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Induced Resistance against *Sclerotinia sclerotiorum* Disease in some Umbelliferous Medicinal Plants as a Possible and Effective Control Mean

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Laboratory and greenhouse experiments were performed to evaluate the antifungal activity of Bion (Bi), Chitosan (Ch), Oxalic acid (OA) and Salicylic acid (SA) at 5 concentrations against *S. sclerotiorum* growth on PDA and to verify its positive efficacy as defence activators (elicitors) in caraway, coriander and fennel seedlings and plants against damping-off and stem rot, respectively in comparison with the fungicide Switch. For damping-off control, seeds were soaked in each treatment and planted in infested soil, while they were used as spray treatment pre-plant stem inoculation for the purpose of stem rot control. Also, efficacy of these elicitors in increasing contents of phenolic compounds and phytoalexin (Iso-flavone) in the sprayed plants were determined.

All treatments except, Ch at low concentration, significantly reduced the *in vitro* fungal growth. Reduction was always increased by increasing concentration SA and OA prevented the fungal growth at the high concentration.

Soaking seeds in each one of the four elicitors or the fungicide tested significantly decreased pre- (in most cases) & post-emergence as well as increased survivals. More than (5-7), (2-8) and (14-20) folds of increases in survivals were recorded in caraway, coriander and fennel plants, respectively. Treatments of SA in caraway and Bi & SA in coriander gave significant increases in survivals than the others. Also, SA in caraway and Bi in coriander were superior with survivals than the fungicide. Height and fresh weight/plant as well as root length and weight/plant were greatly improved in all treated plants.

Spraying the plants (90-days-old), 3 or 7 days before inoculation, with each treatment significantly decreased disease severity. However, disease severity was higher at 14 days than that of 7 days after inoculation. The least severities (%) were realized with SA followed by Bi & the fungicide in caraway & coriander plants and OA followed by Bi & Ch in fennel. Also, phenolic compound contents, in most cases, in caraway and fennel plants were increased than the control. Bi and Ch gave the highest total, (free and conjugated) phenol contents. The presence of the phenolic compound fractions and its frequency (%) were correlated with the kind of the treatment and the sprayed plant. All treatments increased also concentration of phytoalexins (Iso-flavone) in the treated plants than the control except Bi since phytoalexins were not detected in its plant samples. The fungicide, however, recorded the highest increases in all cases followed by OA in caraway and SA in fennel.

Key words: Damping-off, induced resistance, phenol contents, phytoalexins, plant growth parameters, *S. sclerotiorum* and stem rot.

Umbelliferous medicinal plants such as caraway, coriander and fennel are subjected to infection by Scelrotinia rot incited by *Sclerotinia sclerotiorum* (Lib.) de Bary, causing economic losses in plant stand and yield in Egypt (Hilal *et al.*, 1998) as well as in USA (Anonymous, 1960), Argentina (Gaetan *et al.*, 1997), Netherlands (Evenhuis *et al.*, 1995), India (Lakra, 2000) and China (Yu and Chen, 2002). *S. sclerotiorum* is among the most nonspecific omnivorous, and successful of plant pathogen. Plants susceptible to this pathogen encompass 64 families, 225 genera and 361 species (Purdy, 1979). Moreover, this fungus is geographically cosmopolitan and has a broad ecological distribution including hot and dry areas.

Plants can respond to attempted infection by activating a range of defence mechanisms, which can be local (*e.g.* the hypersensitive response, HR) or systemic (*e.g.* systemic acquired resistance, SAR). In SAR, elicitors enhanced the level of one or more translocatable signal chemicals which, in return, results in coordinated induction of genes controlling divers defence pathways in tissues spatially distant from the initial challenge site (Kuc, 1995). As a consequence, the challenged plant exhibits broad-spectrum partial resistance to a range of pathogens (Storbel & Kuc, 1995).

The defence products induced in systemic disease resistance are diverse, reflecting the number of defence pathways stimulated: lignin, pathogensis related (PR) protein, phytoalexins and the small antimicrobial proteins thionin and defensins (Pan *et al.*, 1992; Kessman *et al.*, 1994 and Epple *et al.*, 1997).

The induction of systemic resistance in crops by exogenous application of inducer chemicals represents a potentially valuable component in integrated pathogen management strategies, complementary to conventional control methods (Mitchell & Walters, 1995 and Görlach *et al.*, 1996). It would be particularly useful against pathogens that are currently poorly controlled such as Sclerotinia stem rot, since infection often occurs at flowering, making fungicidal control problematic, while the wide host range of the pathogen makes crop rotation an ineffective control strategy (Purdy, 1979). Effective elicitors of systemic resistance include pathogen-derived elicitors such as glucans (Lyons *et al.*, 1995). Oxalic acid is a pathogensis factor produced by *S. sclerotiorum* (Godoy *et al.*, 1990).

The long-term goal of our research is to develop and evaluate new or existing alternative safe control method for Sclerotinia rot to replace fungicides in medicinal plants production systems.

Materials and Methods

1. Laboratory experiment:

Efficacy of four elicitors namely; Bion, Chitosan, Oxalic acid and Salicylic acid on linear growth of *Sclerotinia sclerotiorum* (isolated from infected caraway plants) on PDA medium was evaluated. Chitosan was dissolved in 0.1% acetic acid in distilled water according to Eikemo *et al.* (2003). The medium was amended with each elicitor just before solidification. The appropriate amount of each elicitor: was prepared to mix in 40 ml medium in each flask to give concentrations of 0.1, 0.2, 0.4, 0.5 and 1.0 g/L of Bion; 4.0, 8.0, 10.0, 12.0 and 15.0 g/L of Chitosan, 1.3, 2.6, 5.2, 7.8 and 15.8 g/L of Oxalic acid and 0.2, 0.4, 0.5, 1.0 and 1.5 g/L of Salicylic acid. Ten ml of each PDA medium was poured in each Petri dish. Then they were inoculated with 5mm discs of fungal growth and incubated at 20°C. The fungal growth was measured when radial growth in control reached its maximum (9 cm).

2. Greenhouse experiments:

The experiments were performed as follows:

A. The fungus tested was grown on liquid PD medium for 4 weeks at 18°C. The fungal propagules of each flask contains 35 ml medium was blended then mixed with the upper soil surface of each pot (25-cm-diamter) containing 3 kg soil of the three replicates containing sterilized soil equally for each treatment. Apparently healthy seeds of caraway, coriander or fennel were soaked in each of the four elicitors tested (Bion, Chitosan, Oxalic acid and Salicylic acid) for 20 min. just before sowing at the rate of 1.0, 15.0, 15.8 and 1.5 g/L water, respectively. Ten treated seeds/pot were sown in soil infested with *S. sclerotiorum* in comparing with seeds of control and seeds treated with Switch fungicide. Soil treated with free medium of the fungus was served as control treatment. Percentages of pre- and post-emergence damping-off were recorded after 15 and 45 days of sowing. Healthy survival plants and their root length & weight and plant height & weight were calculated 90 days of sowing date.

B. Toothpick inoculation (TPI) technique:

Round hard toothpicks (6.5 cm long with tapered ends) were boiled several times in water to remove resin, gum and other toxic substances then washed several times with sterilized distilled water and autoclaved at 121°C for 30 minutes. Sterilized toothpicks were placed in PDA plates inoculated with one disc (5mm) of *S. sclerotiorum* culture. Inoculated plates containing toothpicks were incubated at 20°C for seven days (Thirumalacher *et al.*, 1977). These toothpicks, however, were used to inoculate plant stems (one toothpick/stem).

Caraway, coriander and fennel plants (90-day-old) were sprayed with each of Bion, Chitosan, Oxalic acid, Salicylic acid and the fungicide Switch 3 or 7 days before inoculation at the rate of 1.0, 15.0, 15.8, 1.5 and 0.5 g/L water, respectively. Plants inoculated in its stems with pathogen-free toothpicks were used as control. Five plants were used for each particular treatment (replicates). Inoculated plants, however, were observed daily to follow up disease symptoms. The length (cm) of the elongate lesion on diseased stem was measured 7 and 14 days after inoculation.

Samples were taken to determine phenolic compounds and phytoalexins (Iso-flavone) in plants at the same time of measuring the diseased stem parts.

C. Fraction of phenolic compounds:

Ten grams of fresh tissues of each sample were homogenized with methanol 40% and stirred on a shaker. The extract was filtered through a Whatman filter paper No.1 and the solvent was evaporated in vacuo. The dry residue containing phenolic compounds was dissolved in solution consists of (methanol : water : acetic acid; 40:59.3:0.7, v/v/v) in vials. The method suggested by Gertz (1990) was then used as follows:

HPLC system in Bio-technology Lab., Pl. Pathol., Inst., Agric. Res. Centre was used to detect and determine the phenolic compounds of plant tissues. The extract was passed through membrane filter 0.45µm. The analysis of phenolic compounds was performed on a model (hp 1050) HPLC equipped with UV detector. The separation and determination were performed on C18 column (150x4.6 mm). The mobile phase yielded results of methanol : water : acetic acid; (40 : 59.3 : 0.7, v/v/v). The wave length in the UV detector was 245 nm. Total run time for the separation was approximately 25 min. at a flow rate of one ml/min.

D. Determination of the phytoalexins (iso-flavone):

Ten grams of fresh tissue of each sample were homogenized with methanol : acetic acid (95:5 v/v) and stirred on a shaker. The extract was filtered through filter paper Whatman No. 1 and the solvent was evaporated in vacuo. The dry residue containing phenolic compounds was dissolved in one ml of mobile phase in vials. The method suggested by Gertz (1990) was then used as follows:

HPLC system was used to detect and determine the flavonoides from plant tissues. The extract was passed through membrane filter 0.45 µm. The analysis was performed on a model (hp 1050). HPLC equipped with UV detector. The separation and determination were performed on ODS column (150x4.6 mm). The mobile phase yielded results of methanol:5% acetic acid, 100%A ..>74%B. The wave length in the UV detector was 254 nm. Total run time for the separation was approximately 15 min at a flow rate of one ml/min.

Statistical analysis:

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

Results

I. Effect of some elicitors at five concentrations on the fungal mycelial growth:

The inhibitory effect of the five concentrations of some elicitors on the linear growth of *S. sclerotiorum* was evaluated *in vitro* (Table, 1). All concentrations of the compounds tested significantly reduced linear growth of *S. sclerotiorum* compared with the control. Significant decreases in the fungus growth were recorded by increasing concentrations of all elicitors tested. The fifth concentration of each elicitor gave the highest reduction in mycelial growth, especially Salicylic acid as it completely prevented the mycelial growth of the fungus using the 4th concentration followed by Oxalic acid which gave the same result at 5th concentration. In contrast, Chitosan was the least effective elicitor in decreasing the fungus growth at all concentrations significantly, except that of 5th concentration than the others.

Table 1. The *in vitro* effect of four elicitors on linear growth of *S. sclerotiorum* in PDA medium

Treatment	Linear growth (mm) at concentration of						Mean
	0.0	C1*	C2*	C3*	C4*	C5*	
Bion	90.0	56.1	38.3	12.5	8.2	4.1	34.87
Chitosan	90.0	83.2	74.5	50.3	34.1	4.0	55.99
Oxalic acid	90.0	54.0	42.2	22.7	10.5	0.0	36.57
Salicylic acid	90.0	46.1	36.4	30.1	0.0	0.0	33.77
L.S.D. at 5% for: Treatments (A)= 2.27, Concentrations (B)= 2.67 and A x B= 5.33							

* Concentrations used for each elicitor.

II. Effect of some elicitors and fungicide as seed soaking on:

1. Damping-off:

The efficacy of the elicitors (Bion, Chitosan, Oxalic acid and Salicylic acid) in addition to Swtich fungicide at the rate of 1.0, 15.0, 15.8 and 1.5 g/L, respectively, as seed soaking treatment, in controlling damping-off was evaluated.

Data in Table (2) indicate that all elicitors tested significantly reduced pre- and post-emergence damping-off of caraway, coriander and fennel compared with the controls, except Chitosan, Oxalic acid and Switch as well as Switch in case of coriander and fennel, respectively. As for post-emergence, all treatments gave significant decreases than the control with the three medicinal plants tested. On the other hand, increases in survival plants resulted from each treatment were highly significant than the control. Salicylic acid was significantly superior in increasing healthy survivals than the other tested compounds in case of caraway. While, this result was recorded with Bion followed by Salicylic acid, without significant differences between them, with coriander. Also, Bion and Salicylic acid gave the highest survivals (%) in case of fennel but without significant differences between them and the other treatments. Treated caraway with SA and coriander with Bion, however, gave significant increases in survivals than Switch.

2. Foliage plant growth parameters:

Data presented in Table (3) show that all elicitors used significantly increased plant height and fresh weight per plant than the control, except in case of fresh weight of fennel plants. In contrast, increases than the controls caused by Switch were not significant in all parameters measured. Increases than the controls in plant height, however, ranged between (66.04%-98.11%), (40.11%-52.75%) and (66.41%-106.11%) as well as in fresh weight (142.47%-249.32%), (320.95%-431.76%) and (124.84%-212.42%) for caraway, coriander and fennel plants, respectively, using the four elicitors tested. While, increases were only 16.98%, 23.63% and 49.62% in plant height, 42.47%, 107.43 and 105.10% in fresh weight/plant in case of treated caraway, coriander and fennel treated with the fungicide Switch, respectively. On the other hand, Bion & Chitosan (caraway), Salicylic acid & Bion (coriander) and Salicylic acid & Oxalic acid (fennel) gave the highest increases in plant height and fresh plant weight. There are significant differences between increases yielded with all used elicitors and the fungicide Switch.

Table 2. Effect of four elicitors and a fungicide as seed soaking treatment on pre- and post-emergence damping-off and survivals of some medicinal plants, 15, 45 and 90 days after sowing in soil infested with *S. sclerotiorum*

Medicinal plant	Treatment	Pre-emergence (%)	Post-emergence (%)	Survival (%)	Increase * (%)
Caraway	Bion	16.67	26.67	56.66	749.48
	Chitosan	16.67	36.67	46.66	599.55
	Oxalic acid	20.00	30.00	50.00	649.63
	Salicylic acid	16.67	13.33	70.00	949.48
	Switch	26.67	20.00	53.33	699.55
	Control	43.33	50.00	6.67	--
L.S.D. at 5%:		9.96	9.19	13.15	--
Coriander	Bion	10.00	23.33	66.67	852.43
	Chitosan	33.33	40.00	26.67	281.00
	Oxalic acid	26.67	36.67	36.66	423.71
	Salicylic acid	16.67	26.67	56.66	709.43
	Switch	30.00	26.67	43.33	519.00
	Control	33.00	60.00	7.00	--
L.S.D. at 5%:		11.98	7.91	13.96	--
Fennel	Bion	10.00	20.00	70.00	2002.10
	Chitosan	20.00	26.67	53.33	1501.50
	Oxalic acid	13.33	33.33	53.34	1501.50
	Salicylic acid	13.33	16.67	70.00	2002.10
	Switch	23.33	26.67	50.00	1401.50
	Control	36.67	60.00	3.33	--
L.S.D. at 5%:		14.73	9.19	20.48	--

* Increases relative to the control.

Table 3. Effect of four elicitors and a fungicide as seed soaking treatment on plant height and fresh weight of some medicinal plant growth in soil infested with *S. sclerotiorum* for 90 days

Treatment	Caraway				Coriander				Fennel			
	Plant height (cm)	Increase (%) *	Plant weight (gm)	Increase (%) *	Plant height (cm)	Increase (%) *	Plant weight (gm)	Increase (%) *	Plant height (cm)	Increase (%) *	Plant weight (gm)	Increase (%) *
Bion	21.00	98.11	1.016	247.95	26.20	43.96	1.246	320.95	22.60	72.52	1.614	157.01
Chitosan	20.00	88.68	1.020	249.32	25.50	40.11	1.490	403.38	21.80	66.41	1.412	124.84
Oxalic acid	17.60	66.04	0.708	142.47	23.40	28.57	1.308	341.89	23.40	78.63	1.920	205.73
Salicylic acid	18.60	75.47	0.986	237.67	27.80	52.75	1.574	431.76	27.00	106.11	1.962	212.42
Switch	12.40	16.98	0.416	42.47	22.50	23.63	0.614	107.43	19.60	49.62	1.288	105.10
Control	10.60	--	0.292	--	18.20	--	0.296	--	13.10	--	0.628	--
L.S.D. at 5%	2.97	--	0.312	--	5.02	--	0.580	--	7.21	--	1.319	--

* Increases relative to the control.

3. Root growth parameters:

All elicitors treatments and the fungicide Switch increased the length and weight of roots per the medicinal plants tested (Table, 4). Increases in root length were significantly higher than the control with the four elicitors tested, except Chitosan, Oxalic acid and Salicylic acid in case of caraway. While, root weight significantly increased with Bion & Chitosan (caraway), Bion & Salicylic acid (coriander) and Salicylic acid (fennel). While, all increases with Switch in these parameters were not significant. Bion followed by Chitosan, Salicylic acid followed by Bion and Salicylic acid followed by Oxalic acid were superior than the others in increasing length and weight of caraway, coriander and fennel roots, respectively. On the other hand, Switch was less effective in increasing these root parameters.

Significant increases in root growth parameters were, however, recorded with Bion than Switch (root length) and Bion & Chitosan than the others (fresh root weight) in case of caraway, with Salicylic acid than the others, except Bion (root length) and Salicylic acid than Switch (fresh root weight) in case of coriander and with Salicylic acid than Chitosan and Switch (root length) in case of fennel.

Increases in root length and fresh weight ranged between [(32.26%-135.48%) & (10.53%-242.11%), (87.27%-274.55%) & (278.57%-1157.14%) and (36.11%-127.78%) & (187.69%-416.98%)] for caraway, coriander and fennel, respectively, were recorded.

Table 4. Effect of four elicitors and a fungicide as seed soaking treatment on root length and root weight per medicinal plant, 90 days after sowing in soil infested with *S. sclerotiorum*

Treatment	Caraway				Coriander				Fennel			
	Root length (cm)	Increase (%) *	Root weight (gm)	Increase (%) *	Root length (cm)	Increase (%) *	Root weight (gm)	Increase (%) *	Root length (cm)	Increase (%) *	Root weight (gm)	Increase (%) *
Bion	14.60	135.48	0.260	242.11	15.60	183.64	0.284	914.29	12.60	75.00	0.418	221.54
Chitosan	11.80	90.32	0.258	239.47	13.70	149.09	0.216	671.42	12.50	73.61	0.438	236.92
Oxalic acid	9.60	54.84	0.108	42.11	13.40	143.64	0.194	592.86	13.40	86.11	0.542	316.92
Salicylic acid	9.80	58.06	0.106	39.47	20.60	274.55	0.352	1157.14	16.40	127.78	0.672	416.92
Switch	8.20	32.26	0.084	10.53	10.30	87.27	0.106	278.57	9.80	36.11	0.374	187.69
Control	6.20	--	0.076	--	5.50	--	0.028	--	7.20	--	0.130	--
L.S.D. at 5%	5.67	--	0.059	--	5.97	--	0.209	--	3.89	--	0.470	--

* Increases relative to the control.

III. Effect of spraying with four elicitors and a fungicide before inoculation with S. sclerotiorum on the severity of stem rot on some medicinal plants:

The length of Sclerotinia rot as elongate lesion on caraway, coriander and fennel stems was measured after the plants were sprayed with each of four elicitors and Switch 3 and 7 days before inoculation by *S. sclerotiorum* using toothpick technique.

Data in Table (5) indicate that spraying caraway, coriander and fennel plants (90-day-old) with each of Bion, Chitosan, Oxalic acid, Salicylic acid and Switch, 3 or 7 days before inoculation by *S. sclerotiorum* significantly decreased disease severity, 7 and 14 days after inoculation. Disease severity, however, was always higher on coriander than that on caraway or fennel in all cases with Bion, Chitosan and Oxalic acid. While, this reaction was recorded on fennel than the others with Salicylic acid and Switch. On the other hand, disease severity was always increased when the sprayed, inoculated plants were examined 14 days after inoculation than that found after 7 days of inoculation, except Switch with only caraway. Also, decreases in disease severity were recorded when plants were sprayed 3 days before inoculation than these of 7 days before inoculation. Spraying with Salicylic acid generally realized the least infection severity in all cases, followed by Bion and Switch with caraway and coriander, while the same results were found with Oxalic acid followed by Bion and Chitosan in case of fennel.

IV. Effect of spraying with each one of four elicitors and a fungicide before inoculation with S. sclerotiorum on phenol and phytoalexin (iso-flavone) contents in caraway and fennel fresh foliages:

1. Phenol content:

Total phenol contents (Table, 6) in caraway and fennel plants were increased with each one of the elicitors tested and the fungicide Switch as spraying treatment than the control. Also, the same result was occurred with contents of free and conjugated phenols, except Salicylic acid (free phenol) with caraway & fennel and Switch (conjugated phenol) with only fennel. Increases, however, ranged between (27.835%-261.125%) and (15.880%-134.887%) were recorded in case of total phenol contents with caraway and fennel, respectively. They also were (11.475%-382.787%) and (102.424%-228.182%) as well as (16.236%-114.844%) and (55.156%-311.151%) in case of free and conjugated phenols with caraway and fennel, respectively. On the other hand, the highest contents of total, free and conjugated phenols were found with Bion and Chitosan in case of caraway, while they were recorded with Bion, Chitosan and Oxalic acid with fennel.

Percentages of phenolic compound fractions in caraway and fennel plants were recorded in Table (7). Salicylic acid was found in all examined samples with various percentages in caraway and fennel except in caraway treated with switch. Also, cinnamic acid and vaniline were found in most samples. Although P-cumaric acid reached 100% in caraway samples which was sprayed with Switch, there was no P-cumaric acid in the same treatment in case of fennel plants. The other phenolic compounds (syringic, protocat and ferulic) were not found in all samples, whereas P-hydbenzoic and coffiec acids found in one sample for each them.

2. Phytoalexins (iso-flavone) content:

Data in Table (8) show the concentrations of phytoalexins (iso-flavone) in caraway and fennel plants as reaction to infection by the fungus and spraying with the treatments tested. All elicitors, however, increased the concentrations of phytoalexins than the controls, except Bion as there was no phytoalexins in plant samples of caraway and fennel tested with it. Switch enhanced caraway (71.429%) and fennel (271.429%) plants to produce more phytoalexin than the other treatments. On the other hand, Oxalic acid (16.071% & 57.143%) and Salicylic acid (7.143% & 238.095%) also caused higher increases (%) in phytoalexins of caraway and fennel plants, respectively, followed by Chitosan.

Table 5. Effect of spraying with each of four elicitors and a fungicide, 3 or 7 days before inoculation, on Sclerotinia rot severity on stems of some medicinal plants, 7 and 14 days after inoculation

Treatment	Spraying * period (days)	Days after inoculation	Length of infected area (cm) of medicinal plant			Mean
			Caraway	Coriander	Fennel	
Bion	3	7	1.30	1.40	1.27	1.32
		14	1.30	4.33	2.43	2.69
	7	7	1.40	6.25	2.87	3.51
		14	3.80	6.80	6.00	5.53
Chitosan	3	7	1.53	3.47	1.10	2.03
		14	3.45	5.50	3.45	4.13
	7	7	2.30	3.80	1.77	2.62
		14	5.73	10.37	4.37	6.82
Oxalic acid	3	7	2.23	5.50	1.00	2.91
		14	3.40	6.70	1.83	3.98
	7	7	2.25	6.13	1.50	3.29
		14	4.90	6.93	3.00	4.94
Salicylic acid	3	7	1.00	1.10	2.33	1.48
		14	1.93	3.10	4.13	3.05
	7	7	2.00	2.55	6.77	3.77
		14	6.83	3.83	8.70	6.45
Switch	3	7	1.00	2.70	4.82	2.84
		14	3.20	3.00	8.50	4.90
	7	7	1.00	4.50	6.37	3.96
		14	3.20	5.43	10.00	6.21
Control (untreated)	3	7	12.23	12.00	10.33	11.52
		14	17.80	22.50	19.00	19.77
	7	7	12.50	13.83	11.90	12.74
		14	18.43	20.67	21.73	20.28
L.S.D. at 5% For:						
Treatment (T)		=	0.61	0.63	0.80	
Spraying periods (S)		=	0.21	0.29	0.22	
T x S		=	0.52	0.71	0.54	
Examination periods (E)		=	0.26	0.26	0.34	
T x E		=	0.63	0.63	0.84	
S x E		=	0.36	0.36	0.48	
T x S x E		=	0.89	0.88	1.19	

* Spraying periods before inoculation.

Table 6. Effect of spraying with each one of four elicitors and a fungicide before inoculation with *S. sclerotiorum* on phenol contents in caraway and fennel fresh foliages (mg/gm fresh weight)

Medicinal plant	Treatment	Phenol contents as mg/g fresh weight of plant					
		Total phenol	Increases * or decreases (%)	Free phenol	Increases or decreases (%)	Conjug-ated phenol	Increases or decreases (%)
Caraway	Bion	3.831	134.881	0.589	382.787	3.242	114.844
	Chitosan	2.437	49.418	0.194	59.016	2.243	48.641
	Oxalic acid	1.890	15.880	0.136	11.475	1.754	16.236
	Salicylic acid	2.638	61.741	0.115	(-) 5.738	2.523	67.197
	Switch	2.444	49.847	0.984	706.557	1.460	(-) 3.247
	Control	1.631	-	0.122	-	1.509	-
Fennel	Bion	2.573	121.048	1.083	228.182	1.488	78.417
	Chitosan	4.205	261.125	0.776	135.152	3.429	311.151
	Oxalic acid	3.026	159.966	0.682	106.667	2.341	180.695
	Salicylic acid	1.488	27.835	0.194	(-)41.212	1.294	55.156
	Switch	1.969	69.158	0.668	102.424	1.301	55.995
	Control	1.164	-	0.330	-	0.834	-

* Increases or decreases relative to the control.

Table 7. Effect of spraying with each one of four elicitors and a fungicide before inoculation with *S. sclerotiorum* on phenolic compound fractions (%) in caraway and fennel fresh foliages

Medicinal plant	Treatment	Phenolic compound fractions (%):						
		Cinnamic acid	P-Hydbenzoic acid	Cofffec acid	Vaniline	Cumarin	P-Cumaric acid	Salicylic acid
Caraway	Bion	30.26	-	-	16.69	37.38	-	6.00
	Chitosan	24.23	-	-	61.37	-	-	7.39
	Oxalic acid	28.93	-	-	68.69	-	-	2.36
	Salicylic acid	23.39	-	-	-	63.38	-	5.95
	Switch	-	-	-	-	-	100.00	-
	Control	-	-	-	18.02	35.69	45.12	1.71
Fennel	Bion	16.91	-	-	77.53	-	-	1.88
	Chitosan	-	-	-	84.37	-	-	1.15
	Oxalic acid	-	71.12	-	-	-	16.09	0.70
	Salicylic acid	-	-	-	43.33	-	8.51	3.64
	Switch	15.32	-	9.28	55.54	-	-	8.94
	Control	13.38	-	-	49.76	-	-	6.59

(-) The fraction not detected.

Table 8. Effect of spraying with each one of four elicitors and a fungicide before inoculation with *S. sclerotiorum* on phytoalexins (iso-flavone) in caraway and fennel fresh foliages (μg/gm)

Medicinal plant	Treatment	Phytoalexins (Iso-flavone)	
		Concentration (μg/gm)	Increase * or decreases (%)
Caraway	Bion	0.00	(-) 100.00
	Chitosan	5.7×10^{-3}	1.786
	Oxalic acid	6.5×10^{-3}	16.071
	Salicylic acid	6.0×10^{-3}	7.143
	Switch	9.6×10^{-3}	71.429
	Control	5.6×10^{-3}	--
Fennel	Bion	0.00	(-) 100.00
	Chitosan	2.3×10^{-3}	9.524
	Oxalic acid	3.3×10^{-3}	57.143
	Salicylic acid	7.1×10^{-3}	238.095
	Switch	7.8×10^{-3}	271.429
	Control	2.1×10^{-3}	--

* Increases or decreases relative to the control.

Discussion

Currently, identifying the defence activators (elicitors) that can supplement conventional chemical fungicides is a valuable contribution to medicinal plants disease management, especially for the prevalence and destructive root & stem rot (Sclerotinia rot), caused by *S. sclerotiorum*, which affecting umbelliferous medicinal plants such as caraway, coriander and fennel. The *in vitro* fungal growth on PDA was decreased significantly using the five concentrations of Bion (Bi), Chitosan (Ch), Oxalic acid (OA) and Salicylic acid (SA). The level of the fungal inhibition increased with increasing concentration of each elicitor, except in case of Bi. OA and SA prevented the fungal growth at 4th and 5th concentrations, respectively. In this respect, two models have been proposed to explain the antifungal activity of Ch: first, the interaction with fungal DNA and RNA (Hadwiger and Loschke, 1981) and second, its ability to interfere with the plasma membrane function (Leuba and Stossel, 1986). Also, Ch has also demonstrated fungicidal activity against several fungi (Hadwiger & Beckman, 1980; Stossel & Leuba, 1984; Hirano & Nago, 1989; Benhamou & Theriault, 1992; Wang, 1992; El-Ghaouth *et al.*, 1992; Rabea *et al.*, 2003 and Nawar, 2005). Similar to the present results were also reported by Cheah *et al.* (1997), who concluded that Ch significantly reduced the growth of *S. sclerotiorum* on PDA plates. The *in vitro* growth of *Microdochium nivale* was also reduced with Bi (10-1000 μg/ml) and Ch (2000 μg/ml) (Hofgaard *et al.*, 2005). On the other hand, soaking seeds of caraway, coriander and fennel in each elicitor tested was of great value in decreasing damping-off (pre- & post-emergence) incidence and in increasing survivals. Also, plant growth parameters such as height & fresh weight/plant and root length & weight/plant were greatly improved. SA (caraway) and Bi (coriander) were significantly superior in increasing survivals than the fungicide. Treating seeds of various crops (soaking or other treatments) with the elicitors such as Ch (Jiang *et al.*, 1994; Jaing *et al.*, 1999 and Dasgupta *et al.*, 1998), effectively controlled soilborne diseases and others including *S. sclerotiorum* disease (Yu *et al.*, 1998 & 1999). Treating rice seeds with Ch, however, reduced disease incidences by 91-98% at growth stages (Jiang *et al.*, 1999). Mechanisms of action of the natural disease resistance inducer Ch based on the increase of lignin biosynthesis and plant cell wall lignification and on the effect on enzyme biosynthesis associated with the development of resistance (Tinterev *et al.*, 1996). As for plant growth, Ch was reported by Dasgupta *et al.* (1998) and Yu *et al.* (1999) to enhance a number of plant growth characteristics of other crops similar to the present results.

Spraying caraway, coriander or fennel plants (90-day-old), one or two weeks pre-inoculation with *S. sclerotiorum*, with the elicitors and the fungicide separately significantly decreased disease severity than the control. SA followed by Bi and the fungicide (caraway & coriander) and OA followed by Bi and Ch (fennel) realized the least disease severities. These positive results in decreasing Sclerotinia stem rot severity may due to the induction of systemic resistance similar to that reported against *S. sclerotiorum* in kiwi fruit using SA or OA as inducers (Reglinski *et al.*, 1997) and in oilseed rape using OA (Toal & Jones, 1999). The high level of systemic resistance in oilseed rape, however, was not produced by translocation of OA and its subsequent effect as either a signal or an antifungal molecule; no increases in oxalate concentrations were observed in the distal leaves following OA application. The defence products induced systemic disease resistance, as these in the present study, are divers, reflecting the number of defence pathways stimulated: lignin, pathogensis-related proteins, phytoalexins and the small antimicrobial protein thionin and defensins (Pan *et al.*, 1992; Kessman *et al.*, 1994 and Epple *et al.*, 1997). Also, application with defence product such as Ch may enhance the vitality of plant cells and the plant's ability to degrade the walls of fungi upon entry (Benhamou & Theriault, 1992). The induction of systemic resistance in crops by exogenous application of inducer chemicals would be particularly useful against pathogens that are currently poorly controlled. The infection of the stems of medicinal plants tested usually occurs at flowering, making fungicidal control problematic because of the large plant canopy besides its hazardous effects on human and environment, while the wide host range of the pathogen makes crop rotation an ineffective control strategy. On the other hand, the phenolic compound contents as well as phytoalexins (iso-flavone) in the treated caraway and fennel plant leaves were increased than the control using the elicitors and the fungicide tested in most cases. Bi and Ch gave the highest free and conjugated phenol contents. While, the fungicide switch recorded the highest increases in phytoalexins contents, followed by OA (caraway) and SA (fennel). Similar to the obtained results, treatment with Ch was found to stimulate the accumulation of phenolic compounds (Fajardo *et al.*, 1995 & Reddy *et al.*, 1999) and phytoalexins (Hadwiger *et al.*, 1994 & Pospieszny, 1997) in various plant tissues. Also, Anderson (1988) confirmed that SA was responsible for the accumulation of phytoalexins in viable tissues.

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المقاومة المستحثة ضد مرض الإسكليروتينيا فى بعض نباتات العائلة الخيمية الطبية كوسيلة ممكنة وفعالة فى المقاومة

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أجريت تجارب فى المعمل والصوبة لتقييم فعالية خمسة تركيزات من كل من البيون، الشيتوزان، حمض الأوكساليك وحمض السالسليك ضد نمو الفطر سكليروتينيا سكليروتيورم *S. sclerotiorum* النامى على بيئة البطاطس والدكستروز وكذلك لإثبات الفعالية الإيجابية لها كمنشطات دفاعية (مستحثات) لمقاومة أمراض موت البادرات وعفن الساق على بادرات ونباتات الكراوية ، الكسبرة والشمر مقارنة بالمبيد الفطرى سويتش. تم نقع البذور فى هذه المستحثات كل على حدة قبل زراعتها فى تربة ملوثة بالفطر لمقاومة مرض موت البادرات فى حين إستخدمت رشاً على النباتات قبل حقن السوق بالفطر لمقاومة مرض عفن السوق. كما تم تقدير الزيادة فى محتوى النبات من الفينولات والفيتواليكسينات (أيزو–فلافون) نتيجة رش النباتات بهذه المعاملات.

خفضت كل المعاملات النمو الفطرى فى المعمل ماعدا الشيتوزان فى التركيز المنخفض. زاد معدل الخفض بزيادة تركيزات كل المستحثات المستخدمة باستثناء البيون. ولقد منع حمض الأوكساليك والسالسليك النمو الفطرى كلية عند التركيز العالى.

أدى نقع البذور فى أى من المستحثات او المبيد المستخدم إلى خفض معنوى فى النسبة المئوية لموت البادرات قبل ظهورها (معظم الحالات) والنسبة المئوية لموت البادرات بعد ظهورها فوق سطح التربة كما أدى إلى زيادة النسبة المئوية للنباتات المتبقية حية حيث بلغت الزيادة (٥–٧) أضعاف فى الكراوية ، ٢–٨ أضعاف فى الكسبرة و١٤–٢٠ ضعف فى الشمر عن معاملة المقارنة. فى حين أدى إستخدام حمض السالسيلك فىالكراوية ، البيون فى الكسبرة إلى زيادة معنوية فى النباتات المتبقية حية مقارنة بالمعاملات الأخرى. كما تفوق حمض السالسليك فى الكراوية ، البيون فى الكسبرة فى زيادة النباتات المتبقية حية عن معاملة المبيد الفطرى. ومن ناحية أخرى تشير النتائج إلى حدوث زيادة بدرجة ملحوظة فى قراءات الطول ، الوزن الطازج/نبات وأيضا طول ووزن الجذر/نبات (فى معظم الحالات) عن معاملة المقارنة .

أدى رش النباتات (عمر ٩٠ يوم) – قبل الحقن بالفطر بثلاثة أو سبعة أيام – بكل معاملة منفرده إلى خفض معنوى فى شدة الإصابة بالمرض حيث كانت شدة الإصابة أعلى بعد ١٤ يوم عنه بعد ٧ أيام من العدوى. كما سجلت أقل شدة إصابة مع حمض السالسليك يليه البيون والمبيد الفطرى (الكراوية والكسبرة) وحمض الأوكساليك يليه البيون والشيتوزان (الشمر). ومن ناحية أخرى أدت كل المعاملات المختبرة إلى زيادة محتوى النبات من المركبات الفينولية (فى معظم الحالات) عن معاملة المقارنة. وأعطت معاملتى البيون والشيتوزان أعلى محتوى من الفينولات الكلية (المرتبطة والحرة) ، كما ارتبط وجود الأنواع المختلفة للمركبات الفينولية والنسبة المئوية لتكرار تواجدها بنوع كل من المعاملة والنباتات. أدى إستخدام كل المعاملات إلى زيادة تركيز الفيتوالكسين (ايزو–فلافون) فى النباتات عن معاملة المقارنة باستثناء البيون والذى لم يتم إكتشاف الفيتوالكسينات فى العينات النباتية التى تم رشها به . كما سجل المبيد الفطرى أعلى الزيادات فى الفيتوالكسين (فى معظم الحالات) يليه حمض الأوكساليك (الكراوية) وحمض السالسليك (الشمر).