Induction of systemic resistance in tomato plants against *Fusasium* wilt disease by salicylic acid .

تحفيز المقاومة الجهازية في نباتات الطماطة ضد مرض الذبول الفيوزارمي باستخدام حامض الساليسااليك .

*Waheed. A.Q., *H.H NAWAR, *B.A. Abbas and *M.I.Abdulla.
*Ministry of Science&Technology/Agricultural Research Directorate
Iraq / Baghdad.

Abstract

This study was conducted to evaluate the efficiency of salicylic acid as chemical inducer to stimulate tomato plant resistance against *Fusarium* wilt disease caused by the phytopathogenic fungus *Fusarinm oxysporum* under laboratory , greenhouse and field conditions. Also, the study was led to select the active concentrations of salicylic acid in inhibition the fungus growth (*Fusarium oxysporum*) , three concentrations were studied (50,100 and 200) ppm .

The results showed that tomato seed soaked in multi concentrations from salicylic acid led to inhibition the radial growth and the dry weight of biomass and the number of spores of pathogen , 200 ppm concentration showed the best results which recorded 15 mm,0.18g,$4.66x10^6$ spore/ cm^2as compared to control treatment(0.0 ppm concentration , 43.33 mm , 0.27g, 28.50 x 10^6 spore / cm^2 respectively.

The results of tomato seed treatment with salicylic acid led to a significant increment in percentage of seeds germination, plant height , fresh and dry weight of plant as compared to control treatment under greenhouse conditions . 200 ppm of salicylic acid treatment recorded 94.6%, 54.6 cm, 65.33 g/ plant, 29.0 g / plant respectively , while control treatment recorded 81.6%,42.7 cm,16.3g / plant , 8.4 g/plant respectively .

The results showed that tomato seeds treatment in different concentrations of salicylic acid 50,100,200 ppm gave a significant increment in most tested growth parameters under greenhouse conditions (plant height, root length. no. of leaves /plant , no. of flowers /plant , no. of fruits / plant , average of fruits weight / plant, 200 ppm of salicylic acid treatment significantly increased on the following parameters average which recorded 54.33, 27.33 cm, 14.0 leaves / plant , 11.67 flowers / plant , 3.83 fruits / plant , 62.33 g/ plant) respectively , while control treatment recorded 42.73, 9.93 cm , 6.97 leaves /plant, 3.10 flowers / plant , 1.20 fruits / plant , 16.33 g / plant respectively.

The results of tomato seedlings treatment with salicylic acid led to increment in most the tested growth parameters under field conditions plant height, root length, no. of branches / plant , no. of fruits / plant , average of fruits weight / plant , fresh and dry weight of plant and disease severity as compared to other treatments .

Key words: Systemic resistance , *Fusasium* wilt , Salicylic acid , Tomato .

الخلاصة

اجريت هذه الدراسة لغرض تقييم كفاءة حامض الساليسااليك كمحفز كيمياوي لتحفيز مقاومة نباتات الطمامة ضد مرض ذبول الفيوزارمي المتسبب عن الفطر الممرض *Fusarium oxysporum* تحت الظروف المختبرية والبيت المحمي والحقل . كما هدفت الدراسة الى تحديد التراكيز الفعالة لحامض الساليااليك في كبح وتثبيط نمو الفطر *Fusarium oxysporum* حيث درس تأثير ثلاث تراكيز هي 200,100,50جزء بالمليون . اظهرت النتائج ان نقع بذور الطماطة بتراكيز مختلفة من حامض الساليااليك قد ادى الى تثبيط النمو والوزن الجاف للكتلة الحيوية وعدد السبورات للفطر الممرض ، وقد اعطى التركيز 200 جزء بالمليون افضل النتائج حيث سجل 15ملم ، 0.18 غم ،10^6x4.73سبور / سم^2 بالمقارنة مع معاملة المقارنة التي سجلت صفر جزء بالمليون 43.33 ملم ،0.27غم ، 10^6x28.50 سبور/ سم^2 وعلى التوالي كما بينت النتائج ان معاملة بذور الطماطة بحامض الساليساليك ادى الى زيادة معنوية بنسبة انبات البذور وارتفاع النبات والوزن الطري والجاف للنبات بالمقارنة مع معاملتي السيطرة تحت

ظروف البيت المحمي . تفوقت المعاملة 200 جزء بالمليون من حامض الساليسايك معنوياً على بقية التراكيز المختبرة حيث حققت 94.6% ، 54.6 سم ، 65.33 غم /نبات ، 29.0 غم/نبات وعلى التوالي في حين حققت معاملة السيطرة 81.6%، 42.7 سم، 16.30 غم/نبات ،8.40 غم/ نبات وعلى التوالي.

كما بينت النتائج ان معاملة البذور بالتراكيز المختلفة لحامض الساليسايك 50 ,100 ، 200 جزء بالمليون ادت الى زيادة معظم معايير النمو المدروسة تحت ظروف البيت المحمي ارتفاع النبات ، طول الجذر، عدد الاوراق / نبات ، عدد الازهار / نبات ، عدد الثمار / نبات ، معدل وزن الثمار/نبات وقد تفوقت المعاملة 200 جزء بالمليون من حامض الساليسايك معنوياً على بقية المعاملات حيث حققت 27.33,54.33 سم ، 14.0 ورقة نبات ، 11.67 زهرة / نبات ، 3.83 ثمرة/ نبات ، 62.33 غم / نبات وعلى التوالي في حين حققت معاملة السيطرة 42.73 ، 9.93 سم ، 6.97 ورقة /نبات ، 3.10 زهرة / نبات ، 1.20 ثمرة /نبات ، 16.33 غم / نبات وعلى التوالي . كما اشارت النتائج ان معاملة بادرات الطماطة بحامض الساليسايك ادت الى زيادة معظم معايير النمو المختبرة تحت الظروف الحقلية (ارتفاع بالنبات ، طول الجذر ، عدد الافرع /نبات ، عدد الثمار / نبات ، معدل وزن الثمار و الوزن الطري والوزن الجاف للنبات وشدة الاصابة) بالمقارنة مع بقية المعاملات.

كلمات مفتاحية:Tomato , Salicylic acid , *Fusarium* wilt , Systemic resistance

Introduction

Fusarium wilt , caused by *Fusarium oxysporum* is a widespread disease that occurs in most tomato growing areas (1) . Tomato *Lycopersion esculentum* Mill.is one of the most popular and widely consumed vegetables all over the world (2). *Fusarium* wilt has reduced the tomato production in many areas which cause yield reduction of up to 25% (3). Many strategies for controlling *Fusarium oxysporum* have been introduced over the years e. g. soil culture practices, fungicide treatments etc., but serious losses still occur , largely because the effectiveness of these approaches is variable and often short lived (4). Agrochemicals are commonly used to control this pathogen , but serious losses still occur, largely became the effectiveness of these approaches is variable and of ten short lived (4) . Agro chemises are commonly used of control this pathogen, but are reelbut are relatively expensive and subjected to various environmental constrains . The use of tomato resistant cultivars is one of the most practical and cost efficient strategies for controlling tomato wilt disease (5). The phenomenon of induced resistance has reinforced the potential for pesticide – free disease control strategies (6). Induced plant defense mechanisms involve phytohormones such as salicylic (SA) acid . The role of SA in plant defense against pathogen attack and been elaborately documented . The SA has been used successfully to induced resistance to a wide range of diseases on field crops , tomatoes (2). Chickpea (7), soybean (8), wheat (9) cucumber (10 , 11) .

The present study investigated the role of salicylic acid in basal defiance of tomato plant against *Fusarium oxyspoum* and salicylic acid application can induce resistance to *Fusarium oxysporum* in tomato plants under laboratory , greenhouse and field conditions.

Materials and Methods

Plant materials and growing conditions.

Tomato C. V. Marmand impaired for SA accumulation were grown in sand – clay soil . Tomato seedlings at the five true leaf stage were transplanted to field conditions. Tomato seeds were treated with salicylic acid (SA) at 50 , 100 and 200 ppm for controlling tomato wilt disease caused by the pathogen *F. oxysporum* under artificial inoculation . Tomato seedlings were soaked in the tested solutions for 6 hrs. 100 seedlings / 100 ml test solution .

Fungal pathogen (*Fusarium oxysporum*) :

One isolate of fungal pathogen (*Fusarium oxysporum*) was obtained from Department of Biocontrol for Plant Diseases at Agricultural Researcher Office / Ministry of Science and Technology was used in this study .

Effect of salicylic acid on growth , dry weight of biomass and sporulation of *Fusarium oxysporum* In vitro

Salicylic acid (SA) was used at 50,100 and 200 ppm for studying their effects on liner growth , dry weight of biomass and sporulation of the tested pathogen (*Fusarium oxysporum*) under laboratory conditions .

Linear growth :

Flask 250 ml containing 20 ml potato dextrose agar medium (PDA) were prepared and amended with each concentration of SA , then each flask was poured in sterilized petri dishes 10 cm diam. . In control treatment , non- amended dishes with salicylic acid was used. Dishes were inoculated in the center with disks 5 mm in diam. of the tested pathogen and incubated at 25°C for 7 days . Linear growth of pathogen in treatments was measured and compared to control treatment .

Dry wright of pathogen biomass :

A disk 5mm in diam. from edge of 7- days culture of pathogen was cut and added into 250 ml flasks containing 50 ml of potato dextrose broth medium amount with the tested inducer chemical (SA) . After 7 days of inoculation , the culture medium was passed through filter paper and fungal mass was dried at 80 °c for 24hrs. then the fungal biomass were weighed . Three replicates were used in this study .

Sporulation :

Spore production was estimated after measuring the fungal linear growth on solid medium as follows: one disk 5mm, diam. from each fugal culture , for each treatment was placed in 1 ml distilled water in tube . Tubes were shaken for 2 min. , then kept for 1 hrs. ,haemocytometer slide was used for counting the spores , the number of spores was counted in 16 squares (1/400 mm2) , chosen at random and the average of three slides was calculated (12).

Application of salicylic acid:

Different concentrations 50,100 and 200 ppm of salicylic acid (SA) production of Sigma were prepared in sterilized distilled waters . SA was applied three times 100 ml/ pot at an interval of 5 days and the first application was done when tomato plants were 25-day old .

Effect of tomato seed soaking in salicylic acid on germination , seedling growth under greenhouse conditions .

Healthy tomato seeds were soaked in the tested SA solution at different concentrations 50,100 and 200 ppm for 6 hrs. , then sown in plastic pots 5 kg containing sterilized sand – clay soil . Ten seeds sown in each pot and three pots were used for each test as replicates . After 45 days, Percentage of seed germination , seedling height , fresh and dry weight of plants were recorded. Also, ten seeds treated with distilled water were sown in pots containing soil free or infested with the pathogen (*F. oxysporum*) as control treatment . Percentage of wilt disease were recorded of the 6 weeks after planting .

Effect of seed soaking in salicylic acid on Disease resistance and some growth parameters under field conditions.

Healthy seedlings were removed from pots and soaked in the tested solution of SA(50,100 and 200) ppm for 1 hr.and planted in holes at rate (2 seedlings / hole) on 2 / 10 / 2012 and remained till 30 / 11 / 2012 . The ridges were covered with nylon from 12 November 2012 to 30 /11/ 2012 . Treatments were distributed in field according to Randomized Complete Blocks Design (RCBD) in three replicates (each replicate 10 plants) to evaluate the following treatments :

1-Control . 2-Pathogen (*F. oxysporum*) only. 3-50 ppm of SA. 4-100 ppm of SA. 5- 200ppm of SA .6-50ppm of SA +Pathogen. 7- 100ppm of SA + Pathogen . 8- 200ppm of SA + Pathogen.

Plant were harvested at the end of the experiment ,and the growth parameters (plant height , root length , no. of branches / plant , no. of fruits / plant , average of fruits weight / plant , fresh and dry weight of plant and disease severity) were recorded .

Statistical analysis

The experiments were conducted and analyzed as factorial with three replications using a Completely Randomized Design (CRD) for laboratory experiments , and a Completely Randomized Block Design (CRBD) for greenhouse and field experiments . The mean values were compared by using LSD test at probability of 5 % ($p \leq 0.05$) .

Results and Discussions

Data in table 1 showed that the highest inhibitory effect was noticed when 200 ppm of SA was added to liquid and solid medium. The growth parameters of *F. oxysporum* linear growth and dry weight were decreased to 15.0 mm and 0.18 g in 200 ppm of salicylic acid treatment while the control treatment (0.0 ppm of SA) recorded 43.33 mm and 0.27 g respectively , the lowest inhibitory effect of *F. oxysporum* at 50 ppm which recorded 32.67 mm and 0.24 g . SA greatly inhibited spore formation of *F. oxysporum* , the highest effect was clear of SA at 200 ppm which reduced the spore formation to 28.50×10^6 / cm^2 , while in control to 4.73×10^6 / cm^2 (table 1) .

Table (1) : Impact of salicylic acid on linear growth , dry weight of biomass and sporulation of *Fusarium oxysporum* under laboratory conditions .

Treatment	Linear growth (mm)	Dry weight (g)	Sporulation $\times 10^{6/} cm^2$
Control	43.33	0.27	28.50
50ppm SA	32.67	0.24	21.20
100ppm SA	23.33	0.21	17.57
200ppm SA	15.00	0.18	4.73
LSD(P=0.05)	3.88	0.01	1.47

Data were presented in table 2 showed that seed soaking in SA at 200 ppm gave the highest seed germination 94.6% followed by SA at 100 ppm 91.3% as compared with 81.6% in control. Also tomato seedlings soaked in SA solution at 200 ppm recorded the highest seedling height 54.6 cm as compared with the positive 42.7cm and negative 17.1cm control treatment (table 2) . On the other hand SA at 200 ppm recorded the highest fresh and dry weight per plant (65.33,29.0) g/ plant respectively as compared with 16.30,8.40g/ plant and 7.73,3.67g / plant for both positive and negative control treatment .

Table (2) : Effect of tomato seeds treatment with salicylic acid on response of seedlings to *Fusarium oxysporum* resistance under greenhouse conditions .

Treatment	Seeds germination %	Plant height cm	Fresh weight g/ plant	Dry weight g/ plant
Control	81.6	42.7	16.30	8.40
Pathogen only	34.3	17.1	7.73	3.67
50ppm SA	88.6	49.6	46.33	15.33
100ppm SA	91.3	50.6	55.33	20.67
200ppm SA	94.6	54.6	65.33	29.0
50ppm SA+P	70.6	25.6	21.0	7.50
100ppm SA+P	80.3	36.5	25.67	8.50
200ppm SA+P	84.3	46.6	49.30	20.33
LSD(P=0.05)	3.75	3.05	5.81	2.60

Data in table 3 showed that all the tested concentrations of SA significantly increased tomato growth parameters plant height , root length, no. of leaves and flowers and fruits per plant and average of fruits weight as compared to control treatment , 200 ppm of SA treatment recorded the highest increment in growth parameters54.33 , 27.33 cm , 14.0 leaf/ plant , 11.67 flower / plant , 3.83 fruit/ plant and 62.33 g / plant respectively as compared to control treatment 42.73 , 9.93 cm , 6.97 leaf/plant , 3.10 flower / plant , 1.20 fruit / plant and 16.33 g / plant respectively (table 3) .

Table (3) : Some of tomato growth parameters treatment with salicylic acid artificial infected by *Fusarium oxysporum* under greenhouse conditions .

Treatment	Plant height cm	Root length cm	No. of leaves / plant	No. of flowers / plant	No. of fruits / plant	Average of fruits weight / plant	Wilt disease %
Control	42.73	9.93	6.97	3.10	1.20	16.33	1.53
Pathogen only	17.17	6.33	2.43	0.0	0.0	0.0	69.33
50ppm SA	49.67	21.67	7.80	6.50	2.36	39.0	-
100ppm SA	50.67	24.33	10.67	11.0	3.16	42.33	-
200ppm SA	54.33	27.33	14.0	11.67	3.83	62.33	-
50ppmSA+P	25.67	18.33	6.53	4.20	2.10	35.67	42.67
100ppmSA+P	36.53	20.33	7.83	4.77	3.50	39.0	28.33
200ppmSA+P	46.67	22.33	9.67	8.20	3.83	40.67	14.67
LSD(P=0.05)	3.05	1.86	1.87	1.47	0.61	1.99	2.24

Data in table 4 showed that all the tested treatment significantly increased the tested tomato growth parameters under field conditions plant height , root length , no. of branches / plant , no. of fruits / plant , average of fruits weight / plant , fresh weight and dry weight of plant per plant and decreased the disease severity as compared to both positive and negative control treatment . The highest increment was recorded at 200 ppm of SA and 100 ppm of SA treatments which recorded 89.67 , 81.67 cm , 38.67 , 36.67 cm , 7.67 , 7.33 branches / plant , 21.67 , 15.33 fruits / plant , 145.7 , 99.7 g / plant , 622 , 529 g / plant and 166.3 , 135.7 g / plant respectively as compared to both positive and negative control treatment 46.67 , 29.33 cm , 23.33 , 15.67 cm , 3.67 , 1.33 branches / plant , 5.67 , 0.83 fruits / plant , 81.3 , 23.3 g / plant , 204 , 112 g / plant and 59.7 , 28.3 g / plant respectively (table 4) . These increases in growth, yield quantity and quality may be attributed to elicitors effect on physiological processes in plant such as ion uptake , cell elongation , cell division , enzymatic activation and protein synthesis (14; 15). (16)reported that it has been proposed that salicylic acid acts as endogenous signal molecule responsible for inducing abiotic stress tolerance in plants.

Table (4) : Effect of tomato seedlings treatment with salicylic acid on some growth parameters artificially infected by *Fusarium oxysporum* under field conditions .

Treatment	Plant height cm	Root length cm	No of branches /plant	No. of fruits /plant	Average of weight fruits	Plant Soft weight g/plant	Plant dry weight g/plant	Disease severity %
Control	46.67	23.33	3.67	5.67	81.3	204	59.7	1.07
Pathogen only	29.33	15.67	1.33	0.83	23.3	112	28.3	48.23
50ppm SA	79.33	34.33	6.67	13.33	93.7	390	119.0	-
100ppm SA	81.67	36.67	7.33	15.33	99.7	529	135.7	-
200ppm SA	89.67	38.67	7.67	21.67	145.7	622	166.3	-
50ppm SA+P	69.33	27.67	2.33	10.33	54.3	319	89.0	26.67
100ppm SA+P	76.67	29.33	4.33	11.67	85.3	349	99.3	18.33
200ppm SA+P	23.33	30.33	4.67	13.33	96.7	490	141.7	11.33
LSD(P=0.05)	5.53	2.64	1.17	4.81	17.35	150.40	39.52	2.21

Results of the present studies demonstrated that seeds treatment with SA induced systemic resistance in tomato plants , these findings were in line with earlier studies of tomato plants, where plants developed a systemic induced resistance to *F. oxysporum* infection in response to SA application given by seed treatment (2,13) . (13) noticed that application of SA induced the phenolic accumulation content and defense enzymes in tomato plants infected with *F. oxysporum* .

Treatments that reduced disease severity also increased fresh and dry weight of plants and seed germination . These observation are similar with the findings of (2) where SA reduced severity of the tomato wilt as well as fungal growth and spore formation of *F. Oxysporum* , and increased seed germination and seedling height , fresh and dry weight .

Chemically induced resistance (IR) of plants against pathogens is a widespread phenomenon that has been investigated with respect to the underling signaling pathways as well as to its potential use in plant protection. Elicited by a local infection, plants respond with a salicylic acid dependent signaling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance that is efficient against fungi, bacteria and viruses (17). The tested chemical inducer (SA) might stimulate some defense mechanisms such as phenolic compounds, oxidative enzymes and some metabolites(8; 18).

It could be suggested that SA used as seed soaking or seedling soaking could be used for controlling wilt disease of tomato plants since they are safe , low cost and effective against the disease .

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