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Inducing Resistance Against Sorghum Downy Mildew Disease of Maize by Some Chemical inducers

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ABSTRACT

Five chemical inducers at three different concentrations, *i. e.*, Salicylic acid (SA); Nicotinic acid (NA); Di-basic potassium phosphate (K_2HPO_4) and Bion (BTH) at 2, 4 and 8 mM and Hydrogen peroxide (H_2O_2) at 0.25, 0.50 and 1.0 %, were used to evaluate their capabilities to induce resistance against sorghum downy mildew disease (SDM) of maize under greenhouse and field conditions during two successive seasons (2010/ 2011). In greenhouse and field experiments, all chemical inducers at the tested concentrations obviously decreased disease incidence (DI), showing significant differences with the untreated infected control. Inducer effects in both seasons slightly differ. There is a positive relationship between increasing the concentration of the tested inducers and their effect on disease incidence. In this respect, SA and NA at 8, 4 mM and BTH at 8 mM were the most effective treatments on reducing the disease while H_2O_2 was moderately effective while, K_2HPO_4 was the least effective treatment. Apron fungicide showed the highest effect against SDM disease of maize. Regarding the effect of the aforementioned inducers on grain yield, this study indicated that, all the inducers at the tested concentrations have significantly increased grain yield (Ton/ Fed.) as compared with untreated infected control. The results also indicated that, there is a correlation between induced resistance and biochemical changes in leaf tissues. In this regard, the tested inducers increased the activity of oxidative enzymes and caused greater accumulation of phenolic compounds in leaves of potted maize plants (21 days old). SA at 8 mM give the highest activity of oxidative enzymes [peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL)] as well as increase the amounts of phenols.

Key words: Maize, Downy mildew, Salicylic acid(SA), Nicotinic acid (NA), Di-basic potassium phosphate (K_2HPO_4), Bion (BTH), Hydrogen peroxide (H_2O_2), peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL).

Introduction

Monocots provide the top three staples in the world; rice, wheat and maize. Diseases are Known to reduce yield in these crops and, especially in Wheat, there is a significant reliance on agrochemical to control diseases (Oerke *et al.*, 1994).

Sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* [(weston and Uppal) G. G. Shaw] is a serious disease of maize (*Zea mays* L.), sorghum (*Sorghum spp*.) and grain sorghum [*sorghum bicolor* (L.) Moench]. It occurred in Asia, Africa, the Middle East, North, South, and Central America (Frederiksen, 1977). In Egypt it has been considered a major disease of maize and sorghum (Melchers, 1931).

Control of the disease can be achieved by using fungicides (El-Moghazy, 2003 and El-Shehawy, 2009), resistant cultivars which may loose its efficacy if new pathotypes of the fungus *P. Sorghi* and bio-control agents which help maintain a healthy environment and sustainable production system while reducing the grower's cost.

One of the potential methods is the use of a number of compounds that have not direct antimicrobial activity but increase resistance or at least decrease symptoms, in some host-Pathogen interaction (Kessman *et al.*, 1994). These compound cause induced resistance. Induced disease resistance can be defined as the process of active resistance dependent on the host plants physical or chemical barriers activated by biotic or abiotic agents, (Meena *et al.*, 2001 and Walters *et al.*, 2007). Various chemicals have been discovered that seem to act at various points of the defense. Some compounds *e.g.*, Hydrogen peroxide (H_2O_2), Nicotinic acid, Salicylic acid (SA), Bion (BTH) and K_2HPO_4 have been shown to induce resistance in plants (Mahmoud *et al.*, 2006; Mandal *et al.*, 2009; Hussien, 2011 and Khalifa *et al.*, 2011). Induction of systemic resistance sensitizes the plant to respond rapid after infection. These responses include phytoalexin accumulation, phenols, lignifications and activation of many enzymes such as peroxidase, polyphenoloxidase, phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) (Meena, *et al.*, 2001; Mahmoud *et al.*, 2006 and Hussien, 2011).

The present investigation aimed to evaluate the effectiveness of some chemical inducers to induce resistance against SDM disease of maize under green house and field conditions. Also, to study the effect of the tested compounds on oxidative enzymes and phenolic compounds content.

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Materials And Methods

Five chemical inducers at three different concentrations, *i. e.*, Salicylic acid (SA); Nicotinic acid (NA); Di-basic potassium phosphate (K_2HPO_4) and Bion (BTH) at 2, 4 and 8 mM and Hydrogen peroxide (H_2O_2) at 0.25, 0.50 and 1.0 %, were used to evaluate their capabilities to induce resistance against sorghum downy mildew disease (SDM) of maize under greenhouse and field conditions at Maize, Sorghum and Sugar Crops Diseases Section and Gemmeiza experimental station of Agricultural Research Center (ARC) during two successive seasons (2010/2011).

1. Greenhouse experiment:

A. Preparation of the fungal inoculum:

Shredded infected leaves bearing Oospores were collected from systemically infected plants of sorghum (Sordan 79, used as spreader in the nursery field at Gemmeiza). Infected leaves were cut into short parts, freed by maceration in a Warning Blender and added to the soil at the rate of 1g/ pot (25 cm-dim.) as described by Sadoma, 1995 and modified by the authors.

B. Grains treatment and soil infestation:

The aforementioned five chemical inducers were used as soaking treatment. Grains of Three Way Cross 310 hybrid (T.W.C.-highly susceptible), were soaked for 6 hours in each tested concentration. The wetted grains were left in air cabinet for 12 hour before sowing. Apron (a 35% active ingredient formulation) was used for comparison as soaking application at the recommended dose 0.5 ml/ L. Grains of control plants were soaked in distilled water only. Grains of the susceptible hybrid Three Way Cross 310 were treated with the aforementioned chemicals at the same concentrations as described before. Ten grains were sown in (25 cm-dim) pots filled with soil previously infested with the fungal inoculum at the rate of 1g/ Pot (25cm-dim). Plants were thinned to five plants per/ pot and 5 pots were used per treatment. The experiment was completely randomized design with three replications. Pots were kept under greenhouse conditions.

C. Percentages of treatment efficacy in reducing the disease infection were calculated as follows:

$$\% \text{ Treatment efficiency} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \text{ X } 100$$

$$\% \text{ Chemical inducer efficiency to fungicide efficacy} = \frac{\text{Ch. inducer efficiency}}{\text{Fungicides efficiency}} \text{ X } 100$$

2. Biochemical changes associated with induced resistance:

This study was conducted to identify some biochemical changes associated with induced resistance by the tested inducers. Activity of oxidative enzymes, *i. e.*, peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) as well as phenol content were determined in maize leaves of treated and untreated potted plants under greenhouse conditions. Apron (a 35% active ingredient formulation) was used for comparison as socking application at the recommended dose 0.5 ml/ L.

2. 1. Determination of oxidative enzymes activity:

The extraction procedure was essentially based on the methods described by Biles and Martyn, (1993) to determine the activity of peroxidase (PO) and polyphenoloxidase (PPO) as follow: samples of leaves from each treatment and control plants were collected 21 days after sowing. One gram of leaf tissue was ground with 2 ml of sodium phosphate buffer (pH 6.5), 0.1 M using mortar and pestle. Samples were transferred to Eppendorf tubes, and then centrifuged for 20 min at 12000 rpm at 4°C. Supernatant, was stored at -8°C. Three replicates were prepared for each treatment.

Activity of the oxidative enzymes *i. e.*, Peroxidase, Polyphenoloxidase, Phenylalanine ammonia lyase and Tyrosine ammonia-lyase were determined as described by Hammerschmidt *et al.* (1982); Malik and Singh (1980) and Solecka and Kacperska (2003) respectively. A change of optical density was determined at different intervals according to the tested enzyme using Spectrophotometer, 6300 VIS.

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2.2. Extraction and determination of phenolic compounds:

Samples of leaf, (21 days from sowing) of potted maize plants grown from grains treated with the chemical inducers at three concentrations and, sown in inflected soil, was extracted in soxhlet units using 75 % ethanol for 10-12 hrs then used to determine phenolic compounds as described by **Snell and Snell, (1953)**. The phenolic fraction was calculated as milligrams equivalent of catechol/ g fresh weight of maize leaves.

3. Field experiment:

The highly susceptible Sudan grass [(*Sorghum sudanens*) Sordan 79] was sown in single rows as a spreader at Gemmeiza nursery where soil is highly infested at the beginning of June 2010. The field is annually infested with downy mildew oospores. After one month, the treated grains were sown in alternation with spreader rows under field condition. For each treatment, three replicates were planted, each with fifty plants. The design of the experiment was a randomized complete block design. All agricultural practices were performed as recommended. Disease was rated 45 days after planting, by counting the number of plants with disease symptoms.

4. Effect of the tested inducers on grain yield:

At harvest, three quadrates, each measuring 3X3.5 m of each treatment were used to study the effect of the aforemention chemicals on grain yield/ Feddan. Fears were husked, dried under the sun until 13 % moisture content was attained and then shelled. Reduction in yield was determined by the difference in weight of shelled grains from treated and untreated plots.

5. Statistical analysis:

The data were statistically analyzed by analysis of variance (ANOVA) using the statistical Analysis System (SAS Institute, inc, 1996). Means were separation by least significant difference (L.S.D.) Test at $P \leq 0.05$ level.

Results:

1. Effect of chemical inducers on disease incidence under greenhouse conditions:

Date presented in **Table (1)** show that, all chemical inducers obviously decreased disease incidence (DI), all of the tested concentrations significantly reduced (DI) compared with the untreated infected control. Significant reduction of DI was occurred by increasing concentrations. SA at 8 mM was the most effective treatment followed by each of SA at 4 mM and NA at 8 mM followed by each of NA at 4 mM and BTH at 8 mM, while H_2O_2 and K_2HPO_4 were the least effective treatments. Apron, chemical fungicide showed the highest effect against SDM disease.

Table 1: Effect of soaking maize grains of T.W.C. 310 in different concentrations of five chemical inducers, on downy mildew disease incidence under greenhouse conditions.

Inducers	Conc.	DI	Efficacy %	
			To control	To Apron
Control	…..	78.67	….	…..
SA	2 mM	38.67	50.85	81.07
	4 mM	36.00	54.24	86.48
	8 mM	32.00	59.32	94.58
NA	2 mM	42.67	45.76	72.96
	4 mM	40.00	49.15	78.36
	8 mM	36.00	54.24	86.48
H_2O_2	0.25%	54.67	30.51	48.64
	0.50%	52.00	33.90	54.05
	1.00%	48.00	38.99	62.17
K_2HPo_4	2 mM	58.67	25.42	40.53
	4 mM	56.00	28.82	45.95
	8 mM	53.33	32.21	51.36
Bion	2 mM	46.67	40.68	64.86
	4 mM	42.67	45.76	72.96
	8 mM	40.00	49.15	78.36
Apron 35%	0.5 ml/L.	29.33	62.72	…..
L. S. D. (0.05)				
Treatment		2.091		
Concentration		1.368		
Treat. X Conc.		3.639		

Data in **Table (1)** clearly showed the ability of some tested chemical inducers to be near the fungicide efficiency (Apron) in reducing the disease incidence. In this regard, SA at 8 mM was the nearest one to fungicide efficiency (94.58%) followed by NA at 8 mM which gave 86.48%. While K_2HPo_4 at 8 mM gave the lowest efficiency for fungicide efficiency in reducing the disease severity.

2. Effect of chemical inducers on activity of oxidative enzymes:

Table (2), illustrate the effect of the tested chemical inducers at three different concentrations, used as soaking treatment, on the activity of oxidative enzymes. Data show pronounced increase in oxidative enzymes activity of PO, PPO, PAL and TAL in leaves of potted maize plants (21 days old) grown from treated grains as compared with untreated infected control. Data also show that, SA at 8 mM induced the highest level of oxidative enzymes PO, PPO, PAL and TAL activity followed by H_2O_2 at 1.0%.

Apron fungicide at 0.5 ml/ L decreased the activity of all enzymes than in untreated infected control. Data also show that, increase the concentration of all inducers was accompanied by an increase of activates of the tested enzyme.

Table 2: The effect of five chemical inducers used as soaking treatment on the activity of PO, PPO, PAL and TAL in leaves of potted maize plants (T.W.C. 310) grown in infested soil.

Chemical inducers	Conc.	Enzymes activity*			
		Peroxidase	Polyphenol oxidase	Phenl alanine ammonia lyase	Tyrison ammonia lyase
Control	…..	0.268	0.169	0.557	0.457
SA	2 mM	0.750	0.314	0.798	0.747
	4 mM	0.777	0.363	0.838	0.784
	8 mM	0.801	0.388	0.872	0.826
NA	2 mM	0.513	0.222	0.613	0.634
	4 mM	0.582	0.245	0.654	0.666
	8 mM	0.615	0.276	0.692	0.703
H_2O_2	0.25%	0.693	0.249	0.754	0.711
	0.50%	0.729	0.286	0.783	0.757
	1.00%	0.751	0.315	0.811	0.793
K_2HPO_4	2 mM	0.371	0.160	0.479	0.501
	4 mM	0.391	0.184	0.511	0.548
	8 mM	0.420	0.211	0.535	0.577
Bion	2 mM	0.473	0.193	0.526	0.581
	4 mM	0.497	0.209	0.565	0.614
	8 mM	0.522	0.235	0.593	0.637
Apron 35%	0.5 ml/L	0.172	0.121	0.393	0.359
L. S. D. (5%)					
Treatment		0.023	0.025	0.016	0.029
Concentration		0.015	0.016	0.011	0.019
Treat. X Conc.		0.0398	0.0426	0.0281	0.051

* Determined as a changing of optical density.

3. Effect of chemical inducers on phenol contents:

Date presented in **Table (3)** reveal that, soaking maize grains in the, tested chemical inducers at the three concentrations caused greater accumulation of phenolic compounds (free and total) than those of nontreated infected control. Maximum amounts of phenols were produced in leaves of potted Maize plants (21 days old) grown from grains treated with SA at 8 mM followed by H_2O_2 at 1.0 %, NA at 8 mM and Bion at 8 mM while the lowest content was produced in leaves of potted maize plants grown from grains treated with K_2HPO_4. Data also show that increasing the concentration of the tested inducers led to an increase of phenols content in maize leaves. Apron fungicide al 0.5 ml/ L decreased phenols content than the nontreated infected control.

4. Effect of chemical inducers on disease incidence under Field experiments:

Date presented in **Table (4)** indicate that, inducer effects in both seasons slightly differ. All chemicals at tested concentrations obviously decreased disease incidence (DI) and showing significant differences with the untreated infected control. Data also revealed that, the higher concentrations of application, the greater reduction in disease incidence in both seasons. All concentrations of the chemicals reduce (DI) in both seasons.

SA at 8 mM showed the maximum reduction of the disease followed by each of SA at 4 mM and NA at 8 mM followed by each of NA at 4 mM and BTH at 8 mM. SA at 8 mM reduced DI from 67.33% (untreated infected control) to 28.67 % in 2010 and from 74.0 % (untreated infected control) to 31.33% in 2011. Apron, chemical fungicide, at 0.5 ml/ L followed by Salicylic acid at 8mM exhibited the highest values of effectiveness in controlling SDM disease on maize in both seasons as compared with untreated infected control. The

efficiency of SA at 8 mM increased from 57.42 and 57.66 in 2010, 2011 respectively, to 92.05 and 90.15 as efficiency for fungicide efficiency (**Table 4**). Data also indicate that H_2O_2 and K_2HPO_4 were the least effective inducers. Apron and the aforementioned chemical inducers were more efficient in 2010 growing season than in 2011.

Table 3: Effect of five chemical inducers on phenol contents (mg/g fresh weight) in leaves of potted maize plants (T.W.C. 310) grown in infested soil.

Chemical inducers	Conc.	Phenols content		
		Free	Conjugated	Total
Control	…..	8.81	6.09	14.90
SA	2 mM	17.71	7.61	25.32
	4 mM	20.52	6.80	27.32
	8 mM	23.83	5.54	29.37
NA	2 mM	11.35	4.97	16.32
	4 mM	13.45	5.21	18.66
	8 mM	15.65	5.22	20.87
H_2O_2	0.25%	15.72	4.98	20.70
	0.50%	17.93	4.34	22.27
	1.00%	20.61	4.02	24.63
K_2HPO_4	2 mM	8.69	6.29	14.98
	4 mM	9.13	6.99	16.12
	8 mM	10.45	8.10	18.55
Bion	2 mM	10.12	5.88	16.00
	4 mM	10.99	6.56	17.55
	8 mM	11.25	7.97	19.22
Apron 35%	0.5 ml/L	9.38	5.54	14.92
L. S. D. (5%)				
Treatment		1.148	0.691	1.310
Concentration		0.664	0.211	0.821
Treat. X Conc.		1.932	1.025	2.356

Table 4: Effect of soaking maize grains of T.W.C. 310 in different concentrations of five chemical inducers, on downy mildew disease incidence under field conditions during 2010/2011 growing seasons at Gemmeiza experimental station.

Inducers	Conc.	2010			2011		
		DI	Efficacy %		DI	Efficacy %	
			To control	To Apron		To control	To Apron
Control	…..	67.33	…..	…..	74.00	…..	…..
SA	2 mM	33.33	50.50	80.96	37.33	49.55	77.47
	4 mM	30.67	54.45	87.29	35.33	52.26	81.71
	8 mM	28.67	57.42	92.05	31.33	57.66	90.15
NA	2 mM	37.33	44.56	71.43	40.00	45.95	71.84
	4 mM	34.67	48.51	77.77	38.67	47.74	74.64
	8 mM	32.00	52.47	84.11	35.33	52.26	81.71
H_2o_2	0.25%	45.33	32.67	52.37	45.33	38.74	60.57
	0.50%	42.67	36.63	58.72	43.33	41.45	64.81
	1.00%	39.33	41.59	66.67	41.33	44.15	69.03
K_2HPo_4	2 mM	48.00	28.22	45.24	49.33	33.33	52.11
	4 mM	46.00	31.68	50.79	46.67	36.93	57.74
	8 mM	42.67	36.63	58.72	44.00	40.54	63.38
Bion	2 mM	40.00	40.59	65.07	42.67	42.34	66.20
	4 mM	38.67	42.57	68.24	40.67	45.04	70.42
	8 mM	35.33	47.53	76.19	38.00	48.65	76.06
Apron 35%	0.5 ml/L.	25.33	62.38	…..	26.67	63.96	…..
L. S. D. (0.05)							
Treatment		1.612			1.313		
Concentration		1.053			0.859		
Treat. X Conc.		2.787			2.562		

5. Effect of chemical inducers on grain yield:

Data presented in **Table (5)** show that, all the chemical compounds at the tested concentrations and Apron, chemical fungicide, have significantly increased grain yield (Ton/ Fed.) as compared with untreated infected control but not in-between particular inducer (s) and other in 2011 growing season. Data also show that, there are positive relationship between increasing the concentrations of the tested inducers and their effect on grain yield. The highest grain yield (Ton/ Fed.) in the two seasons was obtained by SA at 8 mM followed by NA at 8 mM and BIH at 8 mM. Results also show no significant differences between the values of SA at 8 mM and Apron at 0.5 ml/ L in both seasons. Hence, a particular recommendation could be valid and SA at 8 mM could be used as seed treatment for SDM disease control to help maintain a healthy environment.

Table 5: Effect of soaking maize grains of T.W.C. 310 in different concentrations of five chemical inducers on grain yield (Ton/Fed) under field conditions during 2010/2011 growing seasons at Gemmeiza experimental station.

Inducers	Conc.	Ton/ Fad.	
		2010	2011
Control	…..	1.409	1.367
SA	2 mM	2.960	2.819
	4 mM	3.308	2.930
	8 mM	3.362	3.057
NA	2 mM	2.812	2.727
	4 mM	2.979	2.838
	8 mM	3.142	2.931
H_2O_2	0.25%	2.459	2.391
	0.50%	2.579	2.519
	1.00%	2.663	2.656
K_2HPO_4	2 mM	2.213	2.127
	4 mM	2.375	2.246
	8 mM	2.456	2.366
Bion	2 mM	2.611	2.613
	4 mM	2.769	2.682
	8 mM	2.863	2.741
Apron 35%	0.5 ml/L	3.547	3.393
L. S. D. (5%)			
Treatment		0.224	0.215
Concentration		0.121	0.112
Treat. X Conc.		0.368	0.346

Discussion:

Five chemical compounds *i. e.* SA, NA, H_2O_2, K_2HPO_4, Bion (BTH) and Apron (chemical fungicide for comparison) were used as seed treatment to evaluate their efficacy to induce resistance against SDM disease of maize. Obtained results clearly indicated that, under greenhouse and field conditions, all tested chemicals have significantly reduced disease incidence (DI) in 2010 but with lower efficacy in 2011. This may be duo to increased pathogen inoculum in the soil in 2011. Also to the variation in agro-metrological conditions. Salicylic acid and Nicotinic acid at 8, 4 mM and Bion at 8 mM were the most effective treatments as compared to the treated infected control (grains were treated with Apron at 0.5 ml/ L). Hydrogen peroxide and di-basic potassium phosphate were moderately effective. Apron fungicide, showed the highest effect against SDM disease. These results were in agreement with those obtained by Morris *et al.*, 1998 who found that, BTH was effective against sorghum downy mildew of maize caused by *Peronosclerospora sorghi* in the field when applied as a seed treatment. El-Moghazy, 2003 indicated that, salicylic acid effectively decreased the percentage of infection of maize plants with downy mildew when compared with the untreated control in the field. Ata *et al.*, 2008 found that Hydrogen peroxide at 1.0 % and 0.5 %, Salicalic acid and di-basic potassium phosphate at 8 mM were the most effective chemicals in controlling rust disease of sugar beet. El-Shehawy, 2009, found that, SA was effective against grain sorghum downy mildew disease in the field when applied as a foliar spray.

Nitric oxide (NO) was suggested to act as a signal molecule mediating responses to biotic and abiotic stress in plants (Durner and Klessig, 1999 and Rashad and Abou-Elalla, 2009). Furthermore, NO was suggested to be involved in the responses to disease resistance (Shi *et al.*, 2005). NO is also considered as a potent antioxidant in plants and direct scavenger reactive oxygen species (ROS) (Beligni and Lamattina, 2002). Additionally, salicylic acid and nitric oxide treatments induced accumulation of defence related genes in plants (Shi *et al.*, 2005). On the other hand, the plant hormones e.g. salicylic acid is involved in the regulation of basal resistance against different pathogens (Ton *et al.*, 2001).

Also, salicylic acid is an important component in the signal transduction pathway and involved in local and systemic resistance to pathogens (Delaney *et al.*, 1995). In addition concentration of endogenous salicylic acid increases at the site of hypersensitive response and acts as a transducer signal for activation of defence response (Delaney *et al.*, 1994). Thus, the exogenous application of salicylic acid is required for the expression of

resistance as well as for the enhancing the defensive capacity of tissues with acquired resistance (Szepesi *et al.*, 2005).

Obtained results also indicated that Apron fungicide was more effective than, the tested chemicals in controlling the disease. The superiority of Apron fungicide in reducing infection may attribute to its toxicity against the pathogen, while the positive effect of chemicals may be due to their action as plant defense activators.

Concerning the effect of the previously mentioned chemicals on grain yield, obtained results showed that, all the chemical inducers at the different concentrations and Apron have significantly increased grain yield (Ton/ Fed.) as compared with untreated infected control but not in-between particular compound (S) and other. The highest grain yield was obtained by SA at 8 mM followed by NA at 8 mM and BIH at 8 mM. Similar results were obtained by Zaky *et al.*, 2006 and El-Shehawy, 2009.

Recent results also showed that, there is a correlation between induced resistance and some biochemical changes in plant tissues like increase in the activity of oxidative enzymes and accumulation of phenols compounds in leaves of potted maize plants (21 days old). This is in agreement with many investigator whose stated that, foliar application of inducer resistance compound led to changes in the activates of many of oxidative enzymes *i.e.* PO, PPO, PAL and TAL and in the contents of phenol compounds (Gusui *et al.*, 1997; Ata *et al.*, 2008; Thabet, 2008 and Khalifa *et al.*, 2011). This biochemical changes became a marker to induce resistance (Reuveni *et al.*, 1992). This is due to the role of peroxidase activity in disease development that has been correlated with the expression of resistance in different host – pathogen system (Cadena-Gomez and Nicholson, 1987). Oxidative enzymes have several functions, which could have an effect on the resistance of a plant such as lignin production and phenol accumulation (Edreva, 1989). Another possible role for oxidative enzymes are the oxidative cross~1inking of pre-existing hydroxyproline-rich structural proteins in the cell wall, making the cell wall more resistant to degradation by microbial enzymes (Bradley *et al.*, 1992). In addition, peroxidase are implicated in an oxidative defense mechanism in elicitor treated (Apostol *et al.*, 1989), and generated hydrogen peroxide which, consider an antimicrobial agent (Peng and Kuc, 1992). Polyphenoloxidases (PPO) generally catalyze the oxidation of phenolic compounds to quinones (antimicrobial compounds) using molecular oxygen as an electron acceptor (Sommer *et al.*, 1994) which are toxic to the invading pathogens and pests (Weir *et al.*, 2004). Polyphenoloxidases are suggested to be indirectly involved in auxin biosynthesis because the o-quinones produced then can react with tryptophan to form indole-3-acetic acid (Mayer and Harel, 1979). Phenylalanine ammonia lyase (PAL) is the primary entry enzyme that leads to phenylpropanoid pathway resulting in the biosynthesis of a diverse array of plant metabolites, such a cinnamic, cumaric, ferulic and caffeic acids, flavonoids, tannins and lignin (Hahlborck and Sheel, 1989). These products consequently protect plants against various abiotic stresses and pathogenic attacks (Jones, 1984). Plants may accumulate phenolics through the phenylpropanoid pathway on activation of (PAL) as a means of passive defense (Barry and Manley, 1986) and the magnitude of the accumulation primarily depends on the supply of the primary precursor, L-Phenylalanine (Da Cunha, 1987).

Results also revealed that soaking maize grains in the tested chemical inducers at different concentrations caused greater accumulation of phenolic compounds in plant leaves than those of untreated infected control. Maximum amount of phenols were produced in leaves of maize potted plant grown from grains treated with SA at 8 mM. These results are in agreement with those of Thabet, 2008 and Ata, *et al.*, 2008. Similar results were obtained by Zaky *et al.* (2006). The role of phenolic compounds in disease resistance was postulated by many authors (Mittelstrass *et al.*, 2006). They indicated that, this role may be attributed to the toxic effect of phenols on the invaded pathogen. Moreover, it was found in the present study that the activity of PPO and PO enzymes were higher in treated leaves, these enzymes act by oxidation of phenolic compounds to quinines that have toxicity reached 100 times than correspondent phenolic compounds (Carrasco *et al.*, 2001). Phenols are essential for the biosynthesis of lignin, which consider an important structure component of plant cell walls (Hahlbrock and Sheel, 1989). Antibiotic phenols have the ability to bind to some proteins *in vitro*, forming soluble and insoluble complexes (Hagerman and Robbins, 1987). These phenolic-protein interactions are thought to be, in part, responsible for putative function of phenolics as plant defense compounds (Coley, 1983).

The obtained data clearly showed the ability of some chemical inducers to have similar efficacy near to the fungicides efficiency (Apron) in reducing SDM disease of maize this may lead to the conclusion that application of chemical inducers is applicable, safe and cost effective method for controlling SDM disease of maize. Also, the use of chemical inducers in agriculture could be a suitable alternative for integration in disease control systems and could act sometimes as main or adjuvant antimicrobial compounds and do not leave a toxic residue in the product moreover, to avoidance of environmental pollution due to the decreased in usage of chemical fungicides.

References

Apostol, I., P.P. Heinstein and P.S. Low, 1989. Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. Plant Physiol., 90: 109-116.

Ata, A.A., M.G. El-Samman, Maysa, A. Moursy and M.H. Mostafa, 2008. Inducing resistance against rust disease of sugar beet by certain chemical compounds. Egypt. J. Phytopathol., 36(1-2): 113-132.

Barry, T.N. and T.R. Manley, 1986. Interrelationships between concentrations of total condensed tannins, free condensed tannin and lignin in *Lotus sp.* and their possible consequences in ruminant nutrition. J. Sci. Food Agric., 37: 248-254.

Beligni, M.V. and A. Lamattina, 2002. Nitric oxide interferes with plant photooxidative stress by detoxifying reactive oxygen species. Plant Cell Environ., 25: 737-748.

Biles, C.L. and R.D. Martyn, 1993. Peroxidase, Polyphenoloxidase and shikimate dehydrognase isozymes in relation to tissue type, maturity and pathogen induction of watermelon seedling. Plant Physiol. Biochem., 31: 499-506.

Bradley, D.J., P. Kjellbom and C.J. Lamb, 1992. Elicitor and wound-induced oxidative cross-linking of a proline-rich plant cell; wall protein: a novel, rapid defense response. Cell, 70: 21-30.

Cadena-Gomez, G. and R.L. Nicholson, 1987. Papilla formation and associated peroxidase activity: A non-specific response to attempted fungal penetration of maize. Physiol. Mol. Plant Pathol., 3l: 51-67.

Carrasco, A.E., M.L. Serrano, J.M. Zapata, B. Sabater and M. Martín, 2001. Oxidation of phenolic compounds from *Aloe barbadensis* by peroxidase activity: Possible involvement in defence reactions. Plant Physiology and Biochemistry, 39: 521-527.

Coley, P.D., 1983. Herbivory and defense characteristics of tree species in a low land tropical forest. Ecol. Monogr., 53: 209-233.

Da Cunha, A., 1987. The estimation of phenylalanine ammonia lyase shows phenyloropanoid biosynthesis to be regulated by L-phenylalanine supply and availability. Phytochemistry, 26: 2723-2727.

Delaney, T.P., L. Friedrich and J.A. Ryals, 1995. *Arabiodopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Nat. Acad. Sci., USA, 92: 6602-6605.

Delaney, T.P., S. Uknes, B. Vernooij, L. Friedrich, K. Weymann, D. Negrotto, T. Gaffney, M. Gutt-Rella, H. Kessmann, E. Ward and J. Ryals, 1994. A central role of salicylic acid in plant disease resistance. Science, 266: 1247-1250.

Durner, J. and N. Klessig, 1999. Nitric oxide as a signal in plants. Curr. Opin. Plants Biol., 2: 369-374.

Edreva, A., 1989. Host-parasite relations: Biochemistry. Pages 105-140 in: Blue Mold of Tobacco. W. E. Mckeen, ed. The American Phytopathological Society, St. Paul, MN.

El-Moghazy, S.M.Y., 2003. Studies on the integrated control of downy mildew disease of maize and sorghum. Ph. D. Thesis, Fac. Agric. Kafr El-Sheikh, Tanta Univ., pp: 128.

El-Shehawy, E.A. Amal, 2009. Studies on control of downy mildew disease of grain sorghum. Ph. D. Thesis, Fac. Agric. Kafr El-Sheikh, Tanta Univ., pp: 188.

Frederiksen, R.A., 1977. Global status of maize downy mildew. Ann. Rev. Phytopathol., 15: 249-275.

Gusui, W.U., B.J. Shortt, E.B. Lawrence, J. Leon, K.C. Fitzsimmons, E.B. Levine, I. Raskin, D.M. Shah and G.S. Wu, 1997. Activation of host defense mechanisms by elevated production of H_2O_2 in transgenic plants. Plant Physiol., 115: 427-435.

Hagerman, A.E. and C.T. Robbins, 1987. Implications of soluble tannin-protein complexes for tannin analysis and plant defense mechanisms. J. Chem. Ecol., 13: 1243-1259.

Hahlbrock, K. and D. Scheel, 1989. Physiology and molecular biology of phenylpropanoid metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol., 40: 347-369.

Hammerschmidt, R., E.M. Nuchles and J. Kuc, 1982. Association of enhanced peroxidase activity with induce systemic resistance of cucumber to *Colletotrichum lagenarium.* Physiol. Plant Pathol., 20: 73-82.

Hussein-Zeinab, N.E., 2011. New approaches for controlling peanut root and pod rots diseases caused by *Rhizoctonia solani*. Ph.D. Thesis, Inst. of African Res. and Studies, Cairo Univ.

Jones, D.H., 1984. Phenylalanine ammonia lyase regulation of its induction and its role in plant development. Phytochemistry, 23: 1349-1359.

Kessman, H., T. Stamb, C. Hofmann, T. Maetzke and J. Herzog, 1994. Induction of systemic acquired disease resistance in plants by chemicals. Annu. Rev. Plant Pathol., 32: 439-459.

Khalifa, M.M.A., M.M. Ibrahim and A.A. Abd-El-Baky, 2011. Induced systemic resistance against Fusarium wilt diseases of sesame by some chemical inducers. Egypt. J. Phytopathol., 39(1): 24-39.

Mahmoud, E.Y., Samia Y.M. Shokry and Zeinab N. Hussin, 2006. Induction of resistance in peanut plants against root rot diseases under greenhouse conditions by some chemical inducers. J. Agric. Sci. Mansoura Univ., 31(6): 3511-3524.

Malik, C.P. and M.B. Singh, 1980. Plant enzymology and histoenzymology. Kalyani Publishers, Iundhiana and Printed in Navin. Shanndara, Delhi, pp: 54-56.

Mandal, S., N. Mallicka and A. Mitraa, 2009. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Plant Physiology and Biochemistry, 47(7): 642-649.

Mayer, A.M. and E. Harel, 1979. Polyphenoloxidases in plants. Phytochemistry, 18: 193-215.

Meena, B., T. Marimuthu and R. Velazhahan, 2001. Salicylic acid induces systemic resistance in groundnut against late leaf spot caused by *Cercosporidium personatum*. J. Mycology Plant Path., 31: 139-145. (C.F. CAB Abstracts 2003).

Melchers, L.E., 1931. Downy mildew of sorghum and maize in Egypt. Phytopathology, 21: 239-240.

Mittelstrass, K., D. Treutter; M. Plessl, W. Heller, E.F. Elstner and I. Heiser, 2006. Modification of primary and secondary metabolism of potato plants by nitrogen application differentially affects resistance to *Phytophthora infestans* and *Alternaria solani*. Plant Biology, 8: 653-661.

Morris, S.W., B. Vernooij, S. Titataran, M. Starrett, S. Thomas, C.C. Wiltse, R.A. Frederiksen, A. Bhandhufalck, S. Hulbert and S. Uknes, 1998. Induced resistance responses in maize. Molecular Plant-Microbe Interaction., 11(7): 643-658.

Oerke, E.C., H.W. Dehne, F. Schonbeck and A. Weber, 1994. Crop production and crop protection. Elsevier Science B. V., Amsterdam.

Peng, M. and J. Kuc, 1992. Peroxidase-generated hydrogen peroxide as a source of antifungal activity *in vitro* and on tobacco leaf disks. Phytopatholoy, 82: 696-699.

Rashad, H.M. and F.M. Abou-Elalla, 2009. Influence of oxygenous nitric oxide on improving drought tolerance of seven hybrids and seven inbreads of maize. Bull. Fac. Agric. Cairo Univ., 60: 398-407.

Reuveni, R., M. Shimoni, Z. Karchi and J. Kuc, 1992. Peroxidase activity as a biochemical marker for resistance of muskmelon (*Cucumis melo*) to *Pseudoperonospora cubensis.* Phytopathology, 82: 749-753.

Sadoma, M.T., 1995. Studies on downy mildew disease of maize in Egypt. M. Sc. Thesis, Fac. Agric. Minufiya Univ., pp: 154.

SAS Institute, Inc, 1996. SAS/ STAT Users Guide, Version 6, 12th Ed. SAS Institute, Inc. Cary, North Carolina., 2: 846.

Shi, S.; G. Wang; Y. Wang and L. Zhang, 2005. Protective effect of nitric oxide against oxidative stress under ultraviolet-B- radiation. J. Nitric Oxide, 13: 1-9.

Snell, F.D. and C.I. Snell, 1953. Colorimetric Methods. Vol. III. D. Van Nostrand Co. Inc., Torento, N. Y., London, pp: 606.

Solecka, D. and A. Kacperska, 2003. Phenylpropanoid deficiency affects the course of plant acclimation to cold. Physiol. Plantarum, 119: 253-262.

Sommer, A., E. Neeman, J.C. Steffens, A.M. Mayer and E. Harel, 1994. Import, targeting and processing of a plant polyphenoloxidase. Plant Physiol., (Lancaster, PA, USA), 105(4): 1301-1311.

Szepesi, A., J. Csiszar, S. Bajkan, K. Gemes, L. Erdei, A. Deer, M. Simon and I. Tari, 2005. Role of salicylic acid pre-treatment on the accumulation of tomato plants to salt and osmotic stress. Acta Biologica Szegediensis, 49(1-2): 123-125.

Thabet, Marian, S., 2008. Induction of resistance in wheat plants against leaf rust disease caused by *Puccinia triticina*. M. Sc. Thesis, Plant Path. Dept., Fac. of Agric., Ain Shams Univ. Egypt, pp: 122.

Ton, J., L.C. Van Pelt and C.M. Pieterse, 2001. Differential effectiveness of salicylate-dependent and jasmonate/ ethylene-dependent induced resistance in *Arabidopsis*. Mol. pl. Microb. Interact., 15: 27-34.

Walters, D., A. Newton and G. Lyon, 2007. Induced Resistance for Plant Defence. Blackwell Publishing Editorial Offices, pp: 269.

Weir, T.L., S.W. Park and J.M. Vivanco, 2004. Biochemical and physiological mechanisms mediated by allelochemicals. Curr. Opin. Plant Biol., 7: 472-479.

Zaky, Wafaa H., M.G.A. Nada and A.A. Hilal, 2006. Evaluation of the efficiency of some environmentally safe means for controlling rust disease of anise (*Pimpinella anisum* L.), as important medicinal plant in Egypt. Egypt J. Phytopathol., 34(2): 103-119.