

SENSITIVITY OF *Alternaria radicina* AND *Alternaria tenuissima* TO SOME ANTIOXIDANT COMPOUNDS

Galal, A.A.; M.M.N. Shaat and A.A. El-Bana

Dept. Plant Pathology, Fac. Agric., Minia Univ., Minia, Egypt.

ABSTRACT

Sensitivity of *Alternaria radicina* and *A. tenuissima* to the following antioxidant compounds ascorbic acid, benzoic acid, hydroquinone, salicylic acid and tannic acid was investigated *in vitro*. Mycelial dry weight of the tested fungi was completely inhibited at 10 mM benzoic acid and salicylic acid. Hydroquinone at 10 mM caused a complete inhibition to the mycelial growth of *A. tenuissima*. At the lowest used concentration (0.1mM), ascorbic acid and salicylic acid stimulated the growth of *A. tenuissima* and inhibited the growth of *A. radicina*. Tannic acid at 0.1mM stimulated mycelial growth of *A. radicina* and inhibited *A. tenuissima*. Spore formation was greatly affected by all the tested chemicals. The reduction in spore formation was enhanced by increasing the chemical concentration. Spore germination of *A. radicina* was more affected than of *A. tenuissima* particularly at 1.0 mM. Viability of conidiospores was greatly affected especially with of *A. radicina* in this respect.

Regarding polygalacturinase (PG) activity, all the tested chemicals reduced PG activity except tannic acid at 0.1 mM. Salicylic acid inhibited PG activity of *A. radicina* greater than of *A. tenuissima*. While, hydroquinone inhibited PG of *A. tenuissima* more than PG of *A. radicina*.

Resistance of 2-year-old pear plants var. Leconte against leaf-spot causing fungi i.e, *A. radicina* and *A. tenuissima* was induced by spraying pear plants with the antioxidant compounds. Salicylic acid caused the highest protection when it was applied 24h. before or after inoculation. Contrary, tannic acid increased the susceptibility of pear plants to both fungi when it was applied 48 hr. after inoculation It showed the least protection (0.7%) in case of *A. radicina* when applied 24h. after inoculation.

Key words: Antioxidants, *Alternaria radicina* and *A. tenuissima*, sensitivity

INTRODUCTION

Application of antioxidants (free radical scavengers) as to increase the resistance of plants against the infection with viruses, bacteria and fungi has been successfully used (Prusky, 1988, Elad, 1992; Galal and Abdou, 1996 and Galal *et al.*, 1997 a and b).

Antioxidants delayed the onset of anthracnose and stem-end decay in avocado after harvest (Prusky, 1988). In 1992, Elad used antioxidant compounds to control diseases of cucumber fruits and found that butylated hydroxytoluene (BHT), tannic acid, ascorbic acid and dimethyl sulfoxide (DMSO) at a concentration of 1.0 mM controlled grey mould of tomato. Galal and Abdou (1996) reported that application of antioxidants to soil was more effective in controlling fusarial diseases of cowpea than seed treatments. They found that ascorbic acid induced protection against *Fusarium*

moniliforme, and *F. oxysporum* *F. solani* individually or in combination. Salicylic acid provided disease control against *F. oxysporum* and *F. solani* but not against *F. moniliforme*. Treatment of sunflower seeds by salicylic acid for 24 h before planting, resulted a resistant of plants against rust fungus, *Puccinia helianthi*, infection (Galal et al., 1997a). Application of ascorbic acid to squash seeds showed resistant plants against cucumber mosaic virus infection (Galal et al., 1997b).

Some of virulence mechanisms in pathogens are due to production of enzymes, plant growth regulators or toxins (Keen, 1992). One of defense mechanisms against pathogens is emanates from the use of specific inhibitors on biosynthetic enzymes (MacKintosh et al., 1994 and Biggs et al., 1997). Thus, the present work was conducted to study a) The *in vitro* effect of antioxidants on fungal growth of pear leaf spots caused by *Alternaria radicina* and *A. tenuissima*. b) Their effect on fungal enzyme(s) as well as in retarding the disease development caused by both fungi

MATERIALS AND METHODS

Effect of the antioxidants on fungal growth

Pure antioxidant compounds were used in this study including ascorbic acid (Riedel-Dehaen, Germany), hydroquinone, benzoic acid, salicylic acid and tannic acid (Prolabo, France). Ascorbic acid, hydroquinone and benzoic acid were dissolved in deionized water while salicylic acid was dissolved in ethanol and applied to Czapek's liquid medium to obtain the desired concentrations (0.1, 1.0 and 10 mM) in order to test their direct effect on fungal growth (Elad, 1992). Each 250-ml Erlenmeyer flask contained 50 ml medium amended or not with antioxidant compounds individually. Each flask received a 5-mm agar disk taken from an actively growing culture of *A. radicina* or *A. tenuissima* which isolated from spotted pear leaves (Galal et al., 1997c). Flasks incubated in dark at 25 C and were evaluated after 7 days. Growth parameters including mycelial dry weight, spore formation, spore germination and spore viability were assessed essentially as described by Tzeng and DeVay (1989) and Elad (1992). Mycelial dry weight was used to measure the effects of antioxidants on growth of test alternarias in Czapeck 's liquid medium amended or not with different concentrations of the antioxidants separately . Fifty milliliter of each medium was distributed into 250 ml -flask and autoclaved at 121 C for 15 min. After cooling, the medium was inoculated with a 5 mm-diameter agar disk removed from the edge of actively growing *Alternaria* culture (7-day-old). The cultures were incubated statically at 25 C. After 7 days cultures were vigorously shaken for 2 hr. and 100 µl were taken from each flask to measure sporulation, while the mycelium was dried before determine mycelial dry weight (70 C for 24 h.). Conidia were also with-drawn from the cultures and plated on water agar to estimate their viability (Tzeng and DeVay, 1989).

The effect of antioxidants on germination of *Alternaria* conidiospores was assayed on plates separately containing different concentrations of antioxidants in distilled water plus 1.5 % agar (15ml reaction mixture/plate). Conidial suspension was prepared (approx. $\times 10^6$ spores/ml) from-7-day-old

cultures grown on PDA medium and plates were seeded to obtain countable spores/microscopic field on the surface of the agar (about 40-70 spores). The seeded plates were incubated at 25 C for 24 h. The percentages of germination were then assayed using light microscope.

Sporulation was measured using a haemocytometer. All experiments were repeated at least 3 times.

Effect of the antioxidants on polygalacturinase activity:

Disks, 5-mm in diameter, of *A. radicina* or *A. tenuissima*, individually grown on PDA, were transferred to stationary 250-ml Erlenmeyer flasks containing 50 ml of Czapek's liquid medium amended or not by antioxidants. The medium was used either in the absence of sucrose after being substituted by 1% of the enzyme substrate, sodium polygalacturonate. pH of the supplemented medium was adjusted to 6.0 with either 0.1 N HCL or NaOH (Biggs *et al.*, 1997) before autoclaving. Flasks were incubated 10 days at 25 C, and filtrates were collected, analyzed immediately for enzyme(s) activity while, polygalacturinase assayed viscometrically (McCarroll and Thor, 1985) while, Three millilitres of crude enzymes preparation was incubated with 3 ml of 1% sodium polygalacturonate and buffered to pH 4.5 with 0.1 M acetate buffer. The mixture was kept in water bath at 30 C. The square of the percentage decrease in viscosity per minute is defined as a unit of enzyme activity (Mc Carroll and Thor, 1985).

Effect of the antioxidants on infection of pear leaves

Trials were conducted in the greenhouse using a 2-year-old apparently healthy pear plants, leconte var., as treatment units. Treatments with 5 mM antioxidant concentration were applied 0, 1 and 2 days intervals before or after inoculation. Antioxidants were applied individually to pear leaves by spraying until fall down, 2 plants / treatment. Leaf inoculation was carried out by inocula prepared from 7-day-old cultures of *A. radicina* or *A. tenuissima* grown on PDA medium in petri dishes at 25 C. Cultures were gently brushed in the presence of 10 ml 0.1% (w/v) sterilized methylcellulose solution to release spores and filtered through muslin then spore suspension was adjusted to obtain 2×10^5 conidia / ml. Spore suspension was sprayed on leaves until fall down. Inoculated plants were covered with plastic bags for 48h to save high humidity. This experiment was repeated twice with two replicates.

Disease assessment :

Unless stated otherwise, 20 days after challenge inoculation the disease index (DI) and disease severity index (DSI) were estimated 4 times at 20 days as described by Liu *et al.*, 1995 as follow: $DSI: \left[\frac{\sum D}{d \times n} \right] \times 100$. Where : d is the disease rating on each plant, damx is the maximum disease rating possible and n is the total number of plants examined in each replicate and values of area under the disease progress curve AUDPC were calculated according to this formula as described by Pandey (1989).

$$\text{AUDPC} = D \cdot 0.5 (Y_1 + Y_2 + \dots + Y_{K-1})$$

Where D = days interval

Y_1 = DS1 of the first reading

Y_K = DS1 of the last reading

Effectiveness of using some antioxidants in controlling leaf spot blight was calculated as $100 - (100 \times y/x)$ where x is the blighted leaf area in inoculated antioxidant-treated plants and y the blighted leaf area in inoculated untreated control plants (Cohen et al., 1993).

Statistical analysis:

Standard error for means (Sem) was used for analysis of variances as described by Little and Hills (1972).

RESULTS AND DISCUSSION

Effect of the antioxidants on fungal growth

Table 1. shows that growth parameters e.g. mycelial dry weight, spore formation, spore germination and spore viability of both fungi *A.rodicina* and *A.tenuissima* were greatly affected by the antioxidant compounds. The antifungal activity for these compounds was more pronounced at highest concentration 10 mM. Benzoic acid and salicylic acid provided a complete inhibition to mycelial growth of both tested *Alternaria* species at 10 mM. Hydroquinone caused complete inhibition to *A. tenuissima* growth only at 10 mM. Ascorbic acid gave least antifungal activity in this respect. However, at 0.1 mM ascorbic acid and salicylic acid exhibited growth stimulation for *A. tenuissima*. Mycelial growth of *A. tenuissima* was affected by tannic acid greater than *A.rodicina*.

Table 1: Mycelial dry weight (MDW), spore formation (SF), spore germination (SG) and spore viability (SV) of *A. radicina* and *A. tenuissima* as affected by different concentrations (mM) of various antioxidant compounds.

Treatments	Chemical Conc. (mM)	<i>A. radicina</i>				<i>A. tenuissima</i>			
		MDW	SF	SG	SV	MDW	SF	SG	SV
Ascorbic acid	0.1	179 ± 6	8.5 ± 0.5	90 ± 4	84 ± 4	309 ± 12	14.2 ± 0.8	94 ± 1	96 ± 1
	1.0	138 ± 9	5.4 ± 0.2	75 ± 3	74 ± 6	200 ± 8	6.7 ±	90 ± 2	88 ± 2
	10	130 ± 8	3.1 ± 0.3	10 ± 2	56 ± 3	85 ± 4	0.5	85 ± 4	87 ± 2
Benzoic acid	0.1	112 ± 8	3.5 ± 0.2	44 ± 2	77 ± 7	147 ± 7	5.3 ±	96 ± 2	97 ± 1
	1.0	49 ± 5	2.5 ± 0.2	40 ± 3	68 ± 5	25 ± 4	0.6	65 ± 4	93 ± 3
	10	0.0	0.0	24 ± 2	0.0	0.0	15.1 ± 0.6	0.0	0.0
Hydroquinone	0.1	166 ± 11	8.5 ± 0.4	44 ± 4	83 ± 3	140 ± 8	12.3 ± 0.2	90 ± 2	100 ±
	1.0	92 ± 4	4.3 ± 0.3	36 ± 6	78 ± 4	61 ± 4	0.0	88 ± 5	86 ± 2
	10	88 ± 7	1.5 ± 0.1	12 ± 2	29 ± 2	0.0	4.5 ± 0.4	6 ± 1	0.0
Salicylic acid	0.1	99 ± 5	9.2 ± 0.5	55 ± 6	44 ± 3	262 ± 11	2.3 ± 0.1	95 ± 2	100 ±
	1.0	38 ± 2	5.0 ± 0.1	48 ± 4	35 ± 3	35 ± 3	0.0	74 ± 6	96 ± 1
	10	0.0	0.0	8 ± 1	0.0	0.0	5.3 ± 0.3	14 ± 3	0.0
Tannic acid	0.1	265 ± 14	8.5 ± 0.4	78 ± 4	86 ± 5	168 ± 10	4.5 ± 0.4	90 ± 4	90 ± 2
	1.0	116 ± 9	7.3 ± 0.4	64 ± 4	84 ± 4	138 ± 8	0.0	90 ± 6	92 ± 2
	10	44 ± 4	4.1 ± 0.3	58 ± 3	36 ± 3	113 ± 9	9.8 ± 0.8	82 ± 3	90 ± 2
Control (water)	0.0	216 ± 8	10.5 ± 0.5	85 ± 4	87 ± 5	243 ± 11	4.2 ± 0.2	96 ± 2	95 ± 2
							1.7 ± 0.2		
							32 ± 5		

Data are means of 3 separate experiments ± Standard error for means (SEM)

MDW : mycelial dry weight mg/50 ml culture medium

SF 3: Spore formation x 10⁴ per ml culture medium

SG: Spore germination % was determined 24h after incubation at 25 C

SV: spore viability % of germinated spores which taken from 7-day-old cultures grown on Czapek's liquid medium amended or not by antioxidants.

Regarding spore formation, data (Table 1) indicate that all tested antioxidants were greatly reduced the fungal ability to form spores. Sporulation of *A. radicina* was more sensitive by benzoic acid than *A. tenuissima* while hydroquinone caused the opposite effect in this respect. However, the reduction in spore formation was increased by increasing the chemical concentration. Spore viability was the least affected to the tested antioxidants (Table 1) particularly *A. tenuissima*. Salicylic acid was more affective on spore viability of *A. radicina* conidiospores. Least effect on spore viability was occurred by tannic acid.

As for spore germination, *A. radicina* conidiospores were more sensitive to all tested antioxidants at all concentrations except 0.1 mM ascorbic acid. Conidiospores germination of *A. tenuissima* was less affected at 0.1 or 1.0 mM ascorbic acid and/or hydroquinone while substantial inhibition in spore germination has revealed at 10 mM concentration. It was noticed somewhat that germination of conidiospores at the highest antioxidant concentrations which caused a complete inhibition of mycelial growth revealed that conidiospores were less sensitive. While the mycelium was greatly affected in this respect. Data indicate that all tested antioxidants have direct antifungal activity depending on fungal structure and chemical concentration as well. Similar antifungal activity for these components was reported on *Colletotrichum gloeosporioides* (Prusky, 1988), *Phytophthora infestans* (Arnoldi *et al.*, 1989), *Rhizopus stolonifer*, *Sclerotinia sclerotiorum* and *Botrytis cinerea* (Elad, 1992) and on *Fusarium moniliforme*, *F. oxysporum* and *F. solani* (Galal and Abdou, 1996).

Effect of the antioxidants on polygalacturinase (PG) activity:

Generally, *A. radicina* secreted PG higher than *A. tenuissima* (Table 2). PG activity is strongly inactivated by all tested antioxidant compounds except tannic acid at 0.1 or 1.0 mM. Tannic acid at 0.1 mM gave stimulatory effect toward PG activity for both fungi but at 1.0 mM tannic acid inactivated PG of *A. radicina* and stimulated PG of *A. tenuissima*. PG of *A. radicina* was more sensitive to salicylic acid than PG of *A. tenuissima*. Ascorbic acid caused the highest inhibitory effect on PG activity of *A. tenuissima*. Data indicated that such given compounds did not essentially affect the enzyme activity of both fungi similarly. This could be due to insimilarity of enzymes from either *A. radicina* or *A. tenuissima*. However, it has been suggested that repression of pectolytic enzymes activity represents a defence mechanism of the host towards the pathogen (McCarroll and Thor, 1985, Ouf *et al.*, 1991 and Biggs *et al.*, 1997).

Effect of the antioxidants on infection of pear leaves:

Data presented (Table 3) show a resistance of antioxidant-treated pear leaves against infection with either *A. radicina* or *A. tenuissima*. Regarding ascorbic acid application, a reduction in pear leaf infection was depended on time of application and pathogen. Highest resistance was provided by ascorbic acid application at the same time of inoculation with *A.*

tenuissima. But under *A. radicina* inoculation, high resistance was pronounced when ascorbic acid applied 2 days before inoculation. While application of ascorbic acid 1 or 2 days after inoculation, a low protection against both *A. radicina* or *A. tenuissima* infection was recorded. Data indicate that the resistance in pear leaves could be induced by ascorbic acid. Similar findings with ascorbic acid were reported on controlling grey mould of tomato (Elad, 1992), fusarial

Table 2. Activity of polygalacturinase (PG) secreted by *A. radicina* and *A. tenuissima* grown in Czapek 's liquid medium supplemented with sodium polygalacturonate and various concentrations of antioxidant compounds.

Treatments	Chemical conc. (mM)	PG. Activity* of	
		<i>A. radicina</i>	<i>A. tenuissima</i>
Ascorbic acid	0.1	4.5 ± 0.2**	1.6 ± 0.1
	1.0	2.3 ± 0.3	0.2
	10	0.4 ± 0.1	0.1
Benzoic acid	0.1	4.8 ± 0.3	3.6 ± 0.3
	1.0	3.2 ± 0.4	2.9 ± 0.3
	10	NA	NA
Hydroquinone	0.1	8.4 ± 0.5	2.1 ± 0.1
	1.0	5.6 ± 0.4	0.5 ± 0.1
	10	1.8 ± 0.2	NA
Salicylic acid	0.1	1.8 ± 0.1	4.2 ± 0.3
	1.0	0.2	2.1 ± 0.1
	10	NA	NA
Tannic acid	0.1	12.4 ± 1.2	6.1 ± 0.4
	1.0	10.8 ± 1.1	4.8 ± 0.5
	10	3.6 ± 0.2	3.2 ± 0.3
Control (water)	0.0	12.2 ± 1.1	4.3 ± 0.2

* PG activity was expressed as enzyme unit per ml culture filtrate

** Data are means of 3 separate experiments run in triplicates ± SEM

NA : No enzyme activity (complete growth inhibition).

Table 3. Mean value of AUDPC¹ caused by *A. radicina* or *A. tenuissima* infection as affected by 5 mM antioxidant sprayed before or after inoculation.

Treatments	Time of Chemical application	<i>A. radicina</i>		<i>A. tenuissima</i>	
		AUDPC	% Protection	AUDPC	% Protection
Ascorbic acid	48 h. pre	172 ² ±8	91.8	255±4	85.8
	24 h. pre	274±5	87.1	133±8	92.6
	0.0	1078±20	49.2	39±2	97.8
	42 h. post	1020±15	51.9	372±8	79.4
	48 h. post	1254±18	40.9	600±6	66.8
Benzoic acid	48 h. pre	1034±18	51.2	780±8	56.8
	24 h. pre	282±6	82.0	246±4	86.4
	0.0	225±6	89.4	377±4	79.1
	42 h. post	308±4	85.5	537±6	70.2
	48 h. post	630±5	70.3	1357±12	24.6
Hydroquinone	48 h. pre	673±7	68.3	2160±14	-19.6
	24 h. pre	561±5	73.5	1450±13	19.7
	0.0	306±4	85.5	1080±11	40.2
	42 h. post	440±4	79.3	928±8	48.6
	48 h. post	575±13	72.9	759±8	57.9
Salicylic acid	48 h. pre	962±10	54.6	656±6	63.7
	24 h. pre	204±6	90.4	101±3	94.4
	0.0	76±4	96.4	16±2	99.1
	42 h. post	195±18	90.8	365±8	79.7
	48 h. post	560±12	73.6	1096±14	39.3
Tannic acid	48 h. pre	1800±15	15.2	1472±12	18.4
	24 h. pre	1950±14	8.1	1510±12	16.3
	0.0	1505±18	29.1	2780±15	-53.9
	42 h. post	2106±12	0.7	1625±14	10.1
	48 h. post	2604±12	-22.6	2170±12	-20
Control (water)		2123	0.0	1806	0.0

1. AUDPC : area under the disease progress curve values.

2. Data are means of one experirun in duplicate ± SD.

diseases of cowpea namely by *F. moniliforme*, *F. oxysporum* and *F. solani* individually or in combination (Galal and Abdou, 1996) and on controlling squash against cucumber mosaic virus (Galal *et al.*, 1997b).

Benzoic acid caused a higher protection of pear leaves against *A. radicina* infection than *A. tenuissima*. Also, the effectiveness of benzoic acid was depended on the time of application and on the pathogen as well.

As for hydroquinone, higher protection was caused against *A. radicina* as compared to *A. tenuissima* infection (Table 3). Application of hydroquinone 2 days before inoculation increased the susceptibility of pear leaves to *A. tenuissima* infection. However, hydroquinone caused resistance against grey mould of tomato (Elad, 1992) and cowpea fusarial diseases

(Galal and Abdou, 1996), but it had no effect on controlling cucumber anthracnose caused by *Colletotrichum lagenarium* (Fought and Kuc, 1996).

The highest reduction in *Alternaria*-leaf spot infection was pronounced when pear leaves were inoculated at the same time of salicylic acid application (Table 3). Increasing time intervals (days before or after) between leaf spraying by salicylic acid and inoculation decreased resistance. In contrast, tannic acid increased the susceptibility of pear leaves to infection with both fungi when applied 48h after inoculation. The highest susceptibility level (53.9% increase in susceptibility than water-treated plants) was induced when tannic acid was applied at the same time of inoculation by *A. tenuissima*. In the light of these data, salicylic acid showed a direct antifungal activity and induced protection against infection with *A. radicina* or *A. tenuissima* individually. Salicylic acid is considered to mediate plant responses to pathogens (Delanay et al., 1994 and De Meyer and Hofte, 1997) and is associated with pathogen induced systemic acquired resistance (Galal and Abdou, 1996; and Galal et al., 1997a). In most cases salicylic acid application to plants has only a local effect on pathogens (Elad, 1992), but in tobacco, radish, *Arabidopsis*, bean, cowpea and sunflower, a systemic resistance to pathogens has been reported after seed, root, or soil treatment with salicylic acid (Enyedi et al., 1992; Leeman et al., 1996; Galal and Abdou, 1996; De Meyer and Hofte, 1997 Galal et al., 1997a and Wisniewska and Chlkoweski, 1999).

More research is needed to determine the best method to utilize some of the more effective antioxidant compounds. On the other hand, *Pseudomonas aeruginosa* which is known to be a salicylic acid-producer (De Meyer and Hofte 1997) could be used as a promising candidate for controlling such disease. Additional research is needed to confirm the results on a large scale.

REFERENCES

- .Arnoldi, A.; Carughi, M.; Farina, G.; Merlini, L. and Parrino, M. G. (1989) Synthetic analogues of phytoalexins: synthesis and antifungal activity of potential free-radicals scavengers. *J. Agric and Food Chem.*, 37:508-512.
- Biggs, A. R.; El-Kholi, M. M.; El-Neshawy, S. and Nickerson, R. (1997) Effects of calcium salts on growth, polygalacturonase activity and infection of peach fruit by *Monilinia fructicola*. *Pl. Dis.*, 81: 399-403.
- Cohen, Y.; Gisi, U. and Niderman, T. (1993). Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology*, 83: 1054-1062.
- Delanay, T. P.; Uknes, S.; Vemooij, B.; Friedrich, L.; Weymann, K.; Negrotto, D.; Gaffney, T.; Gut-Rella, M.; Kessmann, H.; Ward, E. and Ryals, J. (1994) A central role of salicylic acid in plant disease resistance. *Science*, 266:1247-1250.

- DeMeyer, G. and Höfte, M. (1997) Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. *Phytopathol.*, 87:588-593.
- Elad, Y. (1992) The use of antioxidants (free radical scavengers) to control gray mould (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum*) in various crops. *Plant Pathology*, 41: 417-426.
- Enyedi, A. J.; Yalpani, N.; Silverman, P. and Raskin, I. (1992) localization, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. *Proc. Natl. Acad. Sci. USA*, 89:2480-2484.
- Fought, L. and Kuc, J. A. (1996) Lack of specificity in plant extracts and chemicals as inducers of systemic resistance in cucumber plants to anthracnose. *J. Phytopathol.*, 144:1-6.
- Galal, A.A. and Abdou, E. (1996) Antioxidants for the control of fusarial diseases in cowpea. *Egypt. J. Phytopathol.*, 24:1-12.
- Galal, A.A.; Abdel Aziz - Nabila, A.; Hassani, M.H. and Shaat, M.N. (1997b) Induction of systemic resistance in squash plants against cucumber mosaic virus by ascorbic acid. 7th Nat.Con.of Pest and Dis. of Vegetables And Fruits in Egypt, 1:170-182
- Galal, A. A.; Botros -Amal, L.; Shihata, Z. A.; Gazar, A. A. and Ouf, M. F. (1997a) Acquired resistance to *Puccinia helianthi* in sunflower plants. *Egypt. J. Phytopathol.*, 25: 45-54.
- Galal, A. A.; Shaat, M. M. N. and Abdel-Aziz-Nabila. A. (1997c) Occurrence of *Alternaria*-leaf spot on pear in Minia. 7th Nat. Con. of Pest and Dis. of Vegetables and Fruits in Egypt., 1:110-124.
- Keen, N. T. (1992) The molecular biology of disease resistance. *Pl. Mol. Biol.*, 19: 109-122.
- Leeman, M.; den Ouden, F. M., van Pelt, J. A., Dirkx, F. P. M., Steijl, H.; Bakker, P. A. H. M. and Schippers, B. (1996) Iron availability affects induction of systemic resistance to *Fusarium* wilt of radish by *Pseudomonas fluorescens*. *Phytopathol.*,86:149-155.
- Little, T. M. and Hills, F. J.(1972) *Statistical Methods in Agriculture Research*, USD, California, USA. pp. 9 -27.
- Liu, I. ; Kloepper, J.W and Tuzun, S. (1995). Induction of systemic resistance in cucumber against fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathology*, 85: 695-698.
- MacKintosh, C.; Lyon, G. D. and MacKintosh, R. W. (1994) Protein phosphatase inhibitors activate anti-fungal defence responses of soybean cotyledons and cell cultures. *The plant J.*, 5: 137-147.
- McCarroll, D.R. and Thor, E. (1985) Pectolytic, cellulytic and proteolytic activities expressed by cultures of *Endothia parasitica* and inhibition of these activities by components extracted from Chinese and American chestnut inner bark. *Physiol. Plant Pathol.*,26. 367-378.
- Ouf, M. F.; Gazar, A.A.; El-Sadek, S.A.M. and Galal, A. A. (1991) Effect of some plant extracts on growth and enzyme activities of soft rot bacteria. *Egypt. J. Microbiol.*, 26: 157-169.
- Pandy, H.N.; Menon, T.C.M. and Rao, M.V. (1989). A simple formula for calculating area under disease progress curve. *Rachis*, 8: 38-39.

- Prusky, D. (1988) The use of antioxidants to delay onset of anthracnose and stem end decay in avocado fruits after harvest. *Pl. Dis.*, 72: 381-384.
- Tzeng, D.D. and DeVay, J.E. (1989) Biocidal activity of mixture of methionine and riboflavin against plant pathogenic fungi and bacteria and possible mode of action. *Mycologia*, 81: 404-412.
- Wisniewshs, H. and Chelkowschi, J. (1999) Influence of exogenic salicylic acid on *Fusarium* seedling blight reduction in barley. *Acta Physiol. Plant.*, 21: 63-33.

حساسية الفطرين الترناريا راديسينا والترناريا تينوسيميا لبعض المركبات المضادة للأكسدة

أنور عبد العزيز جلال ، محمد محمد نعيم شعت ، على عبد المنعم البنا
قسم أمراض النبات - كلية الزراعة - جامعة المنيا - المنيا - مصر

درس تأثير خمسة مركبات مضادة للأكسدة وهي حمض الأسكوربيك و البنزويك والهيدروكينون وحمض الساليسيليك وحمض التانيك على نمو ونشاط البولجلاكتويورينيز والقدرة المرضية للفطريات المسببة لتبقع أوراق الكمثرى وهما ألترناريا راديسينا وألترناريا تينوسيميا. وتم تقييم قياسات النمو الفطرية والمتمثلة في الوزن الجاف الميسليومي والتجرثم وانبات وحيوية الجراثيم على بيئه تشابكس المدعمه بتركيزات مختلفه من مضادات الأكسدة . أظهرت النتائج حدوث تثبيط كامل لنمو الفطرين عند تركيز ١٠ ملليمول من حمض ساليسيليك بينما عند نفس التركيز من الهيدروكينون حدث تثبيط كامل لنمو الفطر الترناريا تينوسيميا فقط. وتأثر تجرثم كلا الفطرين تأثراً شديداً بحمض الساليسيليك يليه الهيدروكينون وحمض الأسكوربيك. كما تأثر انبات الجراثيم بمضادات الأكسدة خاصة للفطر الترناريا راديسينا وأوضحت الدراسة انخفاض شديد لانتاج البولجلاكتويورينيز بواسطة الفطرين عند تنميتها في وجود مضادات الأكسدة. أدى رش أوراق الكمثرى صنف ليكونت بمضادات الأكسدة الى حدوث وقايه ضد الاصابه بكلا الفطرين حيث أحدث حمض الساليسيليك أعلى وقايه عند استخدامه أثناء اجراء العدوى الصناعيه مباشرة قبل أو بعد العدوى بـ ٢٤ ساعة. وقد شوهدت أقل وقايه على أوراق الكمثرى المعامله بالهيدروكينون بـ ٢٤ ساعة قبل العدوى خاصة عند العدوى بالفطر الترناريا تينوسيميا. على الجانب الآخر فقد أظهر حمض الأسكوربيك تأثيرات متباينه أعتمدت على الكائن الممرض ووقت استخدام الحمض. وعلى العكس فإن رش حمض التانيك بعد العدوى بـ ٤٨ ساعة أدى الى زيادة قابلية نباتات الكمثرى للإصابة بكلا الفطرين.