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EFFICACY OF SYSTEMIC RESISTANCE INDUCERS ON TOMATO FUSARIUM WILT

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ABSTRACT: ***This research was carried out to evaluate the efficiency of chitosan, benzoic acid, salicylic acid and catecol in vitro either linear growth or percentage of inhibition of Fusarium oxysporum f. sp. lycopersici (Fol) the causal pathogen of tomatoes wilt. Also, their effect on disease severity was tested in vivo under greenhouse conditions in order to enhance efficacy of systemic induced resistance (SIR) to such disease. Data obtained showed that resistance inducers at all tested concentrations significantly reduced the mycelium growth of Fol in vitro. The most effective resistance inducers in suppress fusarium wilt of tomato were chitosan and catecol followed by salicylic acid while Benzoic acid was less effective against the disease under greenhouse conditions. However, disease control values were reduced substantially when inducers were applied at the rate of 3g/L water. Tomato cultivars differ in their reaction to fusarium wilt infection showing that Nirouz F1 hybrid was more susceptible but Elotts (E448) hybrid was more resistance as well as Shifa (GS558) F1 hybrid and Cultivar GS12F1were moderately resistant. The maximum increase in the activity of oxidative enzymes peroxidase and polyphenoloxidase due to plant application with chitosan was obtained while using Benzoic acid reduced these activities to a minimum.***

Key words: ***Tomato, fusarium wilt, Fusarium oxysporum f. sp. lycopersici (Fol), systemic induced resistance (SIR), chitosan, benzoic acid, salicyclic acid (SA), catecol, peroxidase, polyphenoloxidase.***

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most popular and widely consumed vegetables all over the world and ranked as the main vegetable crop in Egypt. The tomato growing area was 32% of the total vegetable growing area, represented 35 % of total vegetable production during 2006 (Anon., 2006). It is well accepted that tomato production must be increased considerably in the near future to meet the demands of human population increase. Tomatoes yield is drastically reduced by many fungal diseases. Fusarium wilt caused by *Fusarium oxysporum* f. sp *lycopersici* is one of the most important diseases of tomatoes. Disease control is provided by chemical, biological and cultural methods but chemical control is very expensive and environmentally undesirable. In addition, the pathogen has shown resistance against some fungicides. There is a growing need to

develop alternative approaches for control of soil borne diseases, one approach is being actively pursued involves the use of bioactive substances (Benhamou *et al.* 1994) among of them is chitosan. That led the scientific activities toward systemic induced resistance as a viable method to protect the plants against pathogen using safety substrates. Different studies were subjected to this attitude (Malamy and Klessing, 1992, Rabea *et al*. 2003, Sharaf and Farrag, 2004 and Nawar, 2005).

Many different inducers *i.e.* 3-aminobutyric acid and methyl jasmonate induced resistance of tomato against wilt diseases (Janjun *et al.* 1996). Benzoic acid, salicylic acid and ascorbic acid have significantly reduced damping off of tomatoes when used as a soil drench and they were more effective than tolclofos-methyl (Shahda 2000). Foliage sprays with chitosan, and hydrolysates and menadione sodium bisulfite at the rate of 0.25 ± 0.05 g/litre, respectively suggesting that the induction of systemic resistance plays a major role as defense mechanism of tomato against *Fol*. Both peroxidase and beta-1,3-glucanase activities did respond to both stimuli. Stimulation of peroxidase activity may be recorded 72 h after inoculation. Salicylic acid completely inhibited the mycelial development of Fol *in vitro* at concentrations from 0.6 to 1.0 mM and suppresses fusarium wilt in infected tomato plants (Ozgonen *et al.* 2001). Chitosan at 0.3 and 1.0% used as seed treatment showed very strong inhibitory effects in terms of both symptom expression and plant mortality. In response to infection, treated susceptible plants recorded higher levels of increases in terms of polyphenol oxidase [catechol oxidase], peroxidase and phenylalanine ammonia-lyase activities (Chowdhury and Sinha, 2000). Amendment of PDA with microcrystalline chitosan even at 1000 micro g/ml only slightly inhibited the linear growth of *F. oxysporum*. Also it suppressed the development of *F. oxysporum* f. sp. *dianthi* in peat. Drenching chitosan at concentrations of 500 or 1000 micro g/ml effectively controlled fusarium wilt development and decreased the spread of the pathogen in plant vessels of carnation (Orlikowski and Skrzypczak, 2002).

The objective of this research is to determine and elucidate, *in vitro* and under greenhouse conditions, the inhibitory effect of four resistance inducers on fusarium wilt disease and the impact of this treatment on activity of oxidase enzymes in the treated plants.

MATERIALS AND METHODS

The causal fungus was obtained from tomato seedling showed wilt symptoms, maintained on PDA medium and identified according to Nelson *et al*. (1983). Efficiency of different concentrations of chitosan, benzoic acid, salicylic acid and catechol was tested *in vitro* on the growth of the causal pathogen of tomato fusarium wilt. Also, the effect of products was tested *in vivo* applications under greenhouse conditions.

1. *In vitro* experiment

Three concentrations of compounds from 50, 70 and 100 ppm were individually incorporated in 100 ml of PDA media and poured in Petri dishes. The plates were inoculated with 5mm diameter agar disc containing the fungus. Five plates were used for each concentrations and control. Then, plates were incubated at 25C. Diameters of mycelial colony of Fol were measured daily until control Petri dishes were covered with Fol during the incubation period after 7 days.

2. *In vivo* experiment

To determine the effects of compounds on disease severity of tomato fusarium wilt caused by *Fol*, ten pots contained sterilized soil consisted from sandy soil and peatmoss (3:1 v/v) mixture were used for each tested concentrations. The pot soil was infested with Fol spore suspension at the concentration of $3x10^6$ spore/ml. Ten days period to transplanting 5 tomato seedlings 30 day old of hybrids Elotts (E448), Shifa (GS558) F1, Nirouz F1 and GS12F1. Water solution of compounds was prepared at concentration of 2g. /L and 3g. /L. They were individually added to tomato seedlings grown at the rat of 100 ml/ plot treatments after 10, 20 and 30 days of transplanting. Therefore, 50 plants were sprayed with each tested concentrations of tested substrates then kept under greenhouse conditions for 30 days. In addition, non-inoculated control with the same number of pots was included. Evaluation of disease severity was carried out according to disease symptoms on leaf, vascular discoloration and wilting using the scale proposed by Kesevan and Chounhury (1977) as follows:

0. **No wilting symptoms.**
1. **Plant tightly wilted vascular discoloration only in main root.**
2. **Plant moderately wilted, yellowing of old leaves, spreading of vascular browning.**
3. **Plant severely wilted, dying of all leaves except end leaves.**
4. **Dead plants, seedling entirely wilted.**

The root systems of treated tomato seedlings were removed from infested and control soil, washed in tap water, then the disease severity was calculated using disease scale values. Data obtained were statistically analyzed as completely randomized experimental design, and then tabulated.

3-Determination of oxidative enzymes

Roots of healthy and diseased plants were harvested at different periods after inoculation, by cutting them at the leaf base level for determining the activity of peroxidase and polyphenoloxidase. Enzyme extractions from the roots of inoculated and Non-inoculated plants were prepared as recommended by Maxwell and Bateman (1967). Peroxidase activity was determined according to the method described by Allam and Hollis (1972).

The activity of phenoloxidase was measured by the colorimetric method of Maxwell and Bateman (1967).

RESULTS AND DISCUSSION

Fusarium wilt diseases, caused by pathogenic formae speciales of the soil inhabiting fungus *F. oxysporum* can cause severe losses in a wide variety of crop plants. On tomato the pathogen *F. oxysporum* f. sp. *lycopersici* caused a vascular wilt. For this reason, the most viable method is to protect the plants against pathogen through inducing resistance was used. Consequently, the recent study was enhanced the efficacy of systemic induced resistance on tomato to fusarium wilt *in vitro* and *in vivo*.

1. *In vitro* experiment

Data in Table (1) show the inhibitory effect of inducers on the development of *Fol*. Linear growth increased with the increasing the concentration. Data showed that on the seventh day, the mean of the colony diameter of control plates was 8.15cm. Obtained results revealed that all tested concentrations significantly reduced the linear growth of *Fol* mycelium. Also, increasing the tested concentration of 100 ppm increased the reduction of mycelial growth of *Fol*. Chitosan was the best effective on reduction of colony diameter of *Fol* at concentration 100 ppm followed by Salicylic acid. On the other hand, Catechol was less effective in reduction of colony diameter of *Fol* while Benzoic acid was the least effective one in this respect.

Induced systemic resistance is the phenomenon by which the plant can utilize its specific mechanism to increase the level of resistance. Abiotic inducers include natural or systemic chemicals such as catechol, ethephon, salicylic acids, phosphate and benzoic acid. The nature of this compound is the key to its antifungal properties. Chitosan might have an effect on the synthesis of certain fungal enzymes (Lyon *et al.* 1995 and Lafontaine and Benhamou, 1996). Similar results were also reported by other investigators (Bautista *et al.* 2003; Rabea *et al.* 2003 and Nawar, 2005). The fungicidal activity of chitosan against many fungal pathogens is obtained in the field as well as in the laboratories. Generally the level of inhibition of fungi is highly correlated with chitosan concentration (Chowdhury and Sinha, 2000 and Orlikowski and Skrzypczak, 2002).

Table (1): Effect of different inducers with three concentrations on mycelial linear growth of *Fusarium oxysporum* f. sp. *Lycopersici*, 7 days after incubation at 25± 2C.

Inducers	(ppm)	Colony diameter (cm)	Mycelial growth Inhibition %
Chitosan	50	5.50	37.85
	70	4.00	54.80
	100	3.40	61.58
Benzoic acid	50	6.50	26.00
	70	6.65	24.85
	100	6.10	31.07
Salicylic acid	50	6.00	32.20
	70	6.20	29.94
	100	5.80	34.46
Catechol	50	7.9	10.73
	70	7.15	19.20
	100	6.25	29.37
Control		8.85	0.0

L.S.D. % of Inducers (I) = 0.08
Concentration (C) = 1.00
Interaction (I x C) = 0.22

*Percentage inhibition = (C-T) / C x 100, Where, C = colony diameter (cm) of the control
T = colony diameter (cm) of the test plate.

2. *In vivo* experiment:

The effect of the tested concentrations of different inducer for inducing resistance in tomato plants against fusarium wilt was evaluated after 45 days from treating of the infected plants. Data in Table (2) show that tomato cultivar Nirouz F1 hybrid was more susceptible to fusarium wilt infection but cultivar Elotts (E448) hybrid was more resistance. Shifa (GS558) F1 hybrid and GS12F1 cultivars were moderately resistant. According to the effect of different inducers of resistance on tomato fusarium wilt under greenhouse conditions, it is clear that chitosan was the most effective one on the disease followed by Catechol. The least effective inducer was benzoic acid. It was also obvious that using the studied induce resistance at the rate of 3g/L was more effective than using the rate of 2g/L.

Table (2): Effect of different Inducers of resistance with two concentrations on fusarium wilt severity of four tomato hybrids.

Inducers	Rate of application	Hybrid / Wilt %			
		Elotts (E448)	Shifa (GS558)	Nirouz	GS12
Chitosan	2.0ml./L	15.50	29.00	53.00	40.00
	3.0ml./L	10.00	20.00	40.00	31.00
Benzoic acid	2.0gm/L	16.50	38.00	67.50	54.00
	3.0gm/L	14.00	33.00	55.50	46.00
Salicylic acid	2.0gm/L	16.50	34.00	59.00	47.00
	3.0gm/L	13.00	27.00	46.00	39.00
Catechol	2.0gm/L	13.50	33.00	51.00	39.00
	3.0gm/L	12.90	24.50	43.90	33.00
control	0.0	18.00	41.00	75.00	63.00

LSD at 5% for Inducers (I) : 0.68
Rate of application (RA) : 0.53
Hybrids (H) : 0.61
(I) X (RA) : 1.85
(I) X (H) : 1.37
(RA) X (H) : 1.00
(I) X (RA) X (H) : 2.37

The nature of these above montioned compounds is the key to its antifungal properties. Chitosan might have an effect on the synthesis of certain fungal enzymes which could elicit defensive responses and regulate plant growth (Lyon *et al*. 1995 and Lafontaine and Benhamou, 1996). Induction of systemic resistance plays a major role in defense mechanism of tomato against *Fol* which suggesting when foliage sprays with chitosan (Lafontaine 1996, Benhamou 1998 and Chowdhury and Sinha, 2000), while Ozgonen *et al.* 2001 found that salicylic acid treatment caused a reduction in the fusarium wilt of tomato. Lyon *et al*. (1995) reported that natural disease resistance mechanisms can be induced in many plant species by pre treatment with elicitors.

3-Activity of oxidative enzymes:

Data in Table (3) showed that peroxidase and polyphenoloxidase activities were higher in the roots untreated of cv. Elotts (E448) hybrid than in cv. Nirouz F1 hybrid. It was also indicated that peroxidase and polyphenol-oxidase activities reached its maximum the roots treated by anti-oxidants especially when using catechol followed by salicylic acid. While using Benzoic acid reduced the mentioned activates to a minimum. These results are in agreement with those obtained by Shalaby *et al.* (2001) and Amaresh *et al.* (2001) found that peroxidase and beta-1,3-glucanase activities did respond to both stimuli. Stimulation of peroxidase activity may be recorded 72 h after inoculation and elicitor treatment increased beta -1,3-glucanase activity.

Table (3): Peroxidase and polyphenolaxidae activities in roots of two tomato cultivars treated with different inducers of resistance

Inducer	Hybrid / Enzymes activity			
	Peroxidase		polyphenolaxidae	
	Elotts (E448)	Nirouz	Elotts (E448)	Nirouz
Chitosan	1.030	1.180	0.072	0.057
Benzoic acid	0.650	0.111	0.016	0.011
Salicylic acid	0.948	0.933	0.025	0.019
Catechol	1.000	1.055	0.062	0.052
control	0.799	0.744	0.025	0.041

Beckman (2000), reported that substantial correlative evidence, which these phenolic-storing cells, produced during normal differentiation and strategically located and kinetically poised in xylem parenchyma tissues, serve as a sensing and defense triggering system. In the event of injury or infection, these cells collapse, triggering chemical oxidation of the phenolics, which serves to lignifying and/or suberize the immediate site of disturbance and, in the process, shifts the indoleacetic acid, ethylene and cytokinins balance. These endogenous phytohormonal reactions sound the alarm and mobilize a periderm-like defense in depth, including induced secondary metabolism, for several centimeters beyond the point of immediate danger. Prachi and Singh (2002) reported that, salicylic acid (SA) was used to induce resistance in the callus cultures of *Zingiber offcinale* against culture filtrate of *F. oxysporum* f.sp *zingiber*, as exogenous application of SA resulted in increased activity of peroxidase and B-1,3 glucanase enzymes in the callus culture.

It could be concluded that induction of induced resistance depends up on interaction of different physiological process and not only on one process. This process such as increase in the activity of oxidative enzymes, increase in the activity of PR-proteins, chitinase and β- 1, 3 Glucanase, also accumulation of phenolic compounds, salicylic acid and antifugal compounds (phytoalexins), This is in agreement with Metwally (2004) and Abedel-Kareem, Eman, 2008).

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كفاءة بعض المستحثات على المقاومة الجهازية للطماطم ضد مرض الذبول الفيوزاريومى

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الملخص العربي

يهدف هذا البحث الى دراسة كفاءة بعض المستحثات مثل الكيتوزان وحمض البنزويك وحمض السلسيلك والكاتيكول فى تثبيط النمو الميسليومى للفطر فيوزاريوم أوكسيسبورم ليكوبيرسيساى المسبب لمرض الذبول الفيوزاريومى على الطماطم فى المعمل. أيضا تم دراسة تحسين كفاة المقاومه الجهازية فى التأثير على شدة المرض تحت ظروف الصوبة.

ثبت معمليا الفعل التثبيطى للمستحثات المستخدمه بجميع التركيزات حيث أظهرت النتائج أنها قد قللت بدرجة معنوية من النمو الميسليومى للفطر فيوزاريوم أوكسيسبورم ليكوبيرسيساى على أطباق بترى. وقد كان أكفأ هذة المستحثات فى تثبيط شدة الأصابة بمرض الذبول الفيوزاريومى على الطماطم تحت ظروف الصوبة هى الكيتوزان والكاتيكول حيث أدت الى زيادة درجة المقاومة يليها فى ذلك حمض السلسيلك وحمض البنزويك حيث أظهرا كفاءة أقل ضد المرض. بينما قلت درجة المقاومه معنويا باستخدام المستحثات بتركيز ٣جم / لتر ماء. أختلفت هجن الطماطم فى رد فعلها ضد الأصابه بمرض الذبول الفيوزاريومى حيث أظهر هجين الطماطم (آلوتس٤٤٨) درجة أعلى من المقاومة للمرض عن الهجين (نيروز) الذى أظهر درجة عاليه من الحساسيه وقد أظهر كل من الهجين (شيفا) والهجين (جى اس ١٢) درجة متوسطة من المقاومة. وقد أظهرت معاملة النباتات بالكيتوزان أعلى نشاط للأنزيمات المؤكسدة بيروكسيديز وبولى فينول أوكسيديز بينما عند معاملة النباتات بحمض البنزويك قل هذا النشاط الى الحد الأدنى.