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EFFECT OF SOME CHEMICAL INDUCERS RESISTANCE FOR CONTROLLING DOWNY MILDEW DISEASE OF GRAIN SORGHUM AND ITS IMPACT ON BIOCHEMICAL CHANGES

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ABSTRACT

Four different chemical inducers for resistance and metalaxyl fungicide were tested to control sorghum downy mildew disease (*Sorghum vulgare* L.) caused by *Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw. This evaluation was conducted at Gemmeiza Agricultural Research Station, El-Gharbea Governorate, Egypt in 2006 and 2007 seasons. Combined data reveal that metalaxyl occupied the first rank in controlling the disease. Salicylic acid was the best of the inducers in controlling sorghum downy mildew disease. While, potassium di-phosphate ($K_2 Hpo_4$) was the least effective one in this respect . Seedlings sprayed with salicylic acid gave the highest increase in 1000 grain weight in comparison with non-sprayed. In the same time spraying with salicylic acid and zinc sulphate increased grain sorghum leaves content of chlorophyll, nitrogen, protein and phosphorus. While potassium di-phosphat increased the leaves content of chlorophyll and potassium. Iron sulphate gave the lowest values in grain sorghum leaves content. The enzymatic activity of peroxidase and polyphenoloxidase and phenolic compounds contents in the grain sorghum leaves showed the highest values with salicylic acid and potassium di-phosphate followed by zinc sulphate . While iron sulphate gave the lowest values in this regard. No significant differences were obtained between metalaxl and the other compounds in the plant height and leaf area, while it showed the highest value in head length. However, metalayl followed by salicylic acid showed significant affect in increasing grain yield comparing with the other compounds. salicylic acid, potassium sulphate and metalaxyl showed highest values in increasing activities of phenols and enzymes.

Keywords : Downy mildew, *Sorghum vulgare* , *Peronosclerospora sorghi* , Metalaxyl fungicide , Salicylic acid , Potassium di-phosphate , Zinc sulphate , Iron sulphate, Peroxidase , Polyphenoloxidase , Phenolic compounds.

INTRODUCTION

Sorghum downy mildew, caused by *Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw, is a destructive disease of sorghum (*Sorghum bicolor* L.), grain sorghum (*Sorghum vulgare* L.) and maize (*Zea mays* L.). Soil-borne oospores are the major source of inoculum, and yield loss is directly related to oospores initiated infection (Frederiksen, 1980). Induced systemic resistance is a process of active resistance depending on the host plant's physical or chemical barriers activated by biotic or a biotic agents (Leeman *et al.*, 1996 and El-Sherbeni , *et al*., 2008). Induced systemic resistance (ISR) stimuli were shown to be salicylic acid (De Meyer & Hofte, 1997, Khaleifa, *et al.*, 2007 and Mahmoud, 2007), avirulent pathogens (Kuc

and Richmond,1977), and non-pathogens such as rhizobacteria (Wei *et al.,*1996). Trace elements may play an important role in protection by affecting plant susceptibility to fungal or bacterial phytopathogens (Graham, 1983 and Abd El-Hai *et al*. 2007). They may also affect the predisposition of plants to viral diseases, which have been reported to increase or decrease the resistance (Graham, 1983 and Pennazio and Roggero, 1988). Treatment of cucumber lower leaves with phosphates induced systemic resistance to *Colletotrichum lagenarium* and reduced disease severity (Doubravera *et al*., 1988). Spraying cucumber leaves with salicylic acid and ethyphone (2-chloro ethyl phosphonic acid) reduced the disease area caused by *Pseudoperonospora cubensis* by more of 50% (Okuno *et. al*., 1991). Gamil,(1995) tested different materials as inducers against powdery mildew disease caused by *Sphaerotheca faliginae*, and found that acetyl salicylic acid, cobalt sulphate and di-basic potassium phosphate (K_2 Hpo_4) greatly reduced powdery mildew symptoms on artificially infected plants in comparison with non treated plants.

MATERIALS AND METHODS

The present study was carried out at Gemmeiza Research Station, A.R.C. during 2006 and 2007 growing seasons.

Experimental design:

This experiment was performed in the disease nursery field prepared for evaluation against downy mildew disease. Sorghum cultivar Giza 15(as a highly susceptible) was used in this experiment which sown in plots (8.0 m^2 in area). Each plot contain two rows with 5 meter long and 80 cm apart. This cultivar was obtained from the National Maize Program, Field Crops Research. The field was surrounded by three rows of the highly susceptible variety of Sudan grass (Sordan79) as spreader which sown at least three weeks prior to planting. Three replicates were used for each inducer material in a Complete Randomized Block Design.

Application of inducer materials against sorghum downy mildew:

Four chemical compounds were used as inducers i.e. Salicylic acid, Potassium phosphate dibasic, Zinc sulphate, Iron sulphate These materials were used as spraying on sorghum leaves by three times at 7, 14, and 21 days after planting. Metalaxyl fungicide was used as a recommended check for controlling the disease. Arabic gum was added to each of the tested materials as adhesive material at concentration 1:50 (v: v). The untreated plots (control) was sprayed with water only. Chemical structures and concentration doses are presented in Table (1).

Disease assessment

Downy mildew disease infection was recorded three times after one, two and three months of planting during the season. The infection percentage of sorghum downy mildew disease was recorded as incidence of the total plants (D.I.) using the following equation :

$$\text{Disease incidence (D.I.) \%} = \frac{\text{No. of infected plants}}{\text{No. of total plants}} \times 100$$

Efficiency percentage of the tested materials was calculated according to the equation

$$\text{Efficiency \%} = \frac{\text{(D.I.)\% under control - (D.I.) \% undertreatment}}{\text{(D.I.) \% under control}} \times 100$$

Effect of the tested materials on plant height (cm), head length (cm) and leaf area (cm^2) were recorded 90 days after sowing. After harvest , 1000 grain weight (gm), and the yield/ plot were determined. Increase % of yield/ plot as a result use of each inducer material comparing with the control treatment was calculated as follow :

$$\text{increase \%} = \frac{\text{Yield of treated plants–yield of Control Yield}}{\text{Yield of Control}} \times 100$$

Table (1): Chemical structure and concentration of chemical inducers.

Chemical inducers	Chemical structure	Concentration
Salicylic acid		5mM (0.69 gm/L)
Potassium phosphate dibasic	$K_2 Hpo_4$	5mM (0.87 gm/L)
Zinc sulphate	$Zn So_4.7H_2o$	5mM (1.44 gm/L)
Iron sulphate	$Fe So_4.7H_2o$	5mM (1.39 gm/L)
metalaxyl fungicide	(N-2,6-dimethylphenyl) -N-(methoxyacetyl) -DL- alanine methlester / dicopper chloride-trihydroxide}	1.5 gm/L

Effect of the tested inducers on chemical components of sorghum leaves:

Samples of leaves from each treatment and control plants were calculated, 55 days after sowing, in order to determine chlorophyll, nitrogen, phosphours, potassium, phenolic compound contents, and assay the activity of peroxidase and polyphenol-oxidase.

a) Determination of chlorophyll contents:

Chlorophyll a, b and a & b were determined according to Moran and Porath (1980) using N,N-dimethyl formamide.

b) Determination of nitrogen, phosphours and potassium contents:

Leaves from each treatment and control grain sorghum were dried to constant weight. The dried leaves were grounded to fine powder. Amount of

0.2 gm of the fine powder was digested using sulphuric acid and perchloeic acid (5:1 v/v, respectively) then the solution was completed to 50 ml using distilled water. The final solution was used to determine nitrogen, phosphours and potassium content as follows:

i) Total nitrogen was determined using Kjeldahl method according to Chalmers (1984).

ii) Phosphours and potassium contents were determined using ammonium molybdate and a flame photometer method according to Chapman and Pratt (1961).

c**) Determination of phenolic compound :**

Samples of 5 gm of leaves tissues were cut into small portions and immediately putted in 95 % ethanol in brown bottles and kept in the dark at room temperature until the tissues were colorless then the ethanolic extracts were filtered and evaporated to near dryness on a mild water bath 60 ^{0}C. The extracts were quantitatively transferred into 5 ml of 50 % isopropanol and stored in vials at 1 ^{0}C till determination of phenolic compounds. Total and free phenols were determined using colormetric method of Folin at 250 nm, as described by Snell and Snell (1953). Conjugated phenols were determined from subtracting free phenols from the total phenols.

d) Enzymes activity :

Peroxidase and polyphenoloxidase were determined in the extraction grain sorghum leaves, as described by Maxwell and Bateman (1967).

i) Peroxidase activity was spectrophotometriclly determined by measuring the oxidation of pyrogallol in the presence of H_2O_2 at wave length of 425 nm, according to Allam and Hollis (1972).

ii) Polyphenoloxidase activity was determined using spectrophotometric procedure at 495 nm, as described by Matta and Dimond (1963).

Statistical analysis:

The obtained data were subjected to analysis of variance (Steel and Torrie, 1960) Duncan's multiple rang test (DMRT) was applied for comparing means.

RESULTS AND DISSCUSION

Data in Table (2) reveal that, all of the tested compounds reduced downy mildew disease incidence (D.M.I.) comparing with the control treatment. Metalaxyl fungicide released the highest efficacy and occupied the first rank in reducing disease severity (93.04 %). Salicylic acid at 5 mM concentration was superior in reducing the disease (11.89% disease incidence and 80.34 % Efficiency %). Chen *et al*.(1997) stated that spraying salicylic acid (SA) at 5 mM on leaves of a Lilium hybrid , stargaza, was shown to be effective in inducing resistance against *Botrytis elliptica* before inoculation. Audenaert *et al.*(1999) confermed the role of SA for the induction of induced systemic resistance in tomato to *Botrytis cinerea* to its effect on inducing the plant enzyme phenylalanine ammonia lyase (PAL), a key enzyme in the production of lignin and phytoalexenis. Also, SA involved in an ethelene/ jasmonic acid pathway. Achuo *et al*. (2004) reported that activation

of salicylic acid dependent defense pathway via bedzothiadiazole (BTH) resulted in induced resistance against *O. neolycopersici* in tobacco but not in tomato plants.

The present work demonstrate that the level of protection induced against downy mildew varied slightly from one compound to another but significantly reduced disease incidence as compared with control treatment. Zinc sulphate followed by Iron sulphate (5 mM) were moderate in their efficacy (67.62 and 59.25%). While Potassium phosphate dibasic was the least one in this respect.(45.23%). Abd-EL Kareem (1998) found that sprayinf solutin of KH_2PO_4 at 50, 100 and 150 mM against downy mildew of cucumber reduced disease incidence by 75.8, 74.2 and 77.7%, respectively. Also, Moshe, *et al*.(1997) stated that significantly systemic protection was obtained by K_2HPO_4 or KH_2PO_4 against powdery mildew of cucumber as well as the induced by B, Mn, and Cu. We think that the role of metals in induction systemic induced resistance may be differed according to the disease, density of inoculums and the plant.

Slight significant effect was shown on the treated plant characters as compared with the control. Approximately, Zinc sulphate induced the most response in plant height, head length and leaf area (303.99, 21.58 and 97.44 cm). While, the least one was induced by iron sulphate (296.58, 18.76 and 83.35 cm, respectively). No significant differences were found between the tested materials concerning with 1000 grain weight but it was found between them and the control treatment. Similar results were obtained by Seif El-Eslam *et al.* (2003) who found that asprin (Acetyl salicylic acid) was the best in controlling wilt and rot-root severity of Okra and increased green pod yield compared with the check treatment. No significant differences were obtained between metalaxl and the other compounds in plant height and leaf area, while it showed the highest value in head length (24.17).

Concerning with the effect of inducers on 1000 grain weight, significance differences were detected between metalayl and the inducers compared with the control treatment. Metalayl was the first in increasing % of 1000 grain weight (&&), followed by salicylic acid recorded (9.02 %). Howevere, no significant was obtained between Potassium phosphate dibasic, Zinc sulphate and Iron sulphate (6.18, 6.38 and 5.85 %, respectively).

Table(2): Combined data of four chemical inducers on controlling downy mildew disease (D.M.I.), plant characters , 1000 grain weight of grain sorghum cv. Giza 15 during 2006 and 2007 growing seasons.

Teatments	D.M.I. %	Efficiency %	Plant characters			1000 grian weight (grain index) gm	Increase 1000 grian weight of %
			Plant height cm	Head length cm	Leaf aerea cm^2		
Salicylic acid	11.89^{e}	80.34	307.03^{ab}	20.24^{ab}	90.46^{ab}	49.91^{a}	9.02
Potassium phosphate dibasic	33.11^{b}	45.23	299.37^{ab}	21.58^{a}	96.98^{a}	48.61^{a}	6.18
Zinc sulphate	19.57^{d}	67.62	303.99^{ab}	21.38^{a}	97.44^{a}	48.70^{a}	6.38
Iron sulphate	24.64^{c}	59.25	296.58^{b}	18.76^{bc}	83.35^{bc}	48.46^{a}	5.85
Metalaxyl	1.36^{f}	97.75	312.93^{a}	23.91^{a}	100.00^{a}	50.76^{b}	10.88
Control	60.46^{a}	0.00	280.81^{c}	18.11^{c}	75.33^{c}	45.78^{c}	0.00
LSD at 0.05	4.66	-	8.73	1.90	9.20	1.85	-

Data in Table (3) show that chlorophyll, total nitrogen, protien, phosphours and potassium contents were increased significantly as a result to spray sorghum plants with the tested materials. In general, all the previous contents were increased due to use salicylic acid except with Potassium % . Similar results were obtained by Ismail, *et al*.,(2007) they stated that salicylic acid followed by K_2HPO_4, H_2O_2, Cobalt sulfate, and ascorbic acid decreased soybean damping off disease caused by infestation with *R. solani*,or *S. rolfsii* or *M. phaseolina*. These materials increased number and dray weight of nodules, dry weight of shoot and its N-content and recorded the highest biological yield compared to the control. They added that seed and straw protein % significantly increased by SA, ascorbic acid and cobalt sulfate at 75 day old. Similar results were obtained by Abd El-Hai, *et al*.,(2007) they tested the effect of Zinc, Ferrous, Manganese and Boron on Cowpea rust disease. They showed a significant increase in chlorophyll a and b content by using manganese (2 and 3 g/l), ferrous (3 g/l), boron (0.025 and 0.05 g/l) and zinc (3 g/l) compared with other treatments. No significant differences were obtained between metalaxyl and salicylic acid concerning with the detected components.

Table(3) : Combined data of four chemical inducers on Chlorophyll , nitrogen, Protien, Phosphours and Potassium contents in leaves of susceptible cv. Giza 15, during 2006 and 2007 growing seasons.

Teatments	Chlorophyll contents gm / dm^2			Total nitrogen %	Protien content %	Phosphours %	Potassium %
	Chl a	Chl b	Chl a+b				
Salicylic acid	3.79^{a}	0.92^{a}	4.71^{a}	3.06^{a}	17.60^{a}	0.61^{a}	2.72^{bc}
Potassium di-phosphate	3.59^{ab}	0.71^{b}	4.30^{ab}	2.51^{bc}	14.43^{bc}	0.57^{a}	3.31^{a}
Zinc sulphate	3.28^{b}	0.67^{b}	3.95^{bc}	3.01^{ab}	17.31^{ab}	0.58^{a}	2.99^{ab}
Iron sulphate	2.84^{c}	0.66^{b}	3.50^{c}	2.54^{bc}	14.61^{abc}	0.52^{b}	2.77^{bc}
Metalaxyl	5.27^{a}	1.27^{a}	6.54^{a}	3.48^{a}	20.01^{a}	0.60^{a}	2.92^{a}
Control	1.70^{d}	0.46^{c}	2.16^{d}	2.16^{c}	12.42^{c}	0.41^{c}	2.46^{c}
LSD at 0.05	0.43	0.16	0.50	0.53	3.02	0.05	0.39

Data in Table (4) reveal that, the tested compounds showed some slight changes the systemic induction of total phenolic compounds as well as peroxidase and polyphenoloxidase enzymes were significantly occurred as compared with the control treatment. Spraying with Salicylic acid gave the highest values (8.53, 3.61 and 1.26, respectively) followed by Potassium phosphate dibasic, (7.82, 3.03 and 1.18 respectively), then metalayl fungicide (7.74, 2.30 and 0.92 %). While the contrast was happened with Zinc sulphate and Iron sulphate, since it showed the least values of systemic induction. The role of chemical inducers ,was explained by Raskin,(1992) who reveal that SA stimulated biosynthesis of different families of P-R proteins. While, Tilak *et al.*,(2002) found that it increases the activities of chitinase, peroxidase and B-1,3-glucanase .Also, Reuveni *et al.,*(1995) indicate that treatment with phosphate was associated with systemic resistance to different diseases. Similar results were obtained by Moshe *et al.*(1997). Also, Ismail *et al*.,(2007) stated that cobalt acid (1.00 ppm), Salicylic acid (7.5 mM), ascorbic acid (150 ppm) and K_2HPO_4 (10 mM) caused the highest activity in the peroxidase and polyphenol oxidase. Similar results were obtained by Khaleifa, *et al.*, (2007), they found that Salicylic acid, bion , $FeSo_4$ and K_2HPO_4 considerably increased the activity of oxidative enzymes (Peroxidase and Polyphenoloxidase) and phenolic compounds in Sesame. Metalaxyl showed lowest values in increasing activities of phenols and enzymes .

Table(4): Effect of spraying plants by four inducers on phenolic compounds and Enzymes activity in leaves of susceptible cv. Giza 15 of grain sorghum during 2006 and 2007 growing seasons.

Teatments	phenolic compounds mg/gm fresh weight			Enzymes activity	
	Free	Conjugated	Total	Peroxidase	Polyphenoloxidase
Salicylic acid	4.84[a]	3.69[b]	8.53[a]	3.61[a]	1.26[a]
Potassium phosphate dibasic	3.36[b]	4.46[a]	7.82[b]	3.03[b]	1.18[a]
Zinc sulphate	2.30[c]	3.60[b]	5.90[d]	2.13[c]	0.68[c]
Iron sulphate	1.84[d]	2.93[c]	4.77[e]	1.75[d]	0.62[c]
Metalaxyl	3.24[b]	3.68[b]	6.92[c]	2.29[b]	0.91[b]
Control	1.64[d]	1.80[d]	3.44[f]	1.50[e]	0.47[d]
LSD 0.05	0.43	0.48	0.34	0.19	0.10

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تأثير بعض المستحثات الكيماوية في مقاومة مرض البياض الزغبي في الذرة الرفيعة وتأثيرها على التحولات البيوكيماوية

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تم استخدام أربع مواد كيماوية كمستحثات لمقاومة مرض البياض الزغبي في الذرة الرفيعة والمتسبب عن الفطر بيرونوسكليروسبورا سورجاى ، وكذلك تأثيرها على التحولات البيوكيماوية في النباتات. وكانت هذه المواد هي حامض السالسيليك ، كبريتات البوتاسيوم ثنائية القاعدة ، كبريتات الزنك و كبريتات الحديدوز. وتم إجراء هذه الدراسة بمحطة البحوث الزراعية بالجميزة – بمحافظة الغربية في الموسمين الزراعيين ٢٠٠٦/ ٢٠٠٧ م وأظهرت النتائج الآتي :-

كان حامض السالسيليك يليه كبريتات الزنك ثم كبريتات الحديدوز الأفضل في مقاومة المرض ، بينما كانت كبريتات البوتاسيوم ثنائية القاعدة هي الأقل في هذا المجال .

أعطى رش بادرات نباتات الذرة الرفيعة بحامض السالسيليك أعلى زيادة في وزن محصول ألف حبة (٩,٠٢%) يلية كبريتات الزنك و كبريتات البوتاسيوم ثنائية القاعدة (٦,٣٨% و ٦,١٨%) وأقلهم كبريتات الحديدوز (٥,٨٥%) مقارنة بمعاملة الكنترول . وكذلك حقق حامض السالسيليك و كبريتات الزنك زيادة معنوية في مخزون الأوراق من الكلوروفيل ، النيتروجين والفوسفور. بينما حقق الرش بكبريتات البوتاسيوم ثنائية القاعدة زيادة في مخزون الأوراق من الكلوروفيل و البوتاسيوم فقط . بينما أعطى رش البادرات بكبريتات الحديدوز أقل النسب في هذا المجال. وقد لوحظ عدم وجود فروق معنوية بين المبيد والمستحثات فيما يخص ارتفاع النباتات ومساحة الورقة ووزن الألف حبة بينما أظهر أعلى قيمة في طول الكيزان.

أدى الرش بحامض السالسيليك يليه كبريتات البوتاسيوم ثنائية القاعدة ثم كبريتات الزنك أعلى زيادة في المركبات الفينولية والنشاط الإنزيمي (بيروكسيديز و البوليفينول أكسيديز) . بينما أعطى رش البادرات بكبريتات الحديدوز أقل زيادة في هذا المجال.