml	9.17	81.76	0.29	98.95
Tilt	0.5 ml	18.93	62.35	3.95	85.69
	1.0 ml	13.54	73.07	1.30	95.29
Control	0.0	50.28	--	27.60	--

L.S.D. at 5% for:	1st season	2nd season
Treatment (T) =	0.94	0.97
Concentrations (C) =	1.48	1.05
T x C =	4.66	3.32

* Reduction relative to the control.

pustules per leaf were always occurred by increasing concentration of each compound in both seasons. Differences between the 1st and the 2nd concentrations in reducing number of pustules per leaf were always significant in the 1st season. The highest reduction, however, were generally recorded at the high concentration with IAA, Humic acid, Salicylic acid and Plantvax in both seasons.

C. Size of rust pustule:

Data presented in Table (3) reveal that all compounds tested significantly reduced pustule size than the control in both trial seasons, except IAA, Salicylic acid and Eminent at the 1st concentration in only the 2nd season. Reductions ranged between (16.25-58.00%) and (24.03-74.01%) at 1st and 2nd concentrations, respectively in 2004/2005 season were recorded. While, they were (13.16-57.43%) and (21.34-76.62%) in 2005/2006 season. Increasing in each compound concentration was correlated with significant reduction in pustules size in both seasons. The highest significant reductions in pustule size in both trial seasons, however, were recorded with Plant Guard (4, 2ml/l water), followed by Tilt (1ml/l), Plantvax (1ml/l) and Nicotinic acid (2ml/l).

D. Fruits yield per plant:

All concentrations of the compounds tested (Table, 4) significantly increased fruits yield/plant than the control in both seasons. Increases, however, reached (19.11-218.93%) and (76.43-273.75%) at the 1st and the 2nd concentrations, respectively in 2004/2005 season. While, they were (48.08-264.29%) and (98.08-302.61%) in 2005/2006 season. Fruits yield per plant was always significantly increased by increasing concentration of each compound in both years of investigation. Plantvax (1 m/l water) gave significantly the highest weights of fruits yield/plant than the others in both years of the experiment, followed by Salicylic acid (400 mg/L water) and Plantvax (0.5 ml/L water) with significant differences between them and the others. In contract, the least effective compounds in increasing fruits yield/plant, were Ethephon (200 ppm/l), Tilt (0.5 ml/l) and Plant Guard (2 ml/l).

II. Chitinase enzyme activity:

All compounds tested (Table, 5) increased activity of Chitinase enzyme in leaves of anise plants than the control, except Nicotinic acid and Tilt as they gave reduction in this activity. Increases in this enzyme activity than the control reached, however, 1.924% (Humic acid) to 93.089% (IAA). The highest activities of the enzyme were recorded with IAA(20ppm/l) , followed by Kinetine (20 ppm/l). While, Salicylic acid (400 mg/l) and Plantvax (1 m/l) gave intermediate activity in this respect. While, Humic acid (2.5 ml/l), Eminent (1 ml/l). Plant Guard (4 ml/l) and Ethephon (300 ppm/l) gave the least activity of the enzyme.

III. Peroxidase enzyme activity:

All treatments evaluated (Table, 6) increased Peroxidase activities in anise leaves than the control, except Nicotinic acid since it decreased the enzyme activity than the control. Increase (%) in Peroxidase activities than the control ranged between 11.896% (Humic acid) and 80.733% (Kinetine) were recorded. The highest increases (%), however, were found with Kinetine and Plantvax, followed by IAA, Eminent and Tilt. Whereas, the least enzyme activities were recorded with Humic acid, Salicylic acid, Ethephon and Plant Guard.

Table 3. Effect of spraying anise plants with various compounds on size of rust pustules, 2004/2005 and 2005/2006 seasons

Treatment	Concentration / l water	2004/2005 season		2005/2006 season	
		Pustule size (µm)	Reduction* (%)	Pustule size (µm)	Reduction (%)
Ethephon	200 ppm	480.22	39.19	425.36	42.34
	300 ppm	400.71	49.26	379.13	48.60
IAA	10 ppm	661.44	16.25	640.56	13.16
	20 ppm	600.00	24.03	580.27	21.34
Kinetine	10 ppm	570.45	27.77	420.34	43.02
	20 ppm	405.20	48.69	391.24	46.96
Humic acid	1.5 ml	588.35	25.50	527.52	28.49
	2.5 ml	511.05	35.29	460.53	37.57
Nicotinic acid	1.0 mg	445.33	43.61	516.63	29.96
	2.0 mg	400.00	49.35	353.77	52.04
Salicylic acid	200 mg	660.11	16.41	631.35	14.41
	400 mg	448.77	43.17	415.95	43.61
Plant Guard	2.0 ml	332.11	58.00	314.00	57.43
	4.0 ml	201.31	74.51	172.49	76.62
Eminent	0.5 ml	650.24	17.66	628.00	14.86
	1.0 ml	500.09	36.68	483.14	34.50
Plantvax	0.5 ml	471.13	40.34	438.76	40.52
	1.0 ml	358.32	54.63	330.75	55.16
Tilt	0.5 ml	543.65	31.16	501.56	32.01
	1.0 ml	331.00	58.09	314.00	57.43
Control	0.0	789.73	--	737.65	--

L.S.D. at 5% for:	1st season	2nd season
Treatment (T) =	5.63	60.94
Concentrations (C) =	3.63	36.37
T x C =	11.49	115.00

* Reduction relative to the control.

Table 4. Effect of spraying anise plants with various compounds on fruits yield per plant (g), 2004/2005 and 2005/2006 seasons

Treatment	Concentration / l water	2004/2005 season		2005/2006 season	
		Fruits yield/ plant (g)	Increase* (%)	Fruits yield/ plant (g)	Increase* (%)
Ethephon	200 ppm	7.88	40.71	9.67	68.47
	300 ppm	10.50	87.50	11.37	98.08
IAA	10 ppm	12.14	116.79	13.39	133.28
	20 ppm	14.35	156.25	15.72	173.87
Kinetine	10 ppm	10.33	84.46	12.13	111.32
	20 ppm	15.00	167.86	15.65	172.65
Humic acid	1.5 ml	11.99	114.11	13.72	139.02
	2.5 ml	14.05	150.89	15.24	165.51
Nicotinic acid	1.0 mg	11.44	104.29	14.32	149.48
	2.0 mg	13.98	149.64	15.18	164.46
Salicylic acid	200 mg	15.00	167.86	16.97	195.64
	400 mg	18.13	223.75	20.67	260.10
Plant Guard	2.0 ml	6.95	24.11	8.50	48.08
	4.0 ml	13.35	138.39	15.50	170.03
Eminent	0.5 ml	10.30	83.93	11.71	104.01
	1.0 ml	12.48	122.86	13.82	140.77
Plantvax	0.5 ml	17.86	218.93	20.91	264.29
	1.0 ml	20.93	273.75	23.11	302.61
Tilt	0.5 ml	6.67	19.11	8.73	52.09
	1.0 ml	9.88	76.43	11.67	103.31
Control	0.0	5.60	--	5.74	--

L.S.D. at 5% for:	1st season	2nd season
Treatment (T) =	0.59	1.35
Concentrations (C)=	0.34	0.64
T x C =	1.06	2.03

* Increase relative to the control.

Table 5. Chitinase enzyme activity as N-acetylglucose amine equivalent release/g fresh weight/hr in anise leaves naturally infected by rust and treated with various compounds

Treatment	Concentration / l water	Chitinase activity / hr	Increase * or decrease (%)
Ethephon	300 ppm	3.992	8.184
IAA	20 ppm	7.125	93.089
Kinetine	20 ppm	5.912	60.217
Humic acid	2.5 ml	3.761	1.924
Nicotinic acid	2.0 mg	2.698	(-) 26.884
Salicylic acid	400 mg	4.634	25.583
Plant Guard	4.0 ml	3.809	3.225
Eminent	1.0 ml	3.841	4.092
Plantvax	1.0 ml	4.603	24.743
Tilt	1.0 ml	1.880	(-) 49.052
Control	–	3.690	–

* Increases or decreases relative to the control.

Table 6. Peroxidase enzyme activity (activity/min.) in anise leaves naturally infected by rust and treated with various compounds

Treatment	Concentration / l water	Peroxidase activity/hr	Increase * or decrease (%)
Ethephon	300 ppm	2.657	20.172
IAA	20 ppm	3.422	54.772
Kinetine	20 ppm	3.996	80.733
Humic acid	2.5 ml	2.474	11.896
Nicotinic acid	2.0 mg	1.616	(-) 26.911
Salicylic acid	400 mg	2.517	13.840
Plant Guard	4.0 ml	2.746	24.197
Eminent	1.0 ml	3.218	45.545
Plantvax	1.0 ml	3.728	68.612
Tilt	1.0 ml	3.141	42.062
Control	–	2.211	–

* Increases or decreases (-) relative to the control.

IV. Phenolic compounds content:

Data presented in Table (7) indicate that total phenolic compounds in leaves of anise plants was decreased with the treatments tested, except Salicylic acid and Plantvax since they increased total phenol contents (%) by 13.035% and 10.316%, respectively. The decreases, however, reached 23.601% (Tilt) to 66.771% (Plant Guard). As for contents of free phenols, they were increased than the control with all treatments tested, except Ethephon, Eminent, Tilt and Plant Guard. Increases ranged between 46.693% (Nicotinic acid) and 281.288% (Kinetine) were recorded. The highest increases were found with Kinetine and IAA, followed by Plantvax and Humic acid. On the other hand, conjugated phenol contents were decreased than the control with the treatments tested, except only Salicylic acid. Decreases in these contents (%) reached 11.173% (Plantvax) to 96.693% (Kinetine).

Table 7. Phenolic compounds content (mg/g fresh weight) in anise leaves naturally infected by rust and treated with various compounds

Treatment	Concen. / l water	Phenolic compounds content (mg/g fresh weight)					
		Total phenols (mg)	Increase* or decrease (%)	Free phenols (mg)	Increase or decrease (%)	Conjugated phenols (mg)	Increase or decrease (%)
Ethephon	300 ppm	1.107	(-) 65.395	0.186	(-) 42.945	0.921	(-) 67.943
IAA	20 ppm	1.482	(-) 53.673	1.092	234.969	0.390	(-) 86.425
Kinetine	20 ppm	1.338	(-) 58. 174	1.243	281.288	0.095	(-) 96.693
Humic acid	2.5 ml	2.329	(-) 271.196	0.855	162.270	1.474	(-) 48.695
Nicotinic acid	2.0 mg	1.581	(-) 50.578	0.488	46.693	1.093	(-) 61.956
Salicylic acid	400 mg	3.616	13.035	0.632	93.865	2.984	3.864
Plant Guard	4.0 ml	1.063	(-) 66.771	0.309	(-) 5.215	0.754	(-) 73.756
Eminent	1.0 ml	1.344	(-) 57.987	0.265	(-) 18.712	1.079	(-) 62.443
Plantvax	1.0 ml	3.529	10.316	0.977	199.693	2.552	(-) 11.173
Tilt	1.0 ml	2.444	(-) 23.601	0.280	(-) 14.110	2.164	(-) 24.678
Control	0.0	3.199	–	0.326	--	2.873	--

* Increases or decreases (-) relative to the control.

The presence of phenolic compound fractions in anise leaves and its occurrence (mg/g fresh plant weight) greatly varied according to the kind of the treatment used in spraying program (Table, 8). The treated plant leaves with each one of the treatments tested contained phenol ranged between 4.63% (Ethephon) and 88.34% (Nicotinic acid). The high levels of its presence, however, were found with Nicotinic acid, Kinetine, Eminent and Plantvax, followed by Humic acid and Salicylic acid. In contrast, plant leaves of control treatment were free from phenol. The fraction 2-chlorophenol was also found with all treatments and control, except with Nicotinic

Table 8. Percentages of fractions of phenolic compounds in anise leaves naturally infected by rust and treated with various compounds

Treatment	Concentration / l water	Phenolic compounds fractions (%)					
		Phenol	2-Chloro-phenol	4-nitro-phenol	4-Chloro-phenol	2,4-methy-phenol	4-chloro-3-methyl phenol
Ethephon	300 ppm	4.63	1.10	--	--	6.37	--
IAA	20 ppm	19.40	70.87	--	--	2.00	1.95
Kinetine	20 ppm	86.15	11.01	--	1.70	--	--
Humic acid	2.5 ml	55.66	29.82	13.03	--	--	1.47
Nicotinic acid	2.0 mg	88.34	--	9.89	0.92	--	--
Salicylic acid	400 mg	46.66	7.42	22.70	--	3.61	--
Plant Guard	4.0 ml	29.21	62.31	-	5.28	--	--
Eminent	1.0 ml	79.42	--	8.56	3.02	4.33	--
Plantvax	1.0 ml	78.49	12.26	--	1.80	3.86	--
Tilt	1.0 ml	29.30	61.66	--	3.36	--	--
Control	0.0	--	15.64	16.91	--	10.10	--

* (-) Compounds were not present.

acid and Eminent. The treatments IAA, Tilt and Plant Guard showed the high levels of this fraction. On the other hand, leaves of anise plants sprayed with Humic acid, Nicotinic acid, Salicylic acid or Eminent as well as control were found to contain the fraction 4-nitrophenol. Salicylic acid -or Humic acid- treated plants and water-treated plants (control) contained the high levels of this fraction. Also, anise leaves of all treatments, except Ethephon, IAA, Humic acid, Salicylic acid and the control contained the fraction 4-chlorophenol. Tilt, Plant Guard and Eminent, however, showed the high levels of this fraction. Whereas, the fractions (2, 4-methylphenol) and (4-chloro-3-methylphenol) were found in 6 and 2 treatments, respectively, with approx. low levels. The high level of the first fraction was found in the control treatment (10.10%), followed by Ethephon (6.37%).

Discussion

The high incidence of rust disease on anise plants during recent years in Egypt, possibly caused by the annually increase in inoculum density in seeds, as well as the harmful side effects of the fungicides on humans and environment, promoted this investigation of alternative measures of disease control. The rusted leaf area, number of pustules/leaf and pustule size were significantly minimized than the control (sprayed with water) with all treatments at both concentrations in the two seasons tested. The highest reductions in the former two criteria were recorded with the defense activators Humic acid, IAA and Salicylic acid as well as the fungicide Plantvax. The positive effects of these treatments, except fungicides, may be due to their efficacy as elicitors since localized treatments of plants with biotic or abiotic defense activators can result in a localized or systemic response (den Hond, 1998 and Hammerschmidt, 1999). Several products somewhat similar to the present study, however, have been used as inducers of resistance in plants against pathogens including Salicylic acid analogues (Görlach *et al.*, 1996; Ruess *et al.*, 1997; Benhamou and Belanger, 1998 and Brisset *et al.*, 2000). Such elicitor may provide more effective and safe alternative for human and environment than the application of synthetic fungicides (Kuc, 1991and Wilson *et al.*, 1994). Also, treatment of plant cells with fungal elicitors resulted in the activation of numerous genes leading to the synthesis of structural compounds (Ride, 1983), secondary metabolites (Anderson, 1988) and new proteins (Carr & Klessing, 1989). The positive effects of the elicitors tested in this investigation on the criteria of the rust disease infection on anise plants are in accordance with those reported by Sallam (1997), El-Nagar (1998), El-Nashar (2000), Zaky *et al.* (2002) and Hammouda *et al.* (2003) on other rust diseases in Egypt. On the other hand, fruits yield/plant was significantly maximized than the control with all treatments tested in both seasons. However, the high concentration of each treatment was significantly superior than the low one. Plantvax (1 ml/L) was significantly the highest effective treatment in this respect, followed by Salicylic acid (400 mg/L) and Plantvax (0.5 ml/L). The superiority of Plantvax in increasing plant yield may attribute to its high toxic activity against rust incidence, while the positive efficacy of Salicylic acid may due to its action as defense activator. Reglinski *et al.* (1997) proposed that Salicylic acid operate through the induction of host resistance mechanisms. Also, the present results on plant yield were somewhat similar to those found by El-Nashar (2000). The evidence concerning resistance to diseases in medicinal plants in Egypt, however, is very limited and, to our knowledge, nothing has been published on rust disease on anise.

Spraying anise plants with each one of the treatments tested increased activities of Chitinase and Peroxidase enzymes as well as phenolic compounds accumulation in plant leaves than the control. However, IAA (Chitinase), Kinetine (Peroxidase), Salicylic acid and Plantvax (total phenol content), Kinetine (free phenol content) and Salicylic acid (conjugated phenol content) were the most effective treatments. These results, however, proved the concept of systemic inducing resistance against rust disease. In this respect, Chitinase enzyme has been identified as a pathogenesis-related protein with a potential antifungal activity through inhibiting growth, multiplication and spread of pathogen in plant tissues, enhancing plant defense to necrotizing infection and hydrolyzing chitin, a major cell wall component of many pathogenic fungi such as the rust one (Legrand *et al.*, 1987; Tuzun *et al.*, 1989; Wilson *et al.*, 1994; Sticher *et al.*, 1997 and Hammouda *et al.*, 1999). While, Peroxidase enzyme has been to oxidase the phenolics to more fungitoxic compounds such as quinones (Misaghi, 1982) and inhibit both the germination and fungal growth (Macko *et al.*, 1968). Moreover, Peroxidase was found to participate in synthesis of lignin (Stahmann and Demorest, 1973). On the other hand, the defense activators under study proved to induce a host-plant resistance by enhancing the synthesis and release of high amounts of free phenolic compounds as antifungal substances in the elicited plant cells (Reglinski *et al.*, 1993; Boulot, 1997 and Hammouda *et al.*, 1999), also as precursors for lignin synthesis (Morschbacher *et al.*, 1990).

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تقييم فعالية بعض الوسائل الآمنة على البيئة فى مقاومة مرض صدأ الينسون ، كنبات طبى هام فى مصر

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تم تقييم فعالية الإثيفون (٢٠٠ ، ٣٠٠ جزء فى المليون/لتر) ، الإندول أسيتيك أسد (١٠ ، ٢٠ جزء فى المليون/لتر) ، الكينيتين (١٠ ، ٢٠ جزء فى المليون/لتر) ، حمض الهيوميك (١,٥ ، ٢,٥ مل/لتر) ، حمض النيكوتينك (١ ، ٢ ملجم/لتر) ، حمض السالسيلك (٢٠٠ ، ٤٠٠ ملجم/لتر) ، البلانت جارد (٢ ، ٤ مل/لتر) ، الإمينتت (٠,٥ ، ١مل/لتر) ، البلانتفاكس (٠,٥ ، ١مل/لتر) والتلت (٠,٥ ، ١مل/لتر) فى مقاومة مرض صدأ الينسون باستخدامها رشا فى تجارب حقلية خلال موسمى ٢٠٠٤/٢٠٠٥ ، ٢٠٠٥/٢٠٠٦.

إنخفض معنويا كل من مساحة الورقة المصابة بالصدأ ، عدد البثرات/ورقة وحجم البثرة باستخدام كل تركيزات المعاملات المختبرة فى الموسمين عن معاملة المقارنة باستثناء التركيزات المنخفضة لكل من حمض الإندول أسيتك وحمض السالسيلك ومبيد الإمينتت. علاوة على ذلك كانت هناك فروقا معنوية فى الخفض بين التركيزات المنخفضة والتركيزات العالية بخصوص مساحة الورقة المصابة بالصدأ ، فى حين تحققت هذه النتائج فى معظم المعاملات مع القياسات الأخرى للمرض ، كما تم الحصول على أعلى خفض فى مساحة الورقة المصابة بالصدأ ، عدد البثرات/ورقة باستخدام التركيز العالى لأى من حمض الهيوميك ، حمض الإندول أسيتك ، بلانتفاكس ، حمض السالسيلك. فى حين تم تسجيل أقل خفض فى حجم البثرة باستخدام نفس التركيز لكل من البلانت جارد يليه حمض النيكوتينك ، البلانتفاكس والتلت. ومن ناحية أخرى أدت جميع المعاملات المختبرة فى الموسمين إلى زيادة معنوية فى محصول الثمار لكل نبات. ولقد أدى التركيز العالى لكل المعاملات باستثناء حمض الهيوميك و حمض النيكوتينك إلى حدوث زيادة معنوية فى محصول النبات مقارنة بالتركيز المنخفض لها كما تفوق البلانتفاكس (١مل/لتر) بدرجة معنوية عن باقى المعاملات فى زيادة محصول الثمار/نبات يليه حمض السالسيلك (٤٠٠ملجم/لتر) ، البلانتفاكس (٠,٥مل/لتر).

أدى رش النباتات بالمعاملات المختبرة إلى زيادة نشاط الإنزيمين: الشيتينيز والبيروكسيديز فى أوراق الينسون ، حيث بلغت الزيادة عن المقارنة فى الأول إلى ما بين ١,٩٢٤% (حمض الهيوميك) إلى ٩٣,٠٨٩% (حمض الإندول أسيتك) ، فى حين بلغت الزيادة فى نشاط الثانى ١١,٨٩٦% (حمض الهيوميك) إلى ٨٠,٧٣٣% (كينيتين). ومن ناحية أخرى ، أدت معاملتى حمض السالسيلك والبلانتفاكس فقط إلى زيادة المحتوى الكلى للفينولات فى أوراق النبات بمقدار ١٣,٠٣٥% و ١٠,٣١٦% على الترتيب. فى حين زادت الفينولات الحرة باستخدام كل المعاملات باستثناء الإثيفون ، الإمينتت ، التلت و البلانت جارد ، وعموما تراوحت الزيادة ما بين ٤٦,٦٩٣% (حمض نيكوتينك) و٢٨١,٢٨٨% (كينيتين). كما زادت الفينولات المرتبطة فقط عند استخدام حمض السالسيلك (٣,٨٦٤%). كما احتوت أوراق الينسون عقب رشها بأى معاملة على ثلاثة أو أكثر من المركبات الفينولية الستة التى تم التعرف عليها ، مع اختلاف كبير فى مقدار هذه المركبات (ملجم/جم أوراق طازجة) باختلاف نوع المعاملة المستخدمة. كما خلت معاملة المقارنة كلية من مركبات الفينول ، ٤-كلوروفينول ، ٤ كلورو-٣-ميثايل فينول فى حين وجدت هذه المركبات فى عدد ١٠ (كل المعاملات) ، ٦ ، ٢ معاملة على التوالى.