Minufiya J. Agric. Res. Vol.39 No. 4(1): 1237-1253 (2014) "http://www.mujar.net"

BIOCHEMICAL CHANGES ASSOCIATED WITH INDUCED RESISTANCE OF PEANUT ROOT AND POD ROTS DISEASES

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(Received : Jun. 15 , 2014)

ABSTRACT: *Under greenhouse and field conditions all tested inducers significantly reduced peanut cv. Giza 6 damping-off, root and pod rot incidence. In this respect salicylic acid at 4 mM followed by hydrogen peroxide at 0.25% gave the highest effect on all parameters of disease incidence and consequently increasing percentage of healthy survival plants. Increasing concentration of salicylic acid than 4 mM and hydrogen peroxide than 0.25 % led to a decrease in their effect on reducing the disease incidence. While, the effect of bion on reducing diseases incidence was increased with increasing their concentrations. Results also stated that, there is a correlation between induced resistance and some biochemical changes in root tissues as increasing the activity of oxidative enzymes (catalase, peroxidase and polyphenoloxidase) and accumulation of phenol compounds. Salicylic acid at 4 mM followed by bion at 8 mM. recorded the highest content of phenol compounds while, the highest increase of activity of oxidative enzymes (catalase, peroxidase and polyphenoloxidase) in peanut roots was recorded with salicylic acid at 4 mM and hydrogen peroxide at 0.25 %. There are new protein bands and others increased in their intensity because of chemical inducers treatments. Bion at 4 and 8 mM, and hydrogen peroxide at 0.25 % gave a new protein bands with MW 29, 48 and 54 KD. Salicylic acid treatment in all concentrations showed participation in a new protein band MW 22 KD. While, salicylic acid at 2 and 4 mM and hydrogen peroxide at 0.5% and 1% participated in a new protein band (MW 55 KD). In the same time all concentrations of hydrogen peroxide gave a new protein band with MW 34 and 36 KD.*

Key words: *Peanut- Rhizoctonia solani Damping-off- Root rot- Pod rot- induced resistance – biochemical changes – catalase – peroxidase – polyphenoloxidase - phenols*

INTRODUCTION

Peanut, (*Arachis hypogaea* L.) is one of the most export and locally consumed crops in Egypt. Damping – off, root and pod rot diseases are among the most destructive diseases attacking peanut in Egypt (Yehia *et al.,* 1979 and Mahmoud *et al.,* 2006a).

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 (Kloepper *et al.,* 1992). Induced resistance is characterized by many advantages such as; non–specific, systemic, has durable effect, safe for human and environment, and has a positive effect on plant growth and yield (Kuc, 1982). Various chemicals have been discovered that seem to act at various points of the defense-activating network (Oostendorp *et al.,* 2001). Some compounds *e.g.*, salicylic acid (SA); hydrogen peroxide (H_2O_2) and bezo (1,2,3) thiodiazole-7-carbothioic-methyle ester (Bion), have been shown to induce resistance in plants (Colson, 1998; Mosa, 2002, Mahmoud *et al.,* 2006b and Mahmoud *et al.,* 2009). In peanut Meena *et al.,* (2001) showed that, foliar application of SA at a concentration of 1 mM significantly reduced late leaf spot disease intensity and increased pod yield under greenhouse conditions. While, Mahmoud *et al.,* (2006b) and Hussin (2011) found that, various chemical inducers treatment of peanut plants as a seed soaking were effective in reducing peanut root rot diseases caused by *R. solani, F. solani, M. phaseolina,* and *S. rolfsii,*. Salicylic acid at 4 mM gave the highest effect on incidence of peanut damping – off, wilt, and root rot diseases.

Inducer of systemic resistance sensitizes the plant to respond rapid after infection. These responses include phytoalexin accumulation, lignifications and activation of peroxidase, polyphenoloxidase, chitinase and β-1.3 gluconase (Kuc, 1982, Metraux and Boller, 1986). In addition, many investigators reported that inducers of systemic resistance accumulated or enhanced new proteins in systemically protected leaves (Tuzun and Kloepper 1994). In this respect many investigators indicated that, treated peanut plants with induction compounds led to changes in the activity of phenylalnine ammonialyase, chitinase, beta-1.3- gluconase, peroxidase, polyphenoloxidase and in the contents of phenolic compounds (Meena *et al.,* 2001, Mahmoud *et al.,* 2006 b and Hussin 2011).

MATERIALS AND METHODS

1. Isolation and identification of causal organisms:

The fungal isolates which used throughout this study were previously isolated by the authors from diseased peanut roots and pods and their pathogenic capabilities were confirmed also (Mahmoud, *et, al.,* 2006a).

2. Preparation of fungal inoculum:

Inocula of isolates of *Fusarium solani*, *Fusarium moniliforme, Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii* were prepared using sorghum - coarse sand - water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for 2 hours at 1.5 air pressure. The sterilized medium was inoculated using agar discs, obtained from the periphery of 5-days-old colony of each of the isolated fungi. The inoculated media were incubated at 28°C for 15 days and were then used for soil infestation.

3. Soil Infestation:

Inoculum of each isolate of *F. solani, F. moniliforme, M. phaseolina, R. solani* and *S. rolfsii* was mixed thoroughly with the soil surface of each pot, at the rate of 2% w/w, and was covered with a thin layer of sterilized soil. The infested pots were irrigated and keep for 7 days before sowing.

4. Disease assessment

Disease assessment was recorded as percentage of damping- off (pre- and post emergence) after 15 and 45 days from sowing respectively; using the following formula =

% Pre-emergence =

$$\frac{\text{Number of non germinated seeds}}{\text{Number of sown seeds}} \text{X } 100$$

% Post-emergence =

$$\frac{\text{Number of dead seedlings}}{\text{Number of sown seeds}} \text{X } 100$$

% damping - off = pre- emergence + post emergence

Percentages of infected plants by root-rot and survived healthy plants were estimated after uprooting (120 days from sowing) as follows:

% Root rot =

$$\frac{\text{Number of plants with root - rot}}{\text{Number of sown seeds}} \text{X } 100$$

% Healthy plants =

$$\frac{\text{Number of survived healthy plants}}{\text{Number of sown seeds}} \text{X } 100$$

Plants of individual pot or/plot were dug and inverted based on an optimum maturity index. Pods were threshed, air-dried for three days, weighted and then examined for pod rot incidence. Percentage of pod rot was recorded. Four categories for apparent symptoms of pod rots beside the healthy pods were adopted according to Satour *et al.,* (1978): a) Rhizoctonia rot, pods with dry brown lesion, b) Fusarium rot, pods with pink discoloration and c) complex rot pod with general breakdown resulting from many fungi and all of types were calculated as follows:

Pod rot categories (%) =

$$\frac{\text{Number of rotted pod category}}{\text{Number of total pods}} \text{X } 100$$

Yield loss (%) =

$$\frac{\text{weight of rotted pods}}{\text{weight of total pods}} X100$$

5. Effect of chemical inducers

The effectiveness of three chemical resistant inducers, i.e., hydrogen peroxide (H_2O_2), bion, and salicylic acid, on incidence of root rot and pod rot of peanut were tested under greenhouse and field conditions. Each chemical inducer was used at three different concentrations *i.e.* 2, 4 and 8 mM. for bion and salicylic acid and 0.25, 0.5 and 1% for hydrogen peroxide. Peanut Giza 6 cv. Peanut seeds were individually soaked in the chemicals inducers for 30 min. and the plants were sprayed after 40 days from sowing by the same rates of each compound.

5.1. Greenhouse experiment:

Treated and untreated (control) peanut seeds were sown in pots (50 cm. diam.) contained soil infested with mixture of pathogenic fungi at the rate of 2 % (w /w) as mentioned before. Ten seeds/pot and 5 pots for each treatment were used as replicates. The disease assessment was carried out as mentioned before.

5.2. Field experiments:

Field experiments were carried out during 2012 and 2013 seasons in naturally infested field soil at Ismailia Experimental Station, Agriculture Research Center (ARC). The soil type was sandy loam (77% sand, 11% silt, 12% clay; pH 7.98 and EC 7.2). Treated peanut seeds, with various chemical inducers, were sown in the field on the first week of May during two growing seasons; the experimental unit area was 10.5 m^2 (1/400 fed.). Each unit includes four rows; each row was 3.5m in length and 50 cm width. The experiment was arranged in a completely randomized block design with four replicates.

6. Specific biochemical changes associated with chemical inducer treatments:

Peanut root samples were taken and extracted according to Goldschmidt *et al.* (1968), then activities of the oxidative enzymes *i.e.,* peroxidase (PO); polyphenoloxidase (PPO) and catalase (CAT) were determined according to Allam and Hollis (1972); Matta and Dimond (1963) and Maxwell and Bateman (1967), respectively and assayed using a spectrophotometer at 425, 495 and 240 nm. respectively. The reaction substrate of each oxidative enzyme was pyrogallol, catechol and H_2O_2 for determining the activity of peroxidase; polyphenoloxidase and catalase, respectively. Another samples were 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 phenols contents were also calculated as milligrams equivalent of catechol /g fresh weight of peanut roots.

7. Sodium Dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Changes in the soluble proteins due to treatment with heavy metals were determined in peanut roots using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique described by Broglie *et al.,* (1986). Vertical slab gels (0.75 mm-thick) were cast and electrophoresed using the Bio Rad Mini-Protein II system. Gels were stained with commassie brilliant blue R-250 solution, photographed and scored using gel documentation system manufactured by Alpha Ease FC (Alphaimager 2200), U.S.A. The similarity matrices were done using Gel works 1D advanced software UVP-England Program.

8. Statistical analysis

The data were statistically analyzed by analysis of variance (ANOVA) using the statistical Analysis System (SAS Institute, inc, 1996). Means were separated by LSD Test at $P \leq 0.05$ levels.

RESULTS

Bion, hydrogen peroxide, and salicylic acid was used to evaluate their effects as resistance inducing against peanut damping off, root and pod rot diseases. Such treatments were carried out under greenhouse and artificial soil infestation conditions and field natural soil infestation conditions.

1. Effect of inducer resistance compounds on peanut damping-off and root rot incidence:

1.1. Under greenhouse conditions

Data in Table (1) indicate that, all tested chemical inducers with the tested concentrations showed significant reduction of damping-off and root rots compared to the non-treated control. Generally, salicylic acid at 4 mM followed by hydrogen peroxide at 0.25% gave the highest percentage of survival plants. Hydrogen peroxide at 0.25% showed the highest effect on reducing damping-off incidence especially on pre-emergence damping-off followed by salicylic acid at 4 mM. While, salicylic acid at 4 mM recorded the highest effect on minimizing peanut root rot incidence.

Data also showed that, there is a relation between chemical inducers concentration and their effect on the incidence of the different studied disease parameters. Data clearly indicated that, increasing the concentration of bion led to increase their effect in reducing the incidence of the disease while, increasing concentration of salicylic acid than 4 mM led to a decrease in its effect in reducing the disease incidence. It was obvious that increasing of hydrogen peroxide concentrations gave adverse effect on reducing of damping-off, and root rot diseases.

Table (1): Effect of chemical inducers on peanut damping-off and root rot incidence under artificially inoculation conditions.

Treatment	Conc.	Damping-off		Total	Root rot	Survival
		Pre	Post			
Bion	2 mM.	16	14	30	22	48
	4 mM.	14	10	24	20	56
	8 mM.	12	10	22	16	62
SA	2 mM.	14	12	26	20	54
	4 mM.	10	8	18	12	70
	8 mM.	12	12	24	16	60
H_2O_2	0.25%	6	8	14	20	66
	0.5%	10	10	20	20	60
	1.0%	12	12	24	22	54
Control		18	16	34	22	44

L.S.D. 5%: between

a) Treatments =	3.27	2.74	3.66	3.13	4.91
b) Concentrations =	1.66	1.24	1.98	1.87	2.54
(a) × (b) =	4.89	3.79	5.56	4.94	7.34

1.2. Under field conditions:

Results present in Table (2) illustrated that, chemical inducer resistance treatments gave a significant effect in increasing of survival plants during the two successive seasons 2012 and 2013. Increase of their concentration gave a significant effect on reducing damping-off and peanut root rot incidence. In this respect, the highest reduction of chemical inducer resistance treatments to damping-off and peanut root rots during the two seasons was detected by salicylic acid treatment at 4 mM followed by hydrogen peroxide at 0.25%. While, bion at 2 mM and hydrogen peroxide at 1% recorded the lowest effect on damping-off and peanut root rot incidence during the two growing seasons 2012 and 2013.

With regard to the effect of chemical inducers concentration on damping-off and peanut root rot incidence ,data in field experiment showed the same trend of greenhouse experiment.

2. Effect of inducer resistance treatments on peanut brown pod rot incidence:

2.1. Under greenhouse conditions:

Data in Table (3) indicate that, all chemical inducers with the tested concentrations showed significant effects against the incidence of pod rot categories of peanut compared to control. Salicylic acid at 4 mM gave the maximum reduction of all pod rots categories of peanut incidence and increased apparent healthy pods. While, hydrogen peroxide at 1% was the least effective treatment. Increasing the concentrations of bion led to more in reduction of pod rots incidence while, increasing hydrogen peroxide concentrations showed adverse effect, as well as increasing concentration of salicylic acid than 4 mM which led to a decrease in its effect in reducing the disease incidence.

2.2. Under field conditions:

All concentrations of the chemical inducers gave significant reduction for all categories of pod rot incidence and increased the total apparent healthy pods compared to control treatment during the two experimental seasons 2012 and 2013 (Table 4). The highest effect was detected with salicylic acid treatment (4 mM) followed by hydrogen peroxide (0.25%). While, hydrogen peroxide treatment (1.0%) recorded the lowest effect on all categories of pod rot incidence during the two growing seasons.

On the other hand, increasing the concentrations of bion, and salicylic acid gave positive effect in reducing all categories of pod rot incidence except the highest concentration of salicylic acid and increasing of hydrogen peroxide concentrations which gave reverser effect during the two seasons 2012 and 2013.

3. Effect of inducer resistance on peanut yield and yield losses under field conditions:

Regard to peanut pod yield production and percentage of yield losses data in Table (5) illustrate that, peanut pod yield production significantly varied among the tested inducers during two successive seasons 2012 and 2013. The highest total peanut yield in the two seasons was produced with SA (4 mM). While the lowest yield production was recorded when bion (2 mM) was applied. Salicylic acid (4 mM) gave the lowest percentage of yield loss while, bion (2 mM) and SA (8 mM) and control treatment recorded the highest percentage of yield losses during the two seasons 2012 and 2013.

4. Biochemical changes associated with chemical inducers:

The effect of certain chemical inducers was studied on various biochemical changes *i.e.* phenol content electrophoresis proteins patterns and activity of Catalase (CAT) peroxidase (PO) and polyphenolodixidase (PPO) in peanut plants, grown in artificially infested soil under greenhouse conditions.

TABLE 2

Table (3): Effect of chemical inducer on peanut pod-rot diseases under artificially inoculation:-

Treatment	Conc.	Disease incidence [z)]			Apparent healthy
		Dry brown lesion	Pink discoloration	General breakdown	
Bion	2 mM.	12.14	2.11	14.22	71.53
	4 mM.	10.38	1.89	12.58	75.15
	8 mM.	10.12	0.88	10.98	78.02
SA	2 mM.	12.45	0.66	14.65	72.24
	4 mM.	8.59	0.00	8.79	82.62
	8 mM.	10.52	1.28	10.72	77.48
H_2O_2	0.25%	9.56	0.00	10.76	79.09
	0.5%	11.07	0.59	13.27	74.44
	1.0%	13.09	1.22	15.29	70.40
Control		16.55	2.98	18.23	62.24

L.S.D. 5%: between

a) Treatments =	1.21	0.65	2.17	2.44
b) Concentrations =	0.83	0.35	1.77	1.95
(a) × (b) =	2.01	1.01	3.73	4.29

TABLE 4

Table (5): Effect of chemical inducers on peanut yield and losses yield under field conditions during seasons 2012 and 2013.

Treatment	Conc.	2012		2013	
		Yield (Ton/fed)	Loss of Yield losses (%)	Yield (Ton/fed)	Loss of Yield losses (%)
Bion	2 mM.	1.089	20.44	1.046	23.15
	4 mM.	1.141	17.20	1.109	19.18
	8 mM.	1.141	17.20	1.122	18.38
SA	2 mM.	1.167	15.54	1.129	17.92
	4 mM.	1.205	13.10	1.175	15.01
	8 mM.	1.100	19.78	1.055	22.55
H_2O_2	0.25%	1.164	15.74	1.079	21.04
	0.5%	1.140	17.26	1.109	19.18
	1.0%	1.120	18.52	1.094	20.11
Control		0.982	27.18	0.942	29.76

L.S.D. 5%: between

		2012	2013
a) Treatments	=	0.085	0.081
b) Concentrations	=	0.038	0.032
(a) × (b)	=	0.121	0.110

4.1. Effect of chemical inducers on phenol contents in peanut roots (15-day-old):

Results present in Table (6) indicat that, phenol contents including free, conjugated and total phenols were obviously higher in roots of plants grown from seeds treated with either chemical inducer than the untreated control. The highest phenol contents were induced by salicylic acid (4 mM) and bion (8 mM). Data clearly indicat that, phenol contents were affected by the three tested concentrations of each chemical inducer. Generally, increasing the concentration of H_2O_2 and SA (than 4 mM) led to decrease of phenols content in roots while, increasing of bion concentration caused an increase of phenol contents in peanut roots.

Table (6): Phenol compounds in peanut roots grown under artificial soil infestation conditions.

Inducers	Conc.	Phenol compounds		
		Free	Conjugate	Total
Bion	2 mM.	14.35	5.37	19.72
	4 mM.	15.26	7.36	22.61
	8 mM.	15.57	8.77	24.34
SA	2 mM.	14.08	6.35	20.43
	4 mM.	16.49	9.03	25.52
	8 mM.	12.47	5.49	17.96
H_2O_2	0.25%	15.74	6.96	22.70
	0.5%	13.89	5.56	19.45
	1.0%	9.51	3.39	12.90
Control		9.09	3.43	12.53

4.2. Effect of chemical inducers on catalase (CAT), peroxidase (PO) and polyphenoloxidase (PPO) activities in roots (15-day-old):

Data in Table (7) clear that all tested chemical inducers increased the activity of oxidative enzymes *i.e.* catalase, peroxidase and polyphenoloxidase in peanut roots compared to those grown from untreated seeds (control). In this respect, activity of CAT showed the highest increase when salicylic acid was used at 4 mM followed by hydrogen peroxide at 0.25% and bion at 8 mM. The same trend was recorded for the activities of PPO and PO. Data showed also that, higher concentrations of SA and H_2O_2 was accompanied by a decrease of the activities of the tested enzymes, while the increase of bion concentration led to increase in the activity of the same enzymes.

4.3. Effect of chemical inducers on soluble proteins in peanut leaves:

Data presente Table (8) and Fig. (1) indicate that there are new protein bands and others were increased in their intensity as a result of chemical inducer treatments. In this respect, bion at 4 and 8 mM, hydrogen peroxide at 0.25 % gave new protein bands with MW 29, 48 and 54 KD. Salicylic acid treatment in all concentrations showed participation in a new protein band MW 22 KD. While, salicylic acid at 2 and 4 mM participation in a new protein band with hydrogen peroxide at 0.5% and 1% (MW 55 KD). In the same time hydrogen peroxide at their all concentration, gave new protein bands with MW 34 and 36 KD

Table (7): Catalase, Peroxidase and Polyphenoloxidase activities in peanut roots grown under artificial soil infestation conditions.

Inducer	Conc.	Enzymes activity		
		Catalase (CAT)	Peroxidase (PO)	Polyphenoloxidase (PPO)
Bion	2 mM.	0.576	0.819	0.613
	4 mM.	0.602	1.283	0.672
	8 mM.	0.629	1.364	0.704
SA	2 mM.	0.700	1.284	0.811
	4 mM.	0.725	1.405	1.070
	8 mM.	0.609	0.661	0.520
H_2O_2	0.25%	0.633	1.388	0.982
	0.5%	0.571	0.683	0.637
	1.0%	0.485	0.628	0.561
Control		0.445	0.626	0.334

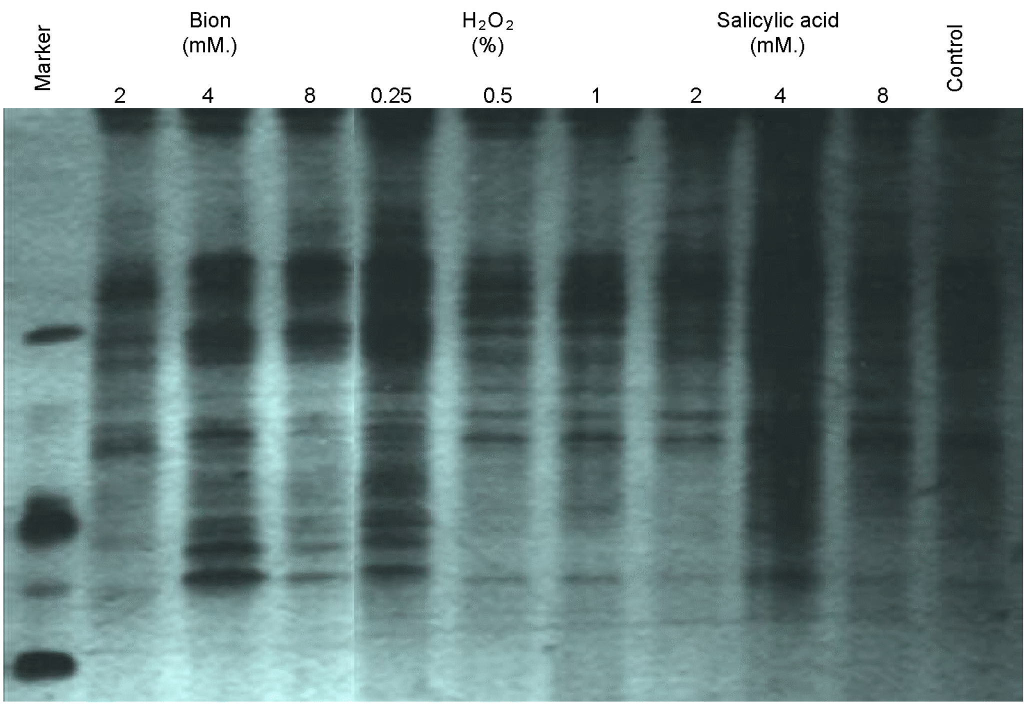

Fig (1): Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of proteins extracted from leaves growing under artificial inoculation conditions.

Table (8): Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of proteins extracted from peanut leaves growing under artificial inoculation conditions.

Molecular weight (KD)	Control	Bion (mM.)			H_2O_2 (%)			Salicylic (mM.)		
		2	4	8	0.25	0.5	1.0	2	4	8
93	==									==
92		==					==			
91						==				
73										
64										
56		==								==
55						==	==	==	==	
54			==	==	==					
50	==		==	==	==					
49									==	
48			==	==	==					
46		==					==			
44				==						
43			==		==					
40	==									
39		==				==	==	==		==
38			==	==	==				==	
37							==	==		==
36					==	==				
34						==	==			
32	==					==	==			==
31		==							==	
30		==				==			==	==
29			==	==	==					
27	==	==				==	==	==		==
26	==		==		==				==	
25										
23	==			==	==					
22								==	==	==
17	==	==	==	==		==	==			==
16	==	==	==	==	==	==	==	==	==	==

y) Peanut leaves were collected after 15 days from sowing.
z) Chemical inducers were treated as a seed soaking for 30 min. before sowing.
== Bands participated with control
== New bands
== Bands may be related with induced of resistance

DISCUSSION

The results in this study indicated that resistance of peanut damping-off, root and pod rot diseases could be induce by chemical treatments. In greenhouse all tested chemical inducers significantly reduced damping-off, root and pod rots disease incidence. This is in agreement with many investigators (Abd El-Kareem *et al.,* 1993, Aly *et al.,* 1993, Colson, 1998, Khalifa, 2003 and Mahmoud, 2004) who studied the effect of these chemical inducers on induction of plants against soilborne fungi. Salicylic acid at 4 mM followed by hydrogen peroxide at 0.25% and bion at 8 mM gave the highest effect on all parameters of diseases resuction incidence whether in roots or peanut pods and consequently increased the percentage of healthy survival plants. While Bion at 2 mM gave the lowest effect in these respects.

Increasing the concentration of Bion compound led to enhancing induced peanut resistance to damping-off, root and pod rot diseases. Although Bion compound has no direct antimicrobial activity against many fungal and bacteria pathogens (Gorlach *et al.,* 1996 and Schweizer *et al.,* 1999) but, it enhanced activities of the defense related enzymes chitinase, *B*-(1,3)-gluconase and peroxidase (Siegrist *et al.,* 1997; Abou-Taleb, 2001 and Mosa 2002). While, in many plants, bion treatment is associated with increase in activities of many classes of pathogenesis-related protein (Gorlach *et al.,* 1996; Schweizer *et al.,* 1999 and Abou-Taleb, 2001).

Peanut seed treatment with hydrogen peroxide (H_2O_2) at the rate of 0.25 % caused reduction to damping-off, root and peanut pod rot diseases. This may be due to the role of hydrogen peroxide in activating an array of host defense mechanisms including the appearance of a large amount of the enzymes as peroxidase and chitinase.

This was accompanied by a significant increase in the lignin and suberin content (Gusui *et al.,* 1997 and Liu *et al.,* 1997). Hydrogen peroxide positively influences the local and systemic accumulation of SA that correlated with the enhancement of peroxidase activity (Martinez *et al.,* 2000). Moreover, hydrogen peroxide inhibits pathogens directly, and/or it may generate other reactive free radicals that are antimicrobial (Peng and Kuc, 1992).

This study provides further evidence that; hydrogen peroxide at 0.25 % concentration enhancement the activity of oxidative enzymes besides increasing the content of phenols compounds. On the other hand, increasing the concentration of hydrogen peroxide led to decrease its effect on damping-off, root and peanut pod rot diseases, this may be due to the role of hydrogen peroxide in rapid generation of active oxygen species (AOS) called the oxidative burst (Lamb and Dixon, 1997). This may be give opposite effect on the physiological processes in the plants when increased its concentration especially the role of hydrogen peroxide in accumulation of salicylic acid (Martinez *et al.,* 2000).

Salicylic acid (SA) as resistant inducer of peanut for damping-off, root and pod rot diseases was increased by increasing its concentration from 2 to 4 mM, which may be increase the activity of oxidative enzymes and content of phenol compounds. This is in agreement with Klessig *et al.,* (1999); Martinez *et al.,* (2000), Khalifa (2003) and Mahmoud (2004), who stated that, there is significant increase in the total peroxidase activity after treatment with salicylic acid. This may be due to the role of salicylic acid in generation of the oxidative burst in incompatible interactions by inducing a rapid transient generation of O_2^{-} which is responsible on regulation of peroxidase activity (Kauss and Jeblick, 1995; Mur *et al.,* 1996 and Rao *et al.,* 1997). On the other hand, the effect of salicylic acid was decreased with increasing its concentration from 4 to 8 Mm. These are in parallel with decreasing in the activities of oxidative enzymes and content of phenols. This is could be explained that salicylic acid at high concentration have damage effects on the plant physiological processes, which includes of inhibition phosphorus uptake and potassium absorption (Glass, 1973 & 1974 and Harper and Balke, 1981), and cause the collapse of the transmembrane electrochemical potential of mitochondria, which have effect on ATP-production (Glass, 1974 and Macri *et al.,* 1986), and reduce of transpiration by effect on stomatal behavior (Larque-Saavedra, 1978 and 1979).

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التغيرات البيوكيميائية المصاحبة للمقاومة المستحثة لأمراض أعفان جذور وقرون الفول السودانى

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الملخص العربى

تحت ظروف الصوبة والحقل اختزلت جميع المستحثات المختبرة أمراض موت بادرات وأعفان جذور وقرون الفول السودانى صنف جيزة ٦ بصورة معنوية . وفى هذا الصدد أعطى حمض الساليسليك (٤ ملليمول) متبوعاً بفوق أكسيد الهيدروجين (٠.٢٥ %) أعلى تأثير على جميع مؤشرات حدوث المرض ، وبالتالى أدى الى زيادة

عدد النباتات السليمة المتبقية . ولم يؤثر زيادة تركيز حمض الساليسليك عن (٤ ملليمول) أو فوق أكسيد الهيدروجين عن (٠.٢٥ %) الى تأثير إيجابى فى نقص الإصابة بالمرض . فى حين أدت زيادة تركيز البيون الى نقص معنوى فى حدوث المرض . وأثبتت النتائج كذلك الى وجود إرتباط قوى بين المقاومة المستحثة والتغيرات البيوكيميائية فى الجذور مثل زيادة نشاط إنزيمات الأكسدة (كتاليز ، بيروأكسيديز ، بولى فينول أكسيديز) وكذلك تراكم المركبات الفينولية . وأدت المعاملة بحمض الساليسليك بتركيز (٤ ملليمول) متبوعا بالبيون بتركيز ٨ ملليمول الى حدوث أعلى محتوى من المركبات الفينولية فى حين كانت أعلى زيادة فى نشاط إنزيمات الأكسدة (كتاليز ، بيروأكسيديز ، بولى فينول أكسيديز) فى جذور نباتات الفول السودانى عند تركيز (٤ ملليمول) من حمض الساليسليك أو (٠.٢٥ %) من فوق أكسيد الهيدروجين . وقد أدت المعاملات الى ظهور حزم بروتين جديدة وزيادة تركيز الأخرى . كاستجابة للمواد المستحثة ، فعلى سبيل المثال أظهر البيون عند تركيزى ٤ ، ٨ ملليمول وفوق اكسيد الهيدروجين ٠.٢٥% الى ظهور حزم بروتين جديدة (MW29 , 48 and 45 KD) وأدت المعاملة بحمض الساليسليك باى تركيز الى ظهور حزمة بروتين جديدة (MW 22 kd) فى حين أدى استخدام حمض الساليسليك بتركيز ٢ ، ٤ ملليمول الى تكوين حزمة بروتين جديدة ، وكذلك فوق اكسيد الهيدروجين ٠.٥ ، ١ % وهى (MW55kd) . وفى نفس الوقت أدى استخدام فوق أكسيد الهيدروجين بجميع تركيزاته الى ظهور حزمة بروتين جديدة (MW 34 and 36 KD) .

Table (2): Effect of chemical inducers on peanut damping-off and root rot incidence under field conditions during 2012 and 2013 growing seasons.

Treatment	Conc.	Season 2012			Season 2013		
		Damping-off	Root rots	Survival	Damping-off	Root rots	Survival
Bion	2 mM.	16.27	12.99	70.74	18.26	14.22	67.52
	4 mM.	14.43	12.57	73.00	15.88	13.14	70.99
	8 mM.	14.43	10.10	75.47	15.65	10.80	73.55
SA	2 mM.	15.88	11.54	72.58	16.52	13.53	69.95
	4 mM.	9.46	7.22	83.32	11.45	8.46	80.09
	8 mM.	12.99	10.10	76.91	13.14	11.84	75.02
H_2O_2	0.25%	8.66	11.54	79.80	10.15	12.53	77.32
	0.5%	10.50	12.99	76.51	12.49	14.18	73.33
	1.0%	12.99	14.43	72.58	14.18	15.88	69.95
Control		18.80	15.51	65.69	20.95	16.92	62.14

L.S.D. 5%: between:						
a) Treatments =	0.95	0.88	2.33	1.01	0.89	2.88
b) Concentrations =	0.67	0.62	1.65	0.71	0.63	2.04
(a) × (b) =	1.54	1.43	3.79	1.65	1.45	4.76

Table (4): Effect of chemical inducers on peanut pod rot incidence under field conditions during 2012 and 2013.

Treatment	Conc.	Season 2012				Season 2013			
		Dry brown lesion	Pink discoloration	General breakdown	Apparent healthy	Dry brown lesion	Pink discoloration	General breakdown	Apparent healthy
Bion	2 mM.	9.74	1.65	11.60	77.01	12.06	1.87	13.92	72.15
	4 mM.	8.17	1.01	10.13	80.69	10.49	1.23	12.45	75.83
	8 mM.	7.94	0.35	8.70	83.01	10.26	0.57	11.02	78.15
SA	2 mM.	10.02	0.66	11.98	77.34	12.34	0.89	13.30	73.47
	4 mM.	6.57	0.00	6.75	86.68	7.89	0.35	9.07	82.69
	8 mM.	8.29	0.00	9.47	82.24	10.61	0.48	11.79	77.12
H_2O_2	0.25%	7.44	0.00	8.51	84.06	9.76	0.00	10.59	79.65
	0.5%	8.78	0.23	10.75	80.24	11.10	0.75	13.07	75.08
	1.0%	9.88	0.68	11.85	77.59	12.20	0.98	13.17	73.65
Control		12.79	1.98	12.91	72.32	14.12	2.01	15.23	68.64
L.S.D. 5%: between									
a) Treatments =		2.01	0.32	2.98	4.26	1.30	0.22	2.39	3.75
b) Concentrations =		1.81	0.28	1.89	2.02	1.13	0.19	1.24	1.95
(a) × (b) =		3.69	0.52	4.60	6.08	2.25	0.32	3.50	5.42