Assiut J. of Agric. Sci., 38 (2) (97-110)

EFFICIENCY OF SALICYLIC ACID AND OXALIC ACID FOR CONTROLLING FUSARIUM WILT DISEASE OF TOMATO

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Abstract: The effect of different concentrations of salicylic acid (SA) or oxalic acid (OA) on linear growth of *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of tomato wilt was studied in *vitro*. SA concentrations ranging from 750 to 2000 ppm have significantly reduced mycelial growth of the pathogen. While, OA significantly inhibited the linear growth at concentrations ranged from 500 to 2000 ppm. The percentage of inhibition varied between the three tested isolates of *F. oxysporum* f. sp. *lycopersici* , the highest inhibition was occurred in FOL isolate 4.

In two successive growing seasons (Summer 2006 and Winter 2006/2007), seedling treatments with SA or OA at concentrations 500 and 2000 ppm were carried out and disease severity as vascular browning and foliar yellowing revealed that SA at concentrations 500 ppm significantly reduced disease severity in both seasons but 2000 ppm of SA reduced vascular browning with nonsignificantly effect. Seedling treatment with OA at concentrations 500 and 2000 ppm significantly reduced the foliar yellowing and vascular browning percent in tomato plants in both tested seasons. The fungal isolates were differed in their virulent FOL isolate 2 was the most virulent one followed by FOL isolate 3, while FOL isolate 4 was the lowest virulent one.

Tomato seedling treated with SA or OA exhibited higher activity of polyphenol oxidase and higher reduction of pectin methyl esterase after 20 days from transplanting compared with untreated plants.

Key words: salicylic acid, oxalic acid, fusarium wilt, Tomato.

Introduction:

Tomato *Lycopersicon esculentum* is an important vegetable crop world wide. Tomato cultivation is affected by vascular wilting diseases which have become one of the serious diseases. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is the most destructive causal agent on tomato, in Egypt (El-Zawahry, Aida, 1984).

Several disease management strategies are available as resistant cultivars, biological control, crop rotation and chemical fungicides. Among the chemical fungicides, many compounds are available, but nearly all are based on direct antibiotic principle.

A second potential principle for chemically mediated disease control could be based on compounds that would induce the resistance as an alternative to fungicide application. The natural protection of plants against pathogens is partially based on a variety of constitutive barriers already present in the plant before the actual attack. Certain natural and synthetic chemical compounds such as salicylic acid (SA) and oxalic acid (OA) can trigger plant responses against pathogens. They may be also fungal inhibitors. Salicylic acid inhibited in *vitro* growth of *Fusarium oxysporum*, the causal agent of onion rot (El-Ganaieny *et. al.*2002).

Exogenous application of SA as seedling treatment reduced significantly Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Attitalla *et. al.* 1998). Also seed treatment with SA enhanced the resistance of seasam plants against root rot and wilt disease (Abdou *et. al.*, 2001).

Some enzymes play an important role in plant defense against many diseases either directly by degradation of cell wall or indirectly by releasing oligosaccharide which could induce additional plant defense (Rose *et. al.*, 2002).

The activity of many polyphenol oxidase are generally higher in the infected tissue of resistance varieties than in infected susceptible ones or in healthy plants based on its ability to oxidase phenolic compounds to quinons which are often more toxic to microorganisms than their original phenols (Hammerschmidt 1999).

The aims of this research were to determine the effect of two synthetic compounds (salicylic acid and oxalic acid) as an alternative to fungicides for controlling Fusarium wilt of tomato and to determine the activities of polyphenol oxidase and pectin methyl esterase enzymes in the treated tomato plants.

Materials and Methods:

Source of fungi:

Three isolates of *F. oxysporum* f.sp. *lycopersici* (FOL 2, 3 and 4) were used throughout this study. Their pathogencity were previously tested by (Eraky, Amal *et. al.* 2006).

Effect of salicylic acid and oxalic acid on *Fusarium oxysporum* f.sp. *lycopersici* (FOL) linear growth in *vitro*:

The direct effect of salicylic acid and oxalic acid on the growth of the fungal isolates was studied in *vitro* as described by El-Ganaieny, *et al.* (2002). Each chemical substance (SA or OA) was dissolved in 5ml ethanol (90%) and adjusted to pH 7 with 1N NaOH. The tested substance was incorporated into sterilized Potato Dextrose Agar (PDA) at concentrations 0, 500, 750, 1000, 1500 and 2000 ppm. PDA

with 5ml ethanol was used as control. A 6-mm- diameter plug from the advancing margins of each fungal isolate was seeded centrally onto 5 plates of each substance concentration and incubated at 27 °C. Results were recorded as a growth reduction when mycelia growth of the control (0 ppm) plates reached to the edge of the plate using the following formula:

$$\%\text{Reduction of growth} = \frac{\text{Growth in control-Growth in treatment}}{\text{Growth in control}} \times 100$$

The experiment was conducted twice.

Effect of salicylic acid and oxalic acid on incidence of tomato Fusarium wilt under greenhouse conditions:

Tomato cv. Prichard (highly susceptible to Fusarium wilt) was used. Tomato seeds were surface sterilized with 2% sodium hypochlorite solution for 2 min., renised in sterile distilled water, then air dried and sowed in tray contained sterilized peat : sand : clay 1:1:1 for 45 days. Nursery was irrigated when needed.

Inoculum for three FOL isolates were (FOL2, FOL3 and FOL4) prepared on barley grain medium was inoculated by each isolate, and incubated at 25°C for 2 weeks. The inoculum was added to sterilized clay- sand soil (2:1) at rate of 3%, thoroughly mixed and potted in sterilized pots (25 cm in diameter) for one week.

Tomato transplants (45 days old) were dug off seedling trays and the root thoroughly washed by running water to remove any adherent particles. Tomato transplants were treated by dipping the root into salicylic acid or oxalic acid solutions at two concentrations 500 and 2000 ppm for 30 min. The treated tomato plants were transferred to the pathogen infested pots. Four replicates were used for each treatment and untreated transplants were transferred to pots with uninoculated Barely grain medium used as control. Plants were irrigated and fertilized when as usual. Disease severity was estimated after 30 days from transplanting, as a foliar yellowing percent and vascular browning percent using the rating scale in which infected plants were classified according to a numerical grades ranging from 0 to 4 as follows:

0 = healthy.

1 = > 25 of plant leaflets are yellow and of vascular root bundles are dark brown.

2 = < 25 – 50 of plant leaflets are yellow and of vascular root bundles are dark brown.

3 = < 50 – 75 of plant leaflets are yellow and of vascular root bundles are dark brown.

4 = <75–100 of plant leaflets are yellow and of vascular root bundles are dark brown.

For calculating the foliar yellowing and vascular browning indices of each plant, the following formulae were used: [(sum of yellowing values /4 x number of leaflets) x100%] and [(sum of vascular browning values /4 x number of internodes) x 100%] (Fakhouri and Buchenaur, 2003). The experiment was carried out twice during summer 2006 and winter 2006/2007 growing seasons.

Effect of salicylic acid and oxalic acid on enzyme activity in tomato plants:

Tomato transplants (45days old) were treated with SA or OA at concentrations 0, 500 and 2000 ppm, and transferred to infested soil (as mentioned above). The samples for enzyme extraction from all treatments were separately harvested 20 days after transplanting date. Plant extracts were prepared by collecting 3 gm fresh samples sectioned from the base of the stems, and cut into 3 mm slices, these slices were homogenized in 25 ml of pH 7.0 phosphate buffer then filtered through cheesecloth and centrifuged to remove debris, and the supernatant considered the plant extract.

a- Polyphenol oxidase (PPO):

Polyphenol oxidase activity was determined using the method described by El-Zawahry, Aida(1984).Two ml of plant extract for each treatment were added to test tube containing 2 ml of 0.1% DOPA (3,4-dihydroxy- phenolalanin) solution. The reaction mixture was incubated at 30°C in water bath for 14 hr., then centrifuged for 14 min. at 3000 rpm, and then measured spectrophotometrically at 400 nm. The test tube contained two ml of DOPA solution + 2ml of phosphate buffer was used as blank. Percentage of increment in enzyme activity was calculated by using the following formula:

$$\% \text{ Increment of enzyme activity} = \frac{ET - EU}{EU} \times 100$$

ET= enzyme activity in treated plants

EU= enzyme activity in untreated plants

b - Pectin methyl sterase (PME):

Pectin methyl esterase was determined using the method described by Abo- Elyousr(1998). The reaction mixture was as follows: 3 ml of plant extract and 20 ml of pure pectin (pH 7). The mixture was incubated at 30°C for 6 hr., and then the pH value was estimated by using pH meter. Control reaction containing 20 ml of 1% pure pectin and 3ml of boild plant extract, and also the pH value was estimated according to the following equation:

Increase in PME =

$$\frac{\text{pH in control samples} - \text{pH in treated samples}}{\text{pH in control samples}} \times 100$$

Statistical analysis

All data were subjected to statistical analysis and means were compared using L.S.D. test (Gomez and Gomez, 1984).

Results

Effect of salicylic acid and oxalic acid on mycelial linear growth of *Fusarium oxysporum* f. sp. *lycopersici,* **in *vitro:***

Data presented in Table 1 and 2 show that the linear growth of the three FOL isolates were inhibited significantly in case of SA at concentrations ranging from 750 to 2000 ppm, while SA at 500ppm showed no effect on mycelial growth. All tested concentrations of OA significantly inhibited the linear growth of FOL where the highest concentration (2000 ppm) was the most effective. The highest inhibition percent in the mycelial growth was observed with FOL isolate 4 when treated by concentration 2000 ppm followed by 1500 and then 1000 ppm with both tested acids.

The percentage of inhibition varied also between the three tested isolates, the highest inhibition occurred in FOL isolate 4 for SA and OA. There is no significant different between FOL isolates 2 and 3 in both tested acids.

Table(1): Inhibition percent of FOL mycelial growth, grown on PDA medium amended with different concentrations of salicylic acid

SA concentration	*F. oxysporum* f.sp. *lycopersici* isolates			Mean
	FOL 2	FOL 3	FOL 4	
0 ppm	0.00	0.00	0.00	0.00
500 ppm	0.00	0.00	1.12	0.38
750 ppm	3.30	2.25	4.37	3.30
1000 ppm	10.00	7.75	12.55	10.10
1500 ppm	19.98	23.30	31.95	25.08
2000 ppm	39.97	33.35	42.75	38.66

L.S.D 0.05

Isolates	1.62
Concentrations	1.88
Interaction	3.26

Table(2): Inhibition percent of FOL mycelial growth, grown on PDA medium amended with different concentrations of oxalic acid

OA concentration	*F. oxysporum* f.sp. *lycopersici* isolates			Mean
	FOL 2	FOL 3	FOL 4	
0 ppm	0.00	0.00	0.00	0.00
500 ppm	3.57	5.47	8.90	5.98
750 ppm	13.30	8.90	31.00	17.73
1000 ppm	24.05	19.40	33.00	25.48
1500 ppm	36.33	38.83	48.60	41.25
2000 ppm	46.33	48.63	61.42	52.13
L.S.D 0.05				
Isolates	1.12			
Concentrations	1.95			
Interaction	3.39			

Effect of SA treatment on disease severity of tomato Fusarium wilt:

Data present in Tables 3 and 4 show that seedling treatment with SA at concentrations 500 and 2000 ppm for two consecutive seasons significantly reduced foliar yellowing percent of tomato plants in both seasons. Vascular browning was signficantly reduced in the plants treated with SA concentrations at 500 and 2000 ppm in summer season 2006, but in winter season 2006/2007, SA treatment at concentration 2000 ppm showed nonsignificantly effect. The results in both seasons exhibited that SA seedling treatment at concentration 500 ppm was more effective than 2000 ppm for controlling the disease.

Table(3): Effect of seedling treatment with salicylic acid on the severity of Fusarium wilt in tomato during summer season 2006

SA concentration	Vascular browning %				Foliar Yellowing %			
	FOL isolates				FOL isolates			
	2	3	4	Mean	2	3	4	Mean
0 ppm	71.88	53.13	18.75	47.92	84.38	62.50	34.38	60.42
500 ppm	43.75	34.38	9.37	29.17	50.00	40.63	18.75	36.46
2000 ppm	53.13	37.50	15.65	35.42	56.25	50.00	21.88	42.71
L.S.D 0.05								
Isolates	8.85			6.83				
Treatment	3.43			8.69				
Interaction	14.62			15.03				

Table(4): Effect of seedling treatment with salicylic acid on the severity of Fusarium wilt in tomato during winter season 2006/2007

SA concentration	Vascular browning %				Foliar Yellowing %			
	FOL isolates				FOL isolates			
	2	3	4	Mean	2	3	4	Mean
0 ppm	50.00	50.00	25.00	41.67	75.00	59.38	40.63	58.33
500 ppm	46.88	37.50	15.63	33.33	53.13	40.63	25.00	39.58
2000 ppm	50.00	46.88	18.75	38.54	62.50	37.50	34.38	44.79
L.S.D 0.05								
Isolates	10.92			6.55				
Treatment	7.63			9.71				
Interaction	13.25			16.8				

Effect of seedling treatment with OA on disease severity of Fusarium wilt of tomato:

Results present in Tables 5 and 6 show that seedling treatment with OA at concentrations 500 and 2000 ppm significantly reduced foliar yellowing and vascular browning percent in tomato plants at the both experimental seasons. The highest disease severity reduction was observed with concentration 2000 ppm and then with 500 ppm, respectively.

Data also show that the fungal isolates were differed in their virulent, the FOL isolate 2 was the most virulent followed by FOL isolate 3 while FOL isolate 4 was the least virulent.

Table(5): Effect of seedling treatment with oxalic acid on the severity of Fusarium wilt in tomato during summer season 2006:

OA concentration	Vascular browning %				Foliar Yellowing %			
	FOL isolates				FOL isolates			
	2	3	4	Mean	2	3	4	Mean
0 ppm	71.88	53.31	18.75	47.92	84.38	62.50	34.34	60.42
500 ppm	50.00	43.75	12.52	35.42	68.75	53.31	28.13	50.00
2000 ppm	46.88	37.50	9.375	31.25	59.38	40.63	21.88	40.63
L.S.D 0.05								
Isolates	7.27			9.84				
Treatment	9.71			9.98				
Interaction	16.84			17.32				

Table(6): Effect of seedling treatment with oxalic acid on the severity of Fusarium wilt in tomato during winter season 2006/2007

OA concentration	Vascular browning %				Foliar Yellowing %			
	FOL isolates				FOL isolates			
	2	3	4	Mean	2	3	4	Mean
0 ppm	62.50	50.00	25.00	45.83	75.00	59.38	40.63	58.33
500 ppm	53.13	43.75	21.88	39.58	65.63	46.88	34.38	48.96
2000 ppm	46.88	34.38	18.75	33.33	53.13	43.75	28.13	41.67

L.S.D 0.05		
Isolates	6.35	12.05
Treatment	6.05	7.29
Interaction	10.54	12.62

Effect of seedling treatment with SA and OA on the enzymes activity in tomato plants

a - Assay of polyphenol oxidase (PPO) activity:

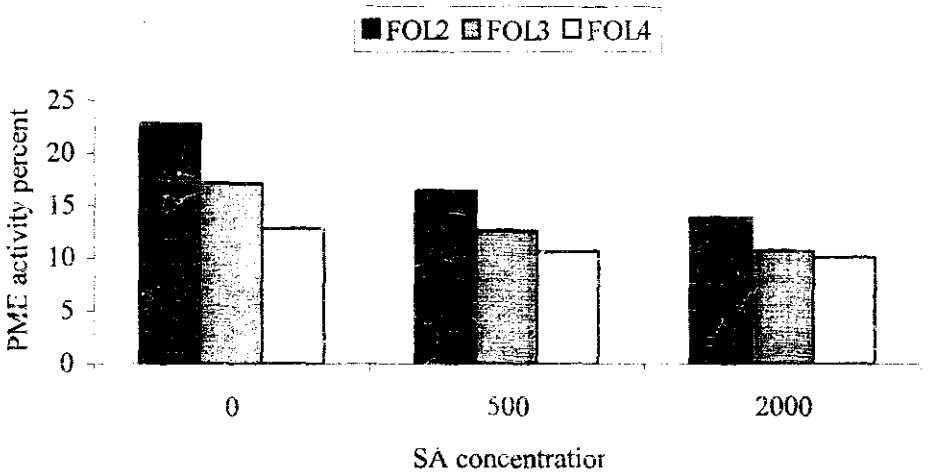
Data in Table 7 indicate that all plants treated with SA or OA and inoculated with FOL isolates exhibited higher increment percent of PPO activity compared with untreated plants. PPO increment percent was increased by increasing concentrations of SA, but plants treated with concentration 500 ppm showed nonsignificant effect compared with plants treated with 2000 ppm. Results also indicate that PPO increment percent was increased by increasing concentrations of OA.

Table(7): Effect of seedling treatment with salicylic acid or oxalic acid on the Polyphenol oxidase activity in infected tomato plants, 20 days after transplanting:

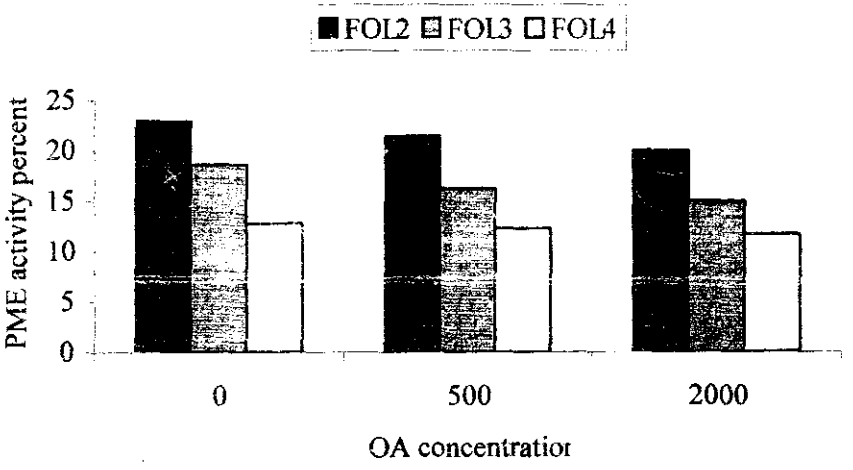
Concentration	SA				OA			
	FOL2	FOL3	FOL4	Mean	FOL2	FOL3	FOL.4	Mean
500 ppm	21.67*	14.12	16.51	17.43	6.76	4.86	2.47	4.69
2000 ppm	20.15	14.88	18.68	17.90	6.91	3.48	7.84	6.07
0ppm (control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* Percent increase in enzyme activity compared with control.

L.S.D 0.05		
Isolates	0.85	0.87
Treatment	1.04	1.31
Interaction	1.80	1.52



Figure(1): Effect of seedling treatment with salicylic acid (SA) on pectin methyle esterase (PME) activity in infected tomato plants, 20 days after transplanting



Figure(2): Effect of seedling treatment with oxalic acid (OA) on pectin methyle esterase (PME) activity in infected tomato plants, 20 days after transplanting

b –Assay of pectin methyl esterase (PME):

Data reported in Figures 1 and 2 indicate that SA and OA decreased percentage of pectin methyle esterase activity in treated tomato plants compared with untreated plants (0 ppm). Data also show that PME activity was decreased by increasing applied chemical concentrations. For all tested isolates, PME were produced in plant tissues, the PME concentrations were ranged from 10.1 % in 2000 ppm in SA treated plants to 22.8 % in untreated plants (0 ppm) and ranged from 11.7 % in OA treated plants to 22.9 % in untreated plants (0 ppm).

Discussion

Using chemicals to induce resistance against plant pathogens have been reported previously (Yalpani *et al.*, 1991, Abo-El yousr *et al.*, 2005 and Asran 2005). Results reported herein indicated that SA and OA significantly inhibited the linear growth of *Fusarium oxysporum* f. sp. *lycopersici*. Also, increase of the concentrations had significantly inhibitory effect on the fungal growth. These results are in agreement with Mahmoud (2005) who found that application of salicylic acid and ascorbic acid in *vitro* significantly inhibited the mycelial growth of *Fusarium oxysporum.*

Seedling treatment with SA at 500 and 2000 ppm significantly reduced the foliar yellowing percent on tomato plants but SA seedling treatment with concentration 500ppm was more effective than 2000 ppm.Other reports mentioned that the high concentrations of SA may have toxic effect on the plant (Van Loon, 1997).

On the other hand OA treatment significantly reduced the disease symptoms and the highest disease severity reduction was observed with concentration 2000 ppm followed by 500 ppm. The mode of action of SA or OA on suppression of disease incidence might be due to their direct effect against the pathogen or its role in induction of resistance. SA- mediated resistance is restricted in only treated tissue that may due to encourgment the creation of β –glucosidase which have a lack of ability to phloem mobility (Enyedi and Raskin, 1993 and Kessmann *et al.* 1994). Many researchers suggested that SA and OA could induce systemic resistance in plants, inhibit catalase enzyme, enhance the PR gene expression in plants and synthesis of expressior. of chitinases which could hydrolyze the wall of many fungi (Dempsy and Klessig, 1994, Narusaka *et al.*, 1999 and Davis *et al.*, 2002) or induction the hypersensitivity response (HR) and expression of some defense genes including genes encoding to

pathogenesis-related PR proteins (Cronje and Bornman, 1999).

As markers of resistance, biochemical changes always appear at certain intervals after application of the inducers agents (Rayls *et al.*, 1996). This study investigates the effect of SA and OA on polyphenol oxidase (PPO) and pectin methyl estrase (PME) in tomato plants inoculated by *Fusarium oxysporum* f. sp. *lycopersici*.

Data reported herein indicate that SA significantly increased increment percent of PPO activity and significantly decreases PME activity in treated tomato plants compared with untreated plants. The importance of PPO activities in disease resistance probably comes from its property to oxidize phenolic compounds to quinines, which are often more toxic to microorganisms than the original phenol (Hammerschmidt, 1999). The activity of PPO was generally higher in infected tissue of resistant varieties than in infected susceptible ones (Abo – Elyousr, 1998). Ferrar and Walker (1993) reported that oxalic acid has inhibitory effect toward activity of diphenol oxidase. Also, an increase in peroxidase and polyphenol oxidase was detected as a results of infection with many pathogens (Clark *et al.*, 2002) or as a result of treatments with different antioxidants (Takahama and oniki, 1994)

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فاعلية إستخدام حمض السلسليك وحمض الأوكساليك فى مقاومة مرض الذبول الفيوزاريومى فى الطماطم

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أجريت هذه الدراسة لمعرفة تأثير حمض السلسليك أو حمض الاوكساليك فىمقاومة فطر *Fusarium oxysporum* f.sp. *lycopersici* المسبب لمرض الذبول الفيوزاريومى فى الطماطم .

معمليا أظهرت النتائج التأثير المثبط لحمض السلسليك على النمو الطولىلثلاث عزلات مختبرة من الفطر بداية من تركيز ٧٥٠ الى ٢٠٠٠ جزء فى المليون حيث وصلت نسبة التثبيط فى النمو الميسلسومى الى ٣٨,٦٦% عند المعاملة بتركيز ٢٠٠٠ جزء فى المليون . وكذلك ثبت أيضا معمليا التأثير المثبط لحمض الاوكساليك على نمو الفطر بداية من تركيز ٥٠٠ – ٢٠٠٠ جزء فى المليون حيث وصلت نسبة التثبيط فى نمو الفطر الى ٥٢,١٣% عند تركيز ٢٠٠٠ جزء فى المليون .

تحت ظروف الصوبة تم دراسة تأثير غمر شتلات الطماطم صنف بريتشارد فى حمض السلساليك أو حمض الاوكساليك بتركيزات ٥٠٠ أو ٢٠٠٠ جزء فى المليون خلال عروتى الزراعة (الصيفى ٢٠٠٦م ، الشتوى ٢٠٠٦/٢٠٠٧م) قد أظهرت النتائج أنخفاض معنوى فى شدة أصابة النباتات للمرض عند أستخدام هذه المواد وقد زادت مقاومة النباتات للمرض بزيادة تركيز حمض الاوكساليك من ٥٠٠–٢٠٠٠ جزء فى المليون بينما أظهر التركيز الاقل (٥٠٠ جزء فى المليون)فاعلية أكبر من ٢٠٠٠ جزء فى المليون وذلك فى حالة غمر الشتلات فى حمض السلساليك .

تم دراسة تأثير المعاملات السابقة على النشاط الانزيمى فى نباتات الطماطم حيث أظهرت النتائج زيادة معنوية فى نشاط أنزيم البولى فينول أوكسيديز وإنخفاضا معنويا فى نشاط إنزيم البكتين ميثيل إستريز فى النباتات المعاملة مقارنة بالنباتات الغير معاملة بالمواد المختبرة وذلك بعد ٢٠ يوم من الشتل.