EFFECT OF MINERAL FERTILIZERS ON *RALSTONIA SOLANACEARUM* FROM DIFFERENT HABITATS

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Abstract

Isolates from tubers were more pathogenic and produced earlier wilt onset compared to the water originated isolates, whereas soil and weed isolates were intermediate in this regard. The effect of certain salts, widely used in plant nutrition in different anionic forms along with certain hydroxides currently used as foliar sprays, on the *in vitro* growth of the pathogen were tried. The tested isolates were more sensitive to NaOH than KOH. The nitrates, KNO_3 (0.6%) caused remarkable increase in *in vitro* growth of the pathogen, being more pronounced for soil and weed isolates. Similar trend could be observed for Ca $(NO_3)_2$, at the same concentration. Differently, chlorides caused deterimental effect on the *in vitro* growth of the pathogen being more pronounced for $FeCl_2.4H_2O$, $CaCl_2$ and $MgCl_2$ Sulphates of ammonium, calcium, copper, potassium and zinc (0.6%) caused a pronounced decrease in growth with the exception of magnesium that increased the growth of potato stems and weed isolates.

Tomato plants treated with KNO_3 and $MgSO_4$ resulted in a decrease in the percentage of wilted plants though the favourable effect on the *in vitro* growth of the pathogen. The same trend was observed for ammonium sulphate. The effect was attributed to an improvement of the plant vigor. Application of KNO_3, $(NH4)_2$ SO_4 along with $MgSO_4$ at the same concentration, however, increased the percentage of scorched plants. It is worth to note that NaOH, KOH and $ZnSO_4$ decreased the *in vitro* growth of the pathogen, and caused a pronounced decrease in the percentage of wilted plants, despite the increase in the percentage of scorched ones.

INTRODUCTION

Potato brown rot caused by *Ralstonia solanacearum* (syn. *Pseudomonas solanacearum*) is an important bacterial disease in warm climates, though it has been reported in the far northern hemisphere and the European continent (stead *et al.*, 1996). The disease was first reported in Egypt by Briton – Jones (1925) based only on symptomatology.

The destructive nature of the pathogen was attributed to different strains, consequently a wide host range, saprophytic survival in soil, and persistence in different habitats (Balabel, 2006). The chemical control of the disease has been

ineffective (Hayward and Hartman, 1994), thus control measures based on agricultural consideration (Farag, 1970 and 1976) and understanding of epidemiological aspects have been suggested (Balabel *et al.,* 2005).

Although there have been many observations as to the relationship of host vigor to disease development, few reports dealing with the relationship of host nutrition to pathogenesis have been carefully analyzed under controlled conditions (Kelman, 1953). Studies on the effect of organic manures revealed retardation of disease onset (Farag, 1976) due to attributes of antibiosis and competitions. The deleterious effect of organic matter on the pathogen *in vitro* studies , on the other hand, has been discussed by Balabel *et al.* (2005).

This work was undertaken to study the effect of certain salts in different anionic forms, being used essentially in mineral fertilization, on the *in vitro* proliferation of the pathogen as well as brown rot disease development.

MATERIALS AND METHODS

1- Isolation, pathogenicity and identification of the pathogen.

Potato plants and tubers with typical disease symptoms were collected from fall plantation season. Samples of weed plants associated with potato fields, soil as well as irrigation water were collected and used in isolation of the pathogen according to the method described by Shehata (2001) and Balabel (2006) using SMSA medium (Elphinstone *et al.,* 1996). Pathogenicity of isolates was made under greenhouse conditions on tomato plants (Shehata, 2001).

2- Salts and Hydroxides tested.

The salts used in this study included ammonium, calcium, magnesium, potassium, copper and zinc sulphates; ammonium, calcium, magnesium, manganese, potassium, ferrous and sodium chlorides; calcium and potassium nitrates as well as potassium and sodium hydroxides.

3- Effect of salts on *in vitro* growth of *R.solanacearum*

The salts in concern were tested at 0.2 and 0.6% for their effect on *R. solanacearum* growth in glucose nutrient broth (g/l 10 glucose, 3 beef extract, 5 peptone, 1000 ml water, pH 7.2). Inoculation was made with standardized suspension of bacteria recovered from potato tubers, potato stems, potato fields,irrigation water and weeds associated with potato plants. Incubation was made at 30° C for 6 days. Control treatments devoiding salts were prepared, in the same way, then the optical density of liquid cultures in all treatments was determined with a spectronic 20 colorimeter at 635 nm.

4- Effect of salts on tomato seedlings growth.

Salts used in this study were selected according to their effect, either stimulative or suppressive, on the *in vitro* growth of the pathogen. The roots of tomato transplants were dipped in 2% solutions of the selected salt for 10 minutes. Ammonium and magnesium sulphate along with potassium nitrate (*in vitro* stimulants) were compared with zinc sulphate as well as sodium and potassium hydroxide (*in vitro* inhibitive)

Inoculation of tomato seedlings was made by dipping the transplants in optically standardized suspension of bacteria (recovered from potato tubers) for 10 minutes. Transplanting was made as previously mentioned after another 10 minutes of partial drying of roots. The same method of inoculation and transplantation was followed for the salt – treated seedlings. Three tomato GS seedlings (4 leaves/plant) were transplanted after salt treatment in each plastic pot (25 cm in diameter) containing clay soil.

Watering of plants was regularly made at 2 days intervals under the greenhouse conditions. The wilt onset in days was recorded and the percentage of infected plants was determined.

RESULTS AND DISCUSSION

1- Pathogenicity and identification.

Stem inoculation of tomato seedlings revealed the pathogenicity of the isolates in concern (Table, 1). The most pathogenic isolates were the tuber isolates that produced the highest number of wilted plants (100%) along with an early disease onset (6 days). The water isolates, however, produced the least number of wilted plants (55.6%) along with delayed disease onset (12days) . Soil and weed isolates, on the other hand, were intermediate in this regard. Such differences between isolates of different origins were also reported by Balabel (2006) who found that isolates, from different habitats, recovered on SMSA medium were all pathogenic to tomato plants showing symptoms 4-12 days after inoculation and emphasized the higher sensitivity of the tomato test compared to the serological immunoflourescent (IF) test for detection of *R. solanacearum.*

In Gram – stained smears all isolates were G(-), non-sporulating short rods. Bipolar staining bodies were often seen that may confirm the identity of *R. solanacearum* (Shehata, 2001), along with the colony morphology on SMSA medium (Elphinstone *et al.,* 1996) which is an important tool in presumptive brown rot diagnosis.

All isolates were similar in colony morphology typical of *R. solanacearum* on SMSA medium. The virulent colony on this medium is irregulary round and mucoid with excessive slime. The avirulent type is butyrous round, evenly red and smaller (Elphinstone *et al.,* 1996). It is important to note that soil and irrigation water samples revealed a noticeable proportion of colonies devoiding the characteristic colony morphology of *R. solanacearum* mixed with the typical colony form of the pathogen.

All isolates from different sources were catalase (+), with oxidative glucose metabolism. Fluorescent pigment is not produced in King's medium B. All isolates produced brown pigments. Poly β-hydroxybutyrate can be seen with Sudan Black B. Acid is produced from glucose, galactose, mannose, fructose, maltose, lactose, and cellobiose.

The biovars of brown rot pathogen are defined based on its ability to oxidize maltose, lactose and cellobiose and inability to oxidize dulcitol, mannitol and sorbitol (Holt *et al.,* 1994). According to the afore-mentioned pathogenic potential and biochemical reactions, the Egyptian isolates conform with those described for race 3 biovar II.

Table 1. Comparative pathogenicity of R. solanacearum isolates from different habitats on tomato seedlings .

Source of isolates	Number of isolates tested	* Percentage of wilted plants	** Disease onset (days)
Tuber isolates	20	100	6
Stem isolates	18	100	10
Weed isolates	7	71.4	6
Water solates	9	55.6	12
Soil isolates	16	87.5	10

* Inoculated plants kept under greenhouse conditions at 28° C for 3 weeks.

** The disease onset was calculated in days for plants just showing the signs of wilting.

2- Effect of salts and hydroxides on *in vitro* growth of *R. solanacearum.*

The effect of certain salts widely used in plant nutrition , in different anionic forms and concentrations, along with certain hydroxides currently used as foliar sprays are shown in Tables (2 a, b, c, and d)

Table (2-a) shows the effect of hydroxides in two different preparations on the *in vitro* growth of *R. solanacearum* isolated from different habitats. Potassium hydroxide (KOH) at 0.6% caused a complete *in vitro* inhibition of the pathogen from different sources, however, the 0.2% allowed a weak growth. Similar trend could be noticed for sodium hydroxide. It is interesting to note that no pronounced differences

in absorbance either for different isolates or the hydroxide(s) used at the aforementioned concentration. It seems probable, however, that the isolates in concern are more sensitive to 0.2% NaOH compared to KOH. The sensitivity of the gram negative bacteria to certain hydroxides as a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining was previously reported by Suslow *et al.* (1982).

Table (2-b) shows the effect of two commonly used nitrate fertilizers on the *in vitro* growth of the brown rot isolates. Potassium nitrate (KNO_3) caused a remarkable increase in growth at 0.6% strength, being more pronounced for isolates recovered from soil. Lower concentration of KNO_3 (0.2%) , on the other hand, caused a variable increase in growth, as the highest readings were recorded for soil and weed isolates.

Calcium nitrate $Ca(NO_3)_2.4H_2O$ at 0.6% caused a slight increase in growth compared to KNO_3. The highest increase was recorded for the soil isolates followed by the weed isolates. Isolates from different sources, however , reacted variably to 0.2% $Ca(NO_3)_24H_2O$. The increase in growth at the latter concentration was recorded only for stem and weed isolates.

Generally, it could be concluded that KNO_3 favoured the growth of *R. solanacearum* at the used concentrations and calcium nitrate had a variable effect in this regard, thus showing the differential effects of cations on bacterial growth.

The first comprehensive investigation of the nutrition of the brown rot pathogen *in vitro* was made by Honing (1912) in Sumatra using mineral salt solution as a basal medium to test a wide range of carbon and nitrogen sources in various combinations. The carbon sources, however, were not utilized when ammonium nitrate was supplied as a source of nitrogen, though potassium nitrate was found to be a suitable source of nitrogen under certain growth conditions (Mushin, 1938).

Table (2-c) shows the effect of the less commonly used salts, as fertilizers i.e., the chlorides, on *in vitro* growth of the brown rot pathogen. This group of salts is currently used in foliar nutrition of plants in different preparations, different composition and different trade names. The limited use of chlorides, in soil application is, however, attributed to some difficulties related to soil chemistry, especially under alkaline conditions .

It is interesting to note that different chlorides used at 0.6% caused a deterimental effect on the *in vitro* growth of *R. solanacearum*, being less pronounced for NH_4Cl, $MnCl_2.4H_2O$, KCl and NaCl. Greater growth inhibition was recorded for $FeCl_2.4H_2O$, $CaCl_2$ and $MgCl_2$, and considerable variation in growth inhibition could be observed between isolates from different sources. Similar trend could be observed for chlorides used at 0.2% concentration.

Table 2. Effect of hydroxides, nitrates, chlorides and sulphates on *in vitro* growth of *R.solanacearum* .

Compounds	Treat.	Tubers		Soil		Water		Stems		Weeds	
		Mean absorbance	%	Mean absorbance	%	Mean absorbance	%	Mean absorbance	%	Mean absorbance	%
a- Hydroxides : Potassium hydroxide (KOH)	0.2 %	0.06	-80	0.05	-80	0.07	-75	0.05	-82	0.05	-81
	0.6%	0.00	-100	0.00	-100	0.00	-100	0.00	-100	0.0	-100
Sodium hydroxide (NaOH)	0.2 %	0.00	-100	0.00	-100	0.00	-100	0.01	- 96	0.01	- 96
	0.6%	0.00	-100	0.00	-100	0.00	-100	0.00	-100	0.00	-100
Control		0.31		0.25		0.28		0.29		0.27	
b- Nitrates : Calcium nitrate $Ca(No_3)_2.4H_2O$	0.2 %	0.09	- 35	0.11	-15	0.14	-12	0.16	+23	0.19	+46
	0.6 %	0.20	+ 42	0.23	+ 76	0.16	0	0.19	+ 46	0.20	+ 53
Potassium nitrate (KNO_3)	0.2 %	0.15	+ 7	0.17	+ 30	0.16	0	0.07	- 46	0,16	+ 23
	0.6 %	0.28	+100	0.27	+107	0.16	0	0.25	+92	0.24	+ 84
Control		0.14		0.13		0.16		0.13		0.13	

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Compounds	Treat.	Tubers		Soil		Water		Stems		Weeds	
		Mean absorbance	%	Mean absorbance	%	Mean absorbance	%	Mean absorbance	%	Mean absorbance	%
c. Chlorides : Ammoinum chloride (NH_4CL)	0.2 %	0.14	- 22	0.16	0	0.15	- 21	0.16	0	0.15	- 11
	0.6%	0.14	- 22	0.14	- 12	0.15	- 21	0.15	- 6	0.15	- 11
Calcium chloride ($CaCl_2$)	0.2 %	0.07	- 61	0.09	- 43	0.10	- 47	0.07	- 56	0.11	- 35
	0.6%	0.08	- 55	0.08	- 50	0.08	- 57	0.10	- 37	0.09	- 47
Ferrous chloride ($Fecl_2.4H_2O$)	0.2 %	0.02	- 88	0.02	- 87	0.03	- 84	0.04	- 75	0.04	- 76
	0.6 %	0.06	- 66	0.06	- 62	0.05	- 73	0.04	- 75	0.04	- 76
Magnesium chloride ($MgCl_2$)	0.2 %	0.11	-38	0.10	- 37	0.19	0	0.08	- 50	0.13	- 23
	0.6 %	0.10	- 44	0.11	- 31	0.12	- 36	0.09	- 43	0.13	- 23
Manganese chloride ($MnCl_2 4H_2O$)	0.2 %	0.14	- 22	0.12	- 25	0.10	- 47	0.13	- 18	0.10	- 41
	0.6 %	0.14	- 22	0.15	- 6	0.15	- 21	0.14	- 12	0.15	- 11
Potassium chloride (KCL)	0.2 %	0.17	- 5	0.15	- 6	0.18	- 5	0.16	0	0.13	- 23
	0.6 %	0.12	- 33	0.16	0	0.18	- 5	0.16	0	0.14	- 17
Sodium chloride (NaCL)	0.2 %	0.08	- 55	0.16	0	0.14	- 26	0.11	- 31	0.16	- 5
	0.6 %	0.18	0	0.16	0	0.19	0	0.16	0	0.17	0
Control		0.18		0.16		0.19		0.16		0.17	

Cont...

Compounds	Treat.	Tubers		Soil		Water		Stems		Weeds	
		Mean absorbance	%	Mean absorbance	%	Mean absorbance	%	Mean absorbance	%	Mean absorbance	%
d. Sulphates : Ammoinum Sulphate $(NH_4)_2\ SO_4$	0.2 %	0.58	+ 41	0.60	+ 76	0.48	+ 29	0.43	+ 16	0.50	+ 42
	0.6%	0.32	- 21	0.29	- 14	0.30	- 18	0.36	0	0.34	- 2
Calcium Sulphate $CaSO_4.\ 2H_2O$	0.2 %	0.34	- 17	0.21	- 38	0.29	- 21	0.23	- 37	0.24	- 31
	0.6%	0.22	- 46	0.23	- 32	0.35	- 5	0.25	- 32	0.27	- 22
Copper sulphate $CuSO_4.\ 5H_2O$	0.2 %	0.03	- 92	0.06	- 82	0.02	- 94	0.05	- 86	0.04	- 88
	0.6 %	0.02	- 95	0.03	- 91	0.06	- 83	0.04	- 89	0.04	- 88
Magnesium sulphate $MgSO_4$	0.2 %	0.55	+ 34	0.40	+ 17	0.44	+ 18	0.47	- 27	0.66	+ 88
	0.6 %	0.34	- 17	0.27	- 20	0.31	- 16	0.64	- 72	0.49	+ 40
Potassium sulphate K_2SO_4	0.2 %	0.16	- 60	0.21	- 38	0.19	- 48	0.13	- 64	0.16	- 54
	0.6 %	0.14	- 65	0.15	- 55	0.14	- 62	0.10	- 72	0.12	- 65
Zinc sulphate $ZnSO_4.\ 7H_2O$	0.2 %	0.00	-100	0.02	- 94	0.01	- 97	0.00	-100	0.02	- 94
	0.6 %	0.00	-100	0.00	- 100	0.00	-100	0.00	-100	0.00	-100
Control		0.41		0.34		0.37		0.37		0.35	

The use of different chlorides as a soil amendment is practically quite limited, under alkaline soil conditions. The use of potassium chloride as a foliar spray was tried in spain in the control of fungal diseases of nectarine (Montero *et al.*, 1985). Application of potassium chloride at the rate of 1 part to 99 parts of soil reduced soil population of *R. solanacearum,* and prevented germination as well (Hanudin – Machmud, 1994). The use of other chlorides may be recognized in different foliar spray preparations under different trade names.

The effect of sulphates on the *in vitro* growth of *R. solanacearum* is shown in table (2-d). Sulphates at 0.6% strength caused pronounced decrease in the *in vitro* growth, being more pronounced with copper sulphate and zinc sulphate. Isolates from different sources did not show much variation in this regard .

It may be observed that all tested salts, namely ammonium, calcium, copper, potassium and zinc sulphates at 0.6% , caused a pronounced decrease in growth with the exception of magnesium sulphate that caused an increased growth in isolates from potato stem and weeds. Moreover, the same salts at 0.2% decreased the *in vitro* growth with the exception of ammonium and magnesium sulphates, where the growth was increased. Such forms of sulphates fertilizers, may be used either as soil dressing or foliar sprays, especially with potatoes (Fahmy and Mohamed, 1990).

Table (3) show the effect of some cations, that increased the *in vitro* growth of *R. solanacearum*, on the performance of tomato plants transplanted in infested soil. Plants treated with potassium nitrate and magnesium sulphate showed decreased percentage of wilted plants to 5.5%. Ammonium sulphate, on the other hand, decreased the percentage of infected plants to 16.7%. Although the aforementioned salts caused remarkable increase of the pathogen *in vitro*, it caused pronounced decrease in the percentage of wilted plants that may be attributed to the effect of those salts on the plant vigor, and consequently plant tolerance.

It is worth noting that the used salts caused a remarkable decrease in the percentage of wilted plants being more pronounced for potassium nitrate and magnesium sulphate followed by ammonium sulphate.

Sodium and potassium hydroxides as well as zinc sulphate decreased the *in vitro* growth of the pathogen, and caused a pronounced decrease in the percentage of wilted plants compared to the control, and increased the percentage of scorched plants . The highest scorching incidence was recorded for sodium hydroxide and zinc sulphate followed by potassium hydroxide.(46.7%, 46.7% and 42.2% respectively). Such influence may be attributed to the direct effect of these elements, at the used concentration, on the plant.

In this regard , it was found that sodium nitrate and ammonium sulphate at the rate of 100 Ibs/acre reduced wilt in tobacco by 51% and 36%, respectively. It was also found that the time of application was important since march application was effective than the October treatment (N.C. Agr. Expt. Sta. 1941). Farag (1970) found that application of nitrogen resulted in early onset of wilt that gradually increased upto plant maturity. He noted also a peak increase in wilt, 23 days after treatment with potassium sulphate *i.e*, earlier than nitrogen and phosphorus treatments. It was reported, however, that balanced fertilizers and soil type, either sandy or organic , had influenced the plant vigor in general that might be correlated with wilt severity (Kelman, 1953). It could be concluded, however, that further investigations are needed on different fertilization regimes recommended for use in brown rot infested area.

Table 3. salts and hydroxide treatments of tomato plants developed in infested soil.

Salts	No. of plants	wilted plants		Scorched Plants	
		No.	Percentage %	No.	Percentage %
Sodium hydroxide	90	44	48.9	42	46.7
Potassium hydroxide	90	13	14.4	38	42.2
Zinc sulphate	90	23	25.5	42	46.7
Ammonium sulphate	90	15	16.7	21	23.3
Magnesium sulphate	90	5	5.5	3	3.3
Potassium nitrate	90	5	5.5	18	20
Control uninoculated	30	0.0	0.0	0.0	0.0
Control inoculated	30	24.0	80.0	0.0	0.0

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تأثير الأسمدة المعدنية على بكتريا " رالستونيا سولاناسيارم " من مصادرها المختلفة

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عزلات البكتيريا رالستونيا سولاناسيارم من مصادر مختلفة أظهرت إختلافاً في قدرتها المرضية. كانت العزلات من درنات البطاطس أكثر تأثيراً من حيث نسبة النباتات المصابة وتاريخ بداية الذبول بالمقارنة مع العزلات الناتجة من مياه الري ، ولكن العزلات من التربة والحشائش كانت متوسطة في هذا الشأن .

درس تأثير بعض الأملاح التى تستخدم على نطاق واسع في تسميد النباتات والتى تختلف فيما بينها في الشق الحامضي وكذلك بعض الهيدروكسيدات التى تستخدم في الرش الورقي على نمو البكتيريا في المعمل ، وقد إتضح أن العزلات موضع الدراسة كانت أكثر حساسية لهيدروكسيد الصوديوم من هيدروكسيد البوتاسيوم وذلك عن طريق التقديرات التى تمت لقياس الكثافة الضوئية.

وقد أوضحت الدراسة أن نترات البوتاسيوم بتركيز (٠,٦%) أدت إلى زيادة ملحوظة في نمو البكتيريا خصوصاً العزلات الناتجة من التربة والحشائش ونفس الشيئ بالنسبة لنترات الكالسيوم . أما الكلوريدات بنفس التركيز كان لها تأثير ضار على نمو البكتيريا خصوصاً كلوريد الحديدوز ، كلوريد الكالسيوم وكلوريد المنجنيز .

ولقد أدت الصور المختلفة لأملاح السلفات خصوصاً سلفات الأمونيوم ، سلفات الكالسيوم ، سلفات النحاس ، سلفات البوتاسيوم ، وسلفات الزنك بتركيز (٠,٦%) أدت كلها إلى إنخفاض في النمو وعلى العكس سلفات المغنسيوم التى أدت إلى زيادة لنمو عزلات الحشائش والسيقان .

وقد أظهرت الدراسة أن بعض الأملاح أدت إلى إنخفاض في نسبة نباتات الطماطم المصابة بالرغم من تشجيعها للنمو الميكروبي في المعمل مثل نترات البوتاسيوم وسلفات المغنيسيوم ونفس الشيء بالنسبة لسلفات الأمونيوم وقد عزى التأثير إلى تحسين هذه الأسمدة لقوة نمو نباتات الطماطم وقد أوضحت الدراسة أن نترات البوتاسيوم وسلفات الأمونيوم وسلفات المغنيسوم قد أدت إلى زيادة في ظهور نسبة النباتات التى ظهر عليها إحتراق على الأوراق.

وأخيراً فإن هيدروكسيد الصوديوم ، هيدروكسيد البوتاسيوم وسلفات الزنك قللت نمو البكتيريا في المعمل وقللت نسبة النباتات المصابة بالذبول بالرغم من زيادتها لنسبة النباتات المحترقة .