EVALUATION OF POTATO RESPONSE TO SOME MANURIAL COMPOUNDS FOR CONTROLLING BROWN ROT DISEASE

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Abstract

Susceptibility to brown rot was determined in five potato cultivars. Valor cultivar showed high susceptibility followed by Spunta. Nicola and Desiree showed low susceptibility, while Daimant was moderate in this respect.

The results of manuring showed that humic acid (2.9%), ammonium sulphate, calcium superphosphate and potassium sulphate decreased the growth of *Ralstonia solanacearum* at the tested concentrations up to 4%.

Greenhouse experiments with Valor cultivar showed that all tested manurial compounds variably reduced the disease incidence. The most effective treatment was calcium superphosphate, followed by potassium sulfate and ammonium sulfate, respectively. Actosol (humic acid, 2.9%) was the least effective in disease control. The increase in tuber yield was recognized at significant level in all used treatments.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in Egypt, for both local consumption and exportation. The brown rot disease caused by *Ralstonia solanacearum* is one of the most important, widespread and notorious diseases of solanaceous crops in warm climates (Farag, 1976 and Shekhawat, 2000) and is principally considered as a soilborne and/ or tuber borne pathogen (Caffier & Expert, 2001).

Susceptibility of some potato cultivars to the disease was evaluated by many investigators (Farag, 1976 and Bringel *et al.,* 2002). Farag, (1970) reported that the Desiree was least susceptible compared with King Edward and Arran Banner and correlated cultivar maturity with wilt severity. The inverse relation between wilt severity and the percentage of infected tubers was also shown. Nemeth *et al.* (2002) found that the percentage of infection in Desiree reached 50%.

Several attempts of disease control were made in Egypt including fertilization and manuring trials (Farag, 1970 and 1976), and in many parts of the world as well. Nunez *et al.* (2002) studied the effects of calcium, Urea and their combination on the survival of *R. solanacearum* cells in laboratory.

The effect of salts widely used in plant nutrition in different anionic forms along with certain hydroxides on the growth of *R. solanacearum in vitro* and *in vivo* was

studied by Gerges (2007). The effects of organic amendments on the severity of the disease and population dynamics of *R. solanacearum* in the soil were evaluated (Farag, 1976 and Hernandez & Bustamante, 2001).

Although humic acid compounds have showed an antibacterial properties (Kuhnert *et al.*, 1991 and Jasek *et al.*, 1993), the application of organic compounds physically containing humic acid had improved disease control (Scheuerell & Mahaffee, 2004). The retardation of disease onset was also reported by Farag (1976).

The efficacy of single or combined application of organic matter and chemical fertilizer (ammonium sulfate, potassium sulfate and Urea) on the potato disease control was determined under greenhouse condition (Abd El- Ghafar and Abd El-Sayed, 2002). They found that chemical fertilizer was more effective than organic matter treatments in reducing disease severity and population of virulent forms of *R. solanacearum*.

The purpose of the present study aimed to evaluate the efficacy of some manurial compounds applied as soil treatment for controlling potato brown rot disease under greenhouse conditions.

MATERIALS AND METHODS

Cultivars susceptibility:

Five potato cultivars currently cultivated in Egypt namely Desiree, Spunta, Valor, Nicola and Daimant were tested for determining their relative susceptibility to the brown rot disease.

Uniform tubers were planted in sterilized pots (25cm in diam.) filled with 4 kg autoclaved Nile silt (one tuber/pot). Inoculation was made with *R. solanacearum* propagated in nutrient glucose broth for four days at 28 ± 2C. The bacterial suspensions were optically adjusted (at 560nm) to 10^8 cfu/ml and infestation was made by adding 50ml of the bacterial suspension for each kg soil. Control pots maintained under the same conditions without inoculation. Five pots were used for each treatment and replicated five times. The disease severity was determined 80 days after planting according to the key proposed by Kempe and Sequeira (1983) using the scale based on the visual observation of the percentage of wilted foliage (0 = no symptoms, 1= up to 25%, 2= 25-50%, 3= 50-75%, 4= 75-100%). The disease index (DI) was calculated using the following formula:

$$DI = \frac{\text{Sum of } (n \times v)}{\text{Total number of sprouts x max. gradings (4)}} \times 100$$

Where: n = number of wilted sprouts in each category.

v= numerical value of each category.

The numbers of total and infected tubers were determined and the percentage of infected tubers was calculated.

Effect of manurial compounds on brown rot disease:

a- Sensitivity of *R. solanacearum* to manurial compounds:

Different concentrations of the selected manurial salts were added to the medium to study their effect on the rate of bacterial growth as follows:

Five sets of tubes containing 9ml of nutrient medium were prepared. The first set served as control without any additions. The other four sets were amended with ammonium sulfate, calcium superphosphate, potassium sulfate and Actosol at various concentrations i.e.0.5, 1,2 and 4% of each compound. All tubes were inoculated with 1ml of *R. solanacearum* cell suspension and then incubated at 28 ± 2 C for four days. Count of *R. solanacearum* was estimated at 1,2 and 4 days after inoculation by using the dilution plate method.

b- Effect of manuring on disease development:

Cultivation (Spunta cv.) and soil infestation were carried out as mentioned before. A pot experiment was designed as a completely randomized design to study the effect of different manurial compounds on the severity of disease and percentage of infected tubers as well as yield. The ammonium sulfate (20.6%N), calcium superphosphate (15%P), potassium sulfate (47%K) and Actosol (2.9% Humic acid) were used at the rate of 1or 2g/ pot, 1or 2g/ pot, 0.5 or 1 g/ pot and 0.03 or 0.06 ml/pot, respectively.

RESULTS

Cultivars susceptibility:

Table (1) shows significant differences between the tested cultivars regarding their reaction to *R. solanacearum*. The results showed higher susceptibility of Valor followed by Spunta (68.8 and 40.7% infection, respectively). Nicola and Desiree, on the other hand, were less susceptible showing 25.7 and 33.3% infection, respectively. Intermediate reaction, however, was recognized for Daimant cultivar showing 38.2% infection. Thus, Nicola and Desiree could be regarded as brown rot tolerant cultivars under Egyptian conditions. The disease index calculated for the above cultivars followed the same trend in the percentage of infection.

Table 1. Reaction of different potato cultivars against *R. solanacearum* infection under greenhouse conditions

Cultivars	Means of:			
	Tubers/ plant	Diseased tubers/ plant	Infection %	Disease index
Valor	3.2	2.2	68.8	45.4
Spunta	5.4	2.2	40.7	24.2
Daimant	6.8	2.6	38.2	13.8
Desiree	6.0	2.0	33.3	10.6
Nicola	7.0	1.8	25.7	8.0
L.S.D at 0.05	0.6	N.S	3.5	2.3

Effect of manurial compounds on brown rot disease:

a- Sensitivity of *R. solanacearum* to manurial compounds:

Data in Table (2) show the densities of *R. solanacearum* growth in response to different tested compounds, and also show the differences in cell numbers between the tested compounds, *i.e.* cell numbers recorded after four days incubation, being 16 x 10^3, 35 x 10^4, 74 x 10^7 and 53 x 10^8 cfu/ml for calcium superphosphate, potassium sulfate, ammonium sulfate and Actosol, at 1% concentration, respectively. There are significant differences among the four concentrations of each tested compounds, as example, the densities recorded after two days incubation were 90 x 10^6, 41 x 10^5, 77 x 10^2 and 21 x 10^2 cfu/ml for calcium superphosphate at 0.5, 1,2 and 4% concentrations, respectively. Generally, the number of *R. Solanacearum* cells was decreased gradually with the increase in the concentrations of tested compounds. Growth was completely inhibited after four days incubation at 2 and 4% concentration when calcium superphosphate was added to the cultures. Thus, calcium superphosphate was the most effective in this respect followed by potassium sulfate, ammonium sulfate and Actosol, respectively.

Table 2. Effect of manurial compounds at different concentrations on the growth of *R. solanacearum*

Manurial Compounds	Concentrations %				
	0.0	0.5	1.0	2.0	4.0
One day incubation					
Calcium superphosphate	149×10^4	30×10^4	65×10^3	117×10^2	30×10^2
Potassium sulfate	149×10^4	38×10^4	89×10^3	160×10^3	59×10^3
Ammonium sulfate	149×10^4	40×10^4	31×10^4	20×10^4	168×10^3
Actosol	149×10^4	92×10^4	63×10^4	34×10^4	18×10^4
Two days incubation					
Calcium superphosphate	137×10^6	90×10^6	41×10^5	77×10^2	21×10^2
Potassium sulfate	137×10^6	97×10^6	115×10^5	26×10^3	13×10^3
Ammonium sulfate	137×10^6	121×10^6	95×10^6	86×10^5	82×10^5
Actosol	137×10^6	134×10^6	102×10^6	85×10^6	67×10^6
Four days incubation					
Calcium superphosphate	132×10^8	69×10^7	61×10^3	-	-
Potassium sulfate	132×10^8	92×10^7	35×10^4	78×10^2	-
Ammonium sulfate	132×10^8	129×10^8	74×10^7	188×10^5	150×10^5
Actosol	132×10^8	130×10^8	53×10^8	39×10^7	147×10^6

b- Effect of manuring on disease development:

Table (3) shows that soil treated with certain compounds under investigation has significantly decreased the brown rot incidence. Calcium superphosphate was the most effective in disease control which resulted the lowest percentage of infection 25.2% and 10.6%, in soil treatment at rate of 1 and 2g/pot, respectively, compared to

100% in untreated control. The same trend was found for other compounds, with the negative correlation between the rate of application and infection. It is clear from the present data that the most efficient applications for controlling brown rot was calcium superphosphate at 2g/pot (showing 89.7% efficacy) followed by potassium sulfate at 1g/ pot (69.7%), ammonium sulfate at 2g/pot (63.3%) and Actosol at 0.06 ml/pot (54.2%), respectively.

Table 3. Effect of manurial compounds on brown rot incidence.

Treatments	Rate of application /pot	Means of :				
		Tubers/plant	Healthy tubers/plant	Infected tubers%	Disease index	Efficacy
Calcium superphosphate	1g	7.0	5.2	25.7	30.6	74.3
	2g	7.8	7.0	10.3	21.4	89.7
Potassium sulfate	0.5g	6.2	4.2	32.3	42.8	67.7
	1g	6.6	4.6	30.3	34.0	69.7
Ammonium sulfate	1g	5.4	3.2	40.7	59.6	59.3
	2g	6.0	3.8	36.7	36.7	63.3
Actosol	0.03ml	4.0	1.4	65.0	77.4	35.0
	0.06ml	4.8	2.6	45.8	70.2	54.2
Control	-	3.2	-	100.0	91.2	-
L.S.D at 0.05	-	0.3	N.S	5.1	7.3	4.2

Yield:

Data in Table (4) show the effect of the tested compounds on tuber yield. The data indicated that there is a positive correlation between the rate of application and the yield, in both inoculated and uninoculated treatments. The tuber yield was found to be 285.6, 153.6, 104.9 and 53.0g per plant for calcium superphosphate, potassium sulfate, ammonium sulfate and Actosol, respectively, in inoculated pots in high dose chemical treatment. The respective value for uninoculated ones were 335.4, 252.0, 202.2 and 134.4g compared to control, where 0.0 and 98.4g/ plant were recorded for inoculated and uninoculated treatments, respectively.

Table 4. Effect of manurial compounds on the tuber yield obtained from soil inoculated with *R. solanacearum* and uninoculated soil

Treatments	Rate of application /pot	Inoculated soil			Uninoculated soil		
		Mean number of healthy tubers/ plant	Mean weight of tubers (g)/ plant	Yield (g)/ plant	Mean number of tubers/ plant	Mean weight of tubers(g)/ plant	Yield (g)/plant
Calcium superphosphate	1g	5.2	36.6	190.3	7.4	38.2	282.7
	2g	7.0	40.8	285.6	7.8	43.0	335.4
Potassium sulfate	0.5g	4.2	30.0	126.0	6.4	30.8	197.1
	1g	4.6	33. 4	153.6	7.0	36.0	252.0
Ammonium sulfate	1g	3.2	24.2	77.4	6.0	26.8	160.8
	2g	3.8	27.6	104.9	6.4	31.6	202.2
Actosol	0.03ml	1.4	18.8	26.3	4.8	22.2	106.6
	0.06ml	2.6	20.4	53.0	5.6	24.0	134.4
Control	-	-	-	-	4.6	21.4	98.4
L.S.D at 0.05	-	N.S	1.4	13.5	N.S	N.S	18.7

DISCUSSION

Potato brown rot caused by *R. solauacearum* is a soil borne disease of substantial economic importance because it became endemic in many parts in the world (Caffier & Expert, 2001). The means of field control of the disease are still limited. It was therefore, of importance to investigate some aspects of disease control.

Cultivars sensitivity of some currently cultivated potato cultivars in Egypt as Spunta, Valor, Desiree, Nicola and Daimant was studied. Results of the present investigation indicated that tested cultivars varied significantly in wilt severity. The highest percentage of infected tubers was obtained in Valor cultivar followed by Spunta. On the contrary, the cultivar Nicola showed the lowest disease incidence followed by Desiree, and Daimant cultivar was moderate. The differences in disease susceptibility is a qualitative genetic character correlated with the degree of earliness of the cultivar (Farag, 1970 and 1976). Cultivars differences in susceptibility was also reported by Farag (1976) and Nemeth *et al* (2002).

As to the sensitivity of *R. solanacearum* to different concentrations of tested manurial compounds, it was clear from the obtained data that there are significant differences among the used concentrations of each compound. Generally, the density of *R. solanacearum* was gradually decreased with the increase in the concentrations of such compounds. Growth was completely inhibited at 2 and 4% concentration when calcium superphosphate was studied *in vitro,* in cultures incubated for four days.

The density of the organism decreased in a medium containing any of these compounds, compared to the control treatment, under the conditions of the experiment. This may be attributed to the detrimental effect of the tested compounds on the growth and/ or the effect of valency of the cations in solution. Calcium superphosphate was the most effective in this respect. Similar attribute was made by Nunez *et al.* (2002) and Gerges (2007).

Application of manure as a mean of controlling plant diseases has been known to be effective in combating diseases. In this investigation, it was found that application of high dose of the tested compounds showed considerable favourable effect in disease control, while lower concentrations were less effective. Obtained results indicated that all used compounds had an obvious influence on wilt index in potato under greenhouse conditions compared with the control treatment. This finding is in accordance with Abd El- Ghafar & Abd El- Sayed (2002), Scheuerell & Mahaffee (2004) and Gerges (2007). It is clear from the present data that the determination of percentage of efficacy as a criterion of the severity of infection, indicated that the

highest efficacy for controlling brown rot was obtained with calcium superphosphate followed by potassium sulfate, ammonium sulfate and Actosol, in a descending order. Tuberization and yield components showed proportional relationship with the efficacy of the compounds. Moreover, the higher rate of application increased the tuber yield as a result of improving of the plant vigor in general and reducing disease in particular. Similar trend was reported by Zinati *et al.* (2001).

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تقييم بعض المركبات السمادية في مقاومة مرض العفن البني على البطاطس

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أظهرت أصناف البطاطس المختبرة في هذه الدراسة اختلافات واضحة فيما بينها من حيث الحساسية لمرض العفن البنى، فقد اتضح أن الصنف فالور أعلى الأصناف حساسية للمرض يليه الصنف سبونتا، وعلى النقيض من ذلك كان الصنف نيقولا أقل الأصناف حساسية يليه الصنف ديزيريه بينما كان الصنف دايمونت متوسط الحساسية.

أجريت هذه الدراسة أيضاً لبيان تأثير بعض المركبات السمادية المختلفة على حدوث مرض العفن البني في البطاطس، حيث أوضحت نتائج المعمل أن كل المركبات السمادية المختبرة سببت نقصاً حاداً في نمو البكتيريا رالستونياسولاناسيرم عند التركيزات المستخدمة (٠,٥% - ١%- ٢%- ٤%)، حيث وجدت علاقة عكسية بين تلك التركيزات وأعداد خلايا البكتيريا.

وقد صممت في الصوبة تجربة أصص منزرعة بالصنف فالور وتبين أن كل المركبات السمادية المختبرة قللت معنوياً من حدوث المرض وذلك بالمقارنة بمعاملة الكنترول، ولكن اختلفت هذه المركبات في مدى تأثيرها على حدوث المرض حيث أوضحت النتائج أن سوبر فوسفات الكالسيوم كان الأكثر تأثيراً في مقاومة المرض يليه كبريتات البوتاسيوم فكبريتات الأمونيوم ثم الاكتوسول (٢,٩% حمض هيوميك)، كما وجد أن هناك زيادة معنوية في محصول الدرنات في جميع معاملات التجربة وذلك مقارنة بمعاملة الكنترول.