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EFFECT OF SOME RESISTANCE INDUCERS AND THE FUNGICIDE TELDOR 50% IN CONTROLLING STRAWBERRY GRAY MOULD CAUSED BY *Botrytis cinerea*

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ABSTRACT

The resistance inducers, *i.e.* Ammonium bicarbonate, Bion, Photophor, Oxalic acid and Salicylic acid as well as the fungicide Teldor 50% caused significant reduction in the linear growth of the fungus *Botrytis cinerea* on PDA medium. This reduction was increased by increasing the concentration of these chemicals. Furthermore, the fungicide Teldor 50% was the most efficient in this regard compared with the tested resistance inducers.

Application of the tested chemicals as dipping, spray and dipping + spray resulted in significant reduction in the gray fruit rot caused by *B. cinerea*. This reduction was, somewhat, in the same trend of the reduction in the linear growth. Also, the reduction in the infection by fruit-rot reflected on the obtained marketable fruit yield.

Application both resistance inducers and the fungicide Teldor 50% caused considerable increases to the activity of oxidation reduction enzymes, i.e. polyphenoloxidase, peroxidase and ascorbic acid.

In general, combined treatment was superior in this regard.

Keywords: Strawberry, fruit-rot, gray mould, *Botrytis cinerea*, inducer resistance, fungicides and oxidative reductive enzymes.

INTRODUCTION

Strawberry (*Fragaria xananossa* Duch.) is one of the most important nontraditional vegetable cash crops in Egypt, and the demand has increased for local consumption and exportation. In addition, the exported amount from strawberry fruits could be increased to European as well as Arab countries if some care is taken into consideration, e.g. packing, firmness of the fruits, pesticide residues and fruit rot ... etc.

Under Egyptian conditions, strawberry fruits are highly liable to infection by rot causing fungi, especially *Botrytis cinerea* (Khafagi, 1982; Abada *et al.*, 2002; Saber *et al.*, 2003; Abada *et al.*, 2005 and Khafagi, 2007). However, using fungicides is recommended for controlling fruit-rots, but some of these fungicides has maintain residue level for a long period (Abada *et al.*, 2005), resulting in a great hazard to human health. Therefore, fruits must be produced with low pesticide residue by using integrated pest management and allowing a suitable PHI for degrading to nontoxic substances.

Therefore, this work was planned to control strawberry fruit-rot caused by *B. cinerea* (the most threatening) by using some resistance inducers and compare their efficiency with the fungicide Teldor 50%.

MATERIALS AND METHODS

1. Effect of some resistance inducers and the fungicide Teldor 50% on the linear growth of *Botrytis cinerea:*

Five resistance inducers chemicals, i.e. Ammonium bicarbonate, Bion, Photophor, Oxalic acid and Salicylic acid as well as the fungicide Teldor 50% (fenhexamid) were tested for their inhibitory effect on the growth of *B. cinerea in vitro*. Different concentrations, i.e. 50, 100, 200, 300, 400, 500, 750, 1000 and 2000 ppm were prepared PDA and autoclaved. Meanwhile the fungicide Teldor 50%, was added to PDA medium after sterilization to obtain the above concentrations. The prepared media were poured in 9 mm Petri-dishes and after solidification, the dishes were inoculated with 5 mm discs taken from 7 days-old cultures of a virulent isolate of *Botrytis cinerea* (kindly provided by Vegetable Crops Dis. Res. Dept., Plant Pathol., Inst., ARC). Five dishes were prepared for each concentration and five dishes without any resistance inducer or fungicide were used as control treatment. The dishes were incubated at 20 ± 1°C for 5 days and the linear growth was measured.

2. Effect of some resistance inducers and the fungicide Teldor 50% on the incidence of fruit rots:

Apparently healthy strawberry transplants (cv. Sweet Charlie) were provided by Strawberry Improvement Center, Fac. Agric., Ain Shams Univ. Solutions of the resistance inducers (500 ppm) and the fungicide Teldor 50% (100 ppm)were prepared. Strawberry transplants were divided into two groups. The first group was soaked for 30 min. in the tested inducers and Teldor 50% just before transplanting. The second group was soaked in water only and transplanted. Two transplants were transplanted in each plastic pot (25 cm in diameter) containing Nile silt soil. At the beginning of the flowering stage, strawberry plants previously soaked in the inducers and Teldor 50% were also divided into two groups. The first one left without any additional treatment and the second was sprayed at the beginning of flowering and two weeks later. Spore suspension of *B. cinerea* (1×10^4 spore/ml water) was prepared from 7 days-old cultures and sprayed on strawberry plants one week after each spray of the inducers or Teldor 50%. Also, strawberry plants previously soaked in the inducers and Teldor 50% were sprayed with the spore suspension of *B. cinerea* concomitantly with the two sprays. Unsoaked and unsprayed plants by inducers or the fungicide were also sprayed with *B. cinerea* and left as control treatment.

The fruits were harvested periodically and the rotted ones were counted and the marketable fruits were weighed and the averages were recorded.

3. Determination of oxidative-reductive enzymes:

Three oxidative-reductive enzymes, *i.e.* polyphenoloxidase, peroxidase and ascorbic acid oxidase were determined at the time of fruiting in the strawberry, fruits of the different treatments. Both polyphenoloxidase and peroxidase were determined according to the method described by Fehrman and Dimond (1967). Meanwhile, determination of ascorbic acid oxidase was assessed according to the method given by Maxwell and Bateman (1967).

Statistical analysis

The obtained data were statistically analyzed according to the standard procedures for split design and complete randomized blocks mentioned by Snedecor and Cochran (1967). The means were compared at the 5% level using least significant differences (LSD) according to Fisher (1948).

RESULTS

1. Effect of some resistance inducers and the fungicide Teldor 50% on the linear growth of *B. cinerea*

Table (1) shows that all the tested inducer resistance, *i.e.* Bion, Photophor, Oxalic acid and Ammonium bicarbonate as well as the fungicide Teldor 50% caused significant reduction in the linear growth of *B. cinerea* compared with control treatment. This decrease was increased by increasing the concentration. In addition, the inhibitory effect of the tested chemicals differed greatly. In this regard, the fungicide Teldor 50% was the most effective causing complete inhibition of linear growth at 100 ppm, followed by Oxalic acid and Salicylic acid (500 ppm). Meanwhile, Ammonium bicarbonate was the least efficient one, which caused complete inhibition at 2000 ppm and by Bion and Photophor at 750 ppm.

Table 1: Effects of some chemicals used as inducer resistance and the fungicide Teldor 50% on the linear growth of *B. cinerea*, 5 days after incubation at 20±1°C.

Chemicals	Linear growth at (ppm)									Mean
	50	100	200	300	400	500	750	1000	2000	
Ammonium bicarbonate	90	90	90	87.4	61.0	50.0	28.0	16.6	0.0	57.0
Bion	86.2	71.4	63.6	52.0	34.2	21.4	0.0	0.0	0.0	36.5
Photophor	79.0	62.8	49.2	35.4	23.2	10.2	0.0	0.0	0.0	28.9
Oxalic acid	80.4	69.2	43.6	27.2	12.0	0.0	0.0	0.0	0.0	25.8
Salicylic acid	83.2	70.8	46.2	30.6	18.4	0.0	0.0	0.0	0.0	27.7
Teldor 50%	38.2	14.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.8
Control	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
Mean	65.3	54.0	41.9	33.2	21.2	10.2	4.7	2.8	0.0	

L.S.D. at 5% for:
Chemicals (C) = 2.7
Linear growth (L) = 2.1
C x L = 3.6

2. Effect of some resistance inducers and the fungicide Teldor 50% on fruit rots

Data presented in Table (2) reveal that all the treatments with the different chemicals caused significant reduction in the percentages of fruit-rots caused by *B. cinerea* with significant increase in fruit yield, compared with the control. Moreover, dipping + spray treatment was the most efficient in the overall meansard, being 13.4% fruit-rot and 174.4 g fruit yield/plant. Meanwhile, dipping treatment was the least efficient one, being 19.9% fruit-rot and 159.8 g fruit yield/plant and spray treatment was of intermediate effect, being 14.8% fruit-rot and 172.7 g fruit yield/plant.

It is noticed that the fungicide Teldor 50% was not efficient when used as dipping treatment (13.4% fruit-rot and 198.2 g fruit yield/plant) and opposite results were found when used as spray, being 2.4% fruit-rot and 218.6 g fruit yield/plant and dipping + spray, being 1.8% fruit-rot and 219.4 g fruit yield/plant.

Furthermore, Salicylic acid was the superior resistance inducer, which gave the lowest figures of fruit-rots and the highest fruit yield when used as dipping (15.1% fruit-rot and 172.8 g fruit yield/plant), spray 11.1% fruit-rot and 180.2 g fruit yield/plant and dipping + spray 9.2% fruit-rot and 186.4 g fruit yield/plant. Control treatment (untreated plants) recorded 41.2% fruit-rot and 86.3 g fruit yield/plant.

Table 2. Effects of dipping, spray and Dipping + spray strawberry plants using some inducer resistance and the fungicide Teldor 50% on the infection by fruit-rot caused by *B. cinerea* and fruit yield, greenhouse experiment.

Chemicals compounds*	% Fruit-rots			Mean	Marketable fruit yields (g)/plant			Mean
	Dipping	Spray	Dipping + spray		Dipping	Spray	Dipping + spray	
Ammonium bicarbonate	18.6	12.8	10.8	14.1	159.0	178.6	181.0	172.9
Bion	17.2	12.0	10.5	13.2	164.2	180.0	183.0	175.7
Photophor	16.8	11.8	10.0	12.9	168.0	182.0	183.2	177.7
Oxalic acid	16.2	12.0	10.0	12.7	170.6	183.0	181.8	178.5
Salicylic acid	15.1	11.1	9.2	11.8	172.8	180.2	186.4	179.8
Teldor 50%	13.4	2.4	1.8	5.9	198.2	218.6	219.4	212.1
Control	41.2	41.2	41.2	41.2	86.3	86.3	86.3	86.3
Mean	19.9	14.8	13.4	--	159.8	172.7	174.4	--

* The resistance inducers were used at 500 ppm and Teldor 50% at 50 ppm.

L.S.D. at 5% for:

Chemicals compounds (C)	= 2.7	= 2.9
Method of application (M)	= 3.1	= 2.8
C x M	= 2.1	= 3.4

3. Determination of oxidative-reductive enzymes

Data shown in Table (3) show that using both resistance inducer and the fungicide Teldor 50% resulted in considerable increases in the activity of polyphenol oxidase, peroxidase and ascorbic acid in the treated plants. This increase was more pronounced when the tested inducers were compared with the fungicide Teldor 50%. Also, the highest activity was recorded when these inducers were used as dipping + spray (0.233) compared with dipping (0.187) and spray (0.228) treatments. In addition, Salicylic acid resulted in the highest figures of the enzymatic activity when used as dipping, spray and dipping + spray for polyphenol oxidase (0.257, 0.401 and 0.412, respectively), peroxidase (0.146, 0.153 and 0.150, respectively) and ascorbic acid (0.297, 0.302 and 0.214, respectively).

Control treatment recorded 0.141, 0.118 and 0.210 for polyphenol oxidase, peroxidase and ascorbic acid, respectively.

In general, polyphenol oxidase recorded the highest activity followed by ascorbic acid then the activity of peroxidase.

Table 3. Activity of polyphenol oxidase, peroxidase and ascorbic acid enzymes in strawberry fruits after treating the plants with the tested chemical compounds.

Chemicals compounds*	Activity of enzymes**									Mean		
	Polyphenol oxidase			Peroxidase			Ascorbic acid					
	Dipping	Spray	Dipping + spray	Dipping	Spray	Dipping + spray	Dipping	Spray	Dipping + spray	Dipping	Spray	Dipping + spray
Ammonium bicarbonate	0.207	0.291	0.307	0.127	0.131	0.136	0.216	0.227	0.238	0.183	0.216	0.227
Bion	0.213	0.314	0.318	0.134	0.139	0.146	0.231	0.280	0.289	0.193	0.244	0.251
Photophor	0.209	0.311	0.309	0.140	0.145	0.147	0.238	0.262	0.268	0.196	0.240	0.241
Oxalic acid	0.231	0.329	0.332	0.142	0.150	0.149	0.233	0.239	0.246	0.202	0.239	0.242
Salicylic acid	0.257	0.401	0.412	0.146	0.153	0.151	0.240	0.297	0.302	0.214	0.284	0.288
Teldor 50%	0.163	0.321	0.327	0.123	0.120	0.128	0.217	0.221	0.225	0.168	0.221	0.227
Control	0.141	0.141	0.141	0.118	0.118	0.118	0.210	0210	0.210	0.156	0.156	0.156
Mean	0.203	0.301	0.306	0.133	0.137	0.139	0.226	0.248	0.254	0.187	0.228	0.233

* The inducer resistance were used at 500 ppm and Teldor 50% at 50 ppm.

** Expressed as absorption after 30 second at appropriate wave length.

DISCUSSION

Nowadays, the world is suffering from a great pollution of the environment by many wastes including agrochemicals, which cause drastic effect on the human health. Therefore, the current strategy of controlling pests, especially of vegetables and fruits depends on using alternative methods of disease management rather than pesticides and/or using safety chemicals as well as which have low residue in the treated plants and/or that transformed inside the treated plants to nontoxic chemical. Hence, this work aimed to manage strawberry fruit-rots caused by *B. cinerea* by using some resistance inducers chemicals and comparing their efficiency with the fungicide Teldor 50%.

The obtained data revealed that all the tested inducers, *i.e.* Ammonium bicarbonate, Bion, Photophor, Salicylic acid and Oxalic acid as well as the fungicide Teldor 50% resulted in different degrees of inhibition to the linear growth of *B. cinerea*. In addition, the fungicide Teldor 50% was the most efficient treatment compared with the tested inducers. The obtained results are expected, as the fungicide Teldor 50% was recommended for controlling *B. cinerea* on different hosts including strawberry fruit-rots (Heltbech *et al.*, 2000 and Khafagi, 2002 and 2007). On the other hand, the low efficiency of the tested inducer resistance as inhibitor to the growth of *B. cinerea* was also expected, where these chemicals one acting indirectly on the pathogen through their effect on the test plant (Lancake, 1981; Dean and Kuc, 1985; Abo-Taleb, 2001; Hilal, 2004 and Khafagi, 2002 and 2007).

It has been found that all the tested inducers and the fungicide Teldor 50% gave different degrees of fruit-rots control and considerable increase in the fruit yield. However, the fungicide Teldor 50% was the most efficient one, especially when used as spray and dipping + spray. Meanwhile, Salicylic acid was the superior inducer resistancee in all treatments, i.e. dipping, spray and dipping + spray. On the other hand, the other inducer resistance were of intermediate effect. The high reduction in fruit-rots due to spraying and/or dipping + spraying Teldor 50% is expected, which this fungicide has direct drastical effect on *B. cinerea* (Brillant and Sauzay, 2000; Legard *et al.*, 2005 and Khafagi, 2002 and 2007). Meanwhile, the effect of the tested antioxidants greatly differed and the best results were obtained when used as dipping + spray, where spraying them increased their activity after their first treatment (dipping at the time of transplanting, about 100 days before spray treatment). However, Dean and Kuc (1985) and Kuc and Rush (1985) indicated that the action of acquired resistance inducer is persistent and are nonspecific for a pathogen. On the other hand, Lancake (1981) reported that unlike elicitors of phytoalexins accumulation, which elicit at the site of application may be responsible for localized protection, inducers of systemic resistance sensitize the plant to respond rapidly after infection. These responses induced phytoalexin accumulation and lignification (Dean and Kuc, 1985; Kuc and Rush, 1985 and Maolepsza *et al.*, 1994) and induced or enhanced activities of chitinase and β gluconase (Metranx and Boller, 1986). Furthermore, Kessmann *et al.* (1994) found that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable, broad spectrum disease control and they could be used preventatively to bolster general plant health, which resulted in long lasting protection.

The obtained results revealed that the application of the tested inducers and the fungicide Teldor 50% to strawberry plants resulted in considerable increase in the activity of the estimated oxidative-reductive enzymes, i.e. polyphenol oxidase, peroxidase and ascorbic acid oxidase compared with untreated control. This increase was more pronounced when the plants were treated by dipping + spraying. Furthermore, Salicylic acid was the most efficient one in this regard. This activation of the estimated enzymes could occurr due to the application of the tested chemicals (inducer resistance and the fungicide Teldor 50%) may play a role in plant resistance. Similar results were reported by Maxwell and Bateman (1967), Ahmed (2001), Ali (2003), Zhang *et al.* (2006), Khafagi (2007) and Abada *et al.* (2008) in different plants. Frakas and Kiraly (1962) and Misaghi (1982) reported that peroxidase enzyme oxidizes the phenolics to more fungal toxic compounds such as quinones, which inhibit both spore germination and fungal growth. Also, peroxidase was found to participate in the synthesis of Legnin. Also, Frakas and Kiraly (1962) mentioned that the participation of an endogenous supply of phenolic compounds in plant disease resistance is dependent upon active polyphenol oxidase system. On the other hand, Hulme (1970) reported that little information is available as to the role of ascorbic acid in plant metabolism.

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تأثير بعض مستحثات المقاومة والمبيد الفطرى تيلدور فى مكافحة مرض العفن الرمادى فى الفراولة المتسبب عن الفطر بوتريتس سيناريا

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أحدثت مستحثات للمقاومة نترات الأمونيوم ، البيون ، الفتوفور ، حامض الأوكساليك و حامض السالسيلك وكذلك المبيد الفطرى تليدور ٥٠% إنخفاضا معنويا للنموّ الطولى للفطر بوتريتس سيناريا على بيئة البطاطس دكستروز أجار. كان هذا النقص يتزايد بتزايد التركيز المستخدم من هذه الكيماويات . علاوة على ذلك ، فقد كان المبيد تيلدور ٥٠% هو الأكثر فعالية فى هذا المضمار عن بقية مستحثات المقاومة.

ادى استخدام هذه المستحثات والمبيد الفطرى تيلدور ٥٠% للنقع ، للرش ، النقع + الرش الى خفض معنوى لعفن الثمار الرمادى المتسبب عن الفطر بوتريتس سيناريا. كان النقص فى الإصابة بالمرض متماشيا مع النقص فى نمو الفطر. أيضاً فإن النقص فى الإصابة بعفن الثمار قد انعكس على محصول الثمار القابل للتسويق بنفس الطريقة.

أحدث استخدام مستحثات المقاومة والمبيد الفطرى تيلدور ٥٠% إلى زيادة ملحوظة فى نشاط إنزيمات الأكسدة والاختزال وهى بولى فينول أوكسيديز ، بيروكسيديز وحامض الإسكوربك.

عموما ، فإن استخدام مستحثات المقاومة والمبيد الفطرى تيلدور ٥٠% عن طريق الغمر + الرش كانت أحسن طريقة للإستخدام.