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Characteristics and Control of *Erwinia carotovora* subsp. *carotovora* Affecting Potato in Behera Governorate, Egypt

M.A. El-Sheikh*; S.A. El-Kazzaz; Eman El-Argawy* and Asia R. Ahmed***

* *Plant Pathol. Dept., Fac. Agric., Damanhour, Alex. Univ.*

** *Plant Pathol. Dept., Fac. Agric., El-Shatby, Alex. Univ.*

Thirteen isolates of *Erwinia carotovora* subsp. *carotovora* (Ecc) (Jones) Bergy *et al.*, were isolated from potato tubers exhibiting soft rot symptoms in a survey conducted in Behera governorate during 2006-2007 growing seasons. The isolates were monomorphic for the different morphological and physiological characteristics tested. All isolates were short rods, non spore forming, gram negative, motile and exhibited positive reaction for catalase activity, gelatin liquefaction, growth on NaCl 6%, growth at 36°C, production of acid from arabinose, lactose, mannose, raffinose and sorbitol. However, the isolates showed negative reaction for hydrolysis of starch, sensitivity to erythromycin, and production of acid from maltose, adonitol and dextrin. More variations between isolates were recorded for their virulence on potato tubers. Four isolates out of the 13 tested were classified as highly virulent (rot covered ≥75% of potato tuber diameter) while 7 isolates were intermediate (rot <75 - >25%) and 2 isolates were weakly virulent. Meantime, the isolates differed in virulence for the host range and even for the same isolate on the different host species studied. The virulent isolates were found to be polygalacturonase (PG) and pectatelyase (PL) active producers. These could be the major factors in the development of soft rot in potato tubers. cv. Nicola of potato was most tolerant to *Ecc* isolates recovered from Behera governorate. Cvs. Diamant and Sponta, however, were highly susceptible while cv. Cara was moderately susceptible. The tests performed on aerial stems of the tested potato cultivars confirmed the obtained results on tubers. A relationship between soft-rot incidence and soil extracts of the four regions surveyed was revealed. Soil extract prepared from Abou El-Matameer region was highly conducive for the population growth of *Ecc* while soil extracts of the other regions (South El-Tahrir, Nubaria and Banger El-Sukar) were not. This could explain the often observed high soft rot bacterial incidence in Abou El-Matameer compared to the other regions in the conducted survey. A high bactericidal inhibition (>90% reduction) was obtained *in vitro* with 100 ppm magnesium sulfate, 200 ppm potassium sulfate, 100 ppm calcium sulfate, 0.3 ppm zinc sulfate, and 0.3 ppm copper sulfate, four days after inoculation. Also, the antibiotics were of significant effect to control *Ecc* isolates affecting potato in Behera governorate. Streptomycin and Ampicillin exhibited a considerable *in vitro* inhibitory effect (16-21%) on *Ecc* particularly at 200 ppm. The integration between such control measures could be the way for a sustainable potato soft rot control in Behera governorate, Egypt.

Keywords: potato, soft rot, *Erwinia carotovora* subsp. *carotovora*, control.

Potato (*Solanum tuberosum* L.) is the fourth most consumed crop in the world with global production of approximately 320,711,961 tons, produced from approximately 19,264,021 hectares (Anonymous, 2007). Meantime Potato is one of the most important vegetable crops in Egypt. Potato production is 2,600,000 tons, produced from 105,000 hectares, making Egypt the Africa's No. 1 potato producer (Anonymous, 2007).

Soft rot erwinias cause diseases in a wide range of plants including many economically important crops (Agrios, 2005). The extent of losses varies from country to country and is affected by the climate as well as the conditions of plant growth and storage. Infections by *Ecc* occur worldwide in potato fields or during storage after harvest. Dispersion of these bacteria occurs naturally by the rain, irrigation water, insects, nematodes and earthworms. Contaminated farm machinery and washing processes are also major means of *Ecc* dissemination. The bacteria can also spread from plant to plant especially in those plants which are vegetatively reproduced (Pérombelon, 2002).

Bacterial populations multiply and secrete a large variety of extracellular enzymes that degrade the celluletic and pectic substances of plant cell walls and of the middle lamella causing maceration of the tissues. The production of such pectinases, cellulases, and proteases, is crucial to the virulence of *Ecc* (Pérombelon, 2002). Unfortunately, Behera governorate, which is an important area for potato cultivation, is badly affected with Erwinia soft-rot in the field and during marketing and storage. However, increasing knowledge about the Erwinia characteristics and control is very important.

Control of soft rot diseases in potato and other plants is difficult. Most of methods for controlling bacterial soft-rots are almost based on sanitary practices. The most important methods to control bacterial soft rot are the use of antibiotics and fungicides (Hseu *et al.*, 2001 and Abd El-Khair and Haggag, 2007). Also, several organic and inorganic salts showed inhibitory effects on growth of *Ecc* and *E. c.* subsp. *atroseptica* (Haggag and Abd El-Khair, 2006; Sullivan, 2001 and Mills *et al.*, 2006).

The present study therefore, was conducted to reveal the characteristics of *Ecc* occurring on potato in Behera governorate, and to study factors affecting its infection and control.

Materials and Methods

1. Characteristics of Erwinia carotovora subsp. carotovora:

1.1. Morphological and physiological characteristics:

Isolation trials were carried out from infected samples of potato tubers showing typical soft rot symptoms collected from fields and storage of potato tubers in Behera governorate during 2006-2007 growing seasons and directly transferred to laboratory for isolation.

Diseased materials were washed thoroughly with tap water. Small portions of the inner tissues of rotted tubers were macerated in small amount of sterile water in test

tube. Loopfull of the resulting suspension was streaked on the surface of plates containing the Nutrient agar medium and incubated for 48h at 28°C. Bacteria were purified through single colony isolation technique.

Identification was carried out according to Bergy's Manual of Systematic Bacteriology (Krieg and Holt, 1984 and Garrity *et al.*, 2005). Morphological, physiological and biochemical characteristics of the bacteria were tested according to Klement *et al.* (1990). The cell shape, sporulation, motility, gram staining, colony type, catalase activity, gelatin liquefaction, starch hydrolysis, growth in 6% NaCl, growth at 36°C, acid and gas production (from maltose, raffinose, adonitol, L-arabinose, sorbitol, mannose and dextrin) and sensitivity to erythromycin were also carried out.

1.2. Pathogenicity test:

- *Sensitivity of tubers to infection with Erwinia carotovora subsp. carotovora isolates:*

Bacterial suspension (10^7 cfu/ml) of about the 13 tested *Ecc* was prepared from 48 h old culture. Tubers about (60 g in weight) of cv. Diamant were thoroughly washed and sterilized with 70% ethanol, left on laminar bench to dry, and inoculated by pricking the fruit (3-4 mm depth) using sterilized needle dipped in standard bacterial suspension (10^7 cfu/ml). Inoculated tubers were kept in sterilized polyethylene bags for 5 days at 28°C. Control tubers were prepared by the same way using sterile distilled water instead of the bacterial suspension. At the end of the 5th day after inoculation, inoculated tubers as well as the control were cut at transverse direction of the inoculated point and the diameters of rotted area were estimated in millimetres according to Hollis and Goss (1950). The mean diameter of rotted tissue produced by each isolate was taken as indication for the rotting ability of isolates. Five replicates of tubers were conducted.

1.3. Host range test:

Healthy potato tubers (cv. Diamant), pepper (cv. California wonder 300), squash (cv. Eskandarany) and eggplant (cv. Black beauty) fruits were inoculated with three virulent isolates of *Ecc* (*Ecc1*, *Ecc2* and *Ecc6*) recovered in the conducted survey. Potato tubers were inoculated as described in the pathogenicity test. Meantime fruits of pepper, squash and eggplant were sterilized with 70% ethanol, left on laminar bench to dry, inoculated by pricking the fruit using sterilized needle that dipped in bacterial suspension prepared as previously described (Saleh and Huang, 1997). The inoculated fruits were placed in sterilized polyethylene bags with piece of cotton wetted with sterile water. The bags were closed and incubated at 28°C for 5 days. The control similarly treated without bacteria. The varietal reaction was determined by estimating diameters of rotten areas (mm) by the inoculated bacteria. Five replicates of each tested host were conducted.

1.4. Activity of pectinolytic enzymes secreted by Erwinia carotovora subsp. carotovora isolates:

- *Bacterial isolates and extracellular enzyme preparation:*

Three isolates highly, moderately and weakly virulent (*Ecc6*, *Ecc10*, and *Ecc12*) recovered in survey of the present study were used to test their ability to

secrete pectinolytic enzymes in relation to pathogensis. The isolates were grown in nutrient broth (NB) medium supplemented with 0.1% pectin as a sole source of carbon for 48 h at 28±2°C. Bacteria were removed from cultures by centrifugation at 12000 rpm for 20 min at 4°C (Universal 32 R centrifuge, Hettich-Zentrifugen, Germany). Ammonium sulfate crystals were added to supernatant to about 60-80% saturation and the mixture was stirred overnight at 4°C. Precipitates were collected by centrifugation re-dissolved in minimal volumes of 0.05 M citrate-phosphate buffer pH 5.5 and dialyzed over night against the same buffer.

- Enzyme assays:

Pectate lyase was assayed by adding the crude enzyme to tubes containing 2.5 ml 0.25% polygalacturonic acid in 0.1 M Tris-HCl, pH 8.5 and one mM $CaCl_2$. The reaction mixture was incubated for 2 h at 30°C and the reaction was stopped by heating the tubes at 100°C for 10 min. After cooling at room temperature the reaction mixture was centrifuged at 12000 rpm for 10 min and 2 ml of the supernatant were mixed with an equal volume of thiobarbituric acid (TBA) reagent (Ayers *et al.*, 1966) and heated at 100°C for 30 min. The activity was determined as an increase in the absorbance (optical density) at 550 nm (OD_{550}). Zero time reaction mixture containing active enzyme was used as control.

Polygalacturonase assay was similar to that applied for Pectate lyase assay except the reaction mixture contained 0.5% polygalacturonic acid in 0.05 M sodium acetate buffer, pH 5.5 and 0.1 M NaCl (Nasuno and Starr, 1966). The activity was determined as an increase in the absorbance (optical density) at 515 nm (OD_{515}). Zero time reaction mixture containing active enzyme was used as control.

- Factors affecting the in vitro activity of Pectate lyase and Polygalacturonase enzymes:

- Effect of incubation temperature:

Standard assay procedure at different temperatures ranging from 20 to 70 °C was performed. Prior to the addition of the enzyme, the substrate (polygalacturonic acid) was pre-incubated at the respective temperature for 10 minutes. The relative activities as percentages were expressed as the ratio of the PG and PL activity at a certain temperature to the maximum activity at the given temperature range.

- Effect of incubation time:

Standard assay procedure at different incubation times (0.5-4 h) was performed. The relative activities as percentages were expressed as the ratio of the PG and PL activity at a certain incubation time to the maximum activity at the given temperature range.

2. Control Studies:

2.1. Potato varietal resistance:

- Tubers resistance:

Healthy potato tubers of four cultivars (Nicola, Diamant, Cara, and Sponta) were inoculated, as previously described, with three isolates of *Ecc* (*Ecc6, Ecc2* and *Ecc1*) proven to be most virulent in the pathogenicity test. Each treatment was represented by five tubers. After five days of incubation at 28°C, all tubers were cut at transverse

direction and the diameter of rotted areas was estimated in millimetres. Control treatments were prepared by the same way using distilled sterile water instead of the bacterial suspension.

- Stem resistance:

Potato tubers of the four cultivars Nicola, Diamant, Cara and Sponta were surfaces sterilized with (1% sodium hypochlorite) for 5 min, washed with sterile water, and planted in 20 cm diameter plastic pots filled with sterile peat moss (one tuber per pot). When plants reached 15-20 cm length, stems were inoculated by pricking the stem (3-4 mm depth) using sterilized needle that dipped in bacterial suspension (10^7 cfu/ml) prepared as previously described, at 5 cm above the soil surface according to Prior and Steva (1990). Control treatments were prepared by the same way.

Severity of wilting was rated on the scale of He *et al.* (1983) as follows: 1= no symptoms, 2= one leaf wilted, 3= two or three leaves wilted, 4= four or more leaves wilted, and 5= plant dead. Results were recorded two weeks after inoculation.

2.2. Effect of soil extracts:

Samples of soils were collected from regions where potato soft rot was severe as well as from regions where potato soft rot was rare based on the disease history in the surveyed regions. Soil extracts were prepared according to Rhoades (1982). Chemical analysis of the soil samples were conducted in National Research Centre (Soil Testing and Plant Analysis Laboratory) and presented in (Table 1).

Flasks 250 ml containing 50 ml of freshly prepared soil extracts (recovered in the present survey) were inoculated with 0.5 ml of bacterial suspension (10^7 cfu/ml) of virulent isolate (*Ecc6*) and incubated at 28°C with agitation 150 rpm for 10 days. Population of *Ecc* in soil extracts were determined every 2 days using diluted method and pour plate technique (Haggag and Abd El-Khair, 2006). Bacterial counts were recorded as colony forming units (cfu) per millilitre (ml) per plate. Replicates were used and percentages were determined compared with the control distilled water.

2.3. Effect of mineral salts:

Bacterial isolate (*Ecc6*) was grown in 250 ml flasks containing 50 ml of nutrient broth (NB) medium amended with sulphate salts of calcium, magnesium, potassium, zinc, manganese and copper at different concentrations in the range detected in the soil analysis (Table 1). Each salt was added separately to the medium to obtain the tested concentration. Three concentrations of each salt was used, for Ca (100, 200 and 400 ppm); Mg and K (50, 100 and 200 ppm); Zn, Mn and Cu (0.1, 0.2 and 0.3 ppm) or unamended (control). The flasks were inoculated with 0.5 ml of bacterial suspension (10^7 cfu/ml), and the inoculated flasks were incubated at 28°C with agitation (150 rpm) for 4 days. Population of *Ecc6* in medium was determined after (1, 2, 3, and 4 days) after inoculation using diluted method and pour plate technique (Haggag and Abd El-Khair, 2006).

2.4. Sensitivity of Erwinia carotovora subsp. carotovora isolates to antibiotics:

Three virulent isolates of *Ecc* (*Ecc6, Ecc1* and *Ecc2*) were tested for sensitivity to streptomycin, tetracycline, ampicillin, and chloramphenicol using the disc-diffusion method according to Gould and Bowie (1952). Nutrient agar plates (10 ml/plate) were inoculated by 0.1 ml of 48h nutrient broth cultures, and evenly spread, on the surface of the agar and filter paper disc 1 cm in diameter (Difco) containing each antibiotic tested with concentrations (25, 50, 75, 100, 150 and 200 ppm) are placed onto the inoculated agar plates. Four discs of each antibiotic were used. Plates were incubated at 28°C for 2 days, then, inhibition zones were recorded according to (Klement *et al.,* 1990). Inoculated plates with discs amended with sterile water were served as control.

- Statistical analysis:

This was conducted using SAS program (Anonymous, 2000). Least significant differences (LSD) were used to separate mean differences and to rank isolates.

Results

1. Characteristics of Erwinia carotovora subsp. carotovora isolates occurring on potato in Behera governorate:

1.1. Morphological and physiological characteristics:

Thirteen isolates of *Ecc* were recovered from diseased potato tubers, showed soft-rot, collected from different fields in Behera governorate. All isolates were rods, non-sporic, gram negative and motile .The isolates exhibited positive reaction for catalase activity, gelatin liquefaction, and production of acid from arabinose, lactose, mannose, raffinose, sorbitol, as well growth on NaCl 6%, and at 36°C. However, the isolates showed negative reaction for hydrolysis of starch, sensitivity to erythromycin and unable to produce of acid from maltose, adonitol and dextrin.

Table 1. Chemical analysis of soil samples collected from four regions where soft rot was occurring on potato in Behera Governorate

Location	N	P	K	Ca	Mg	Na	Mn	Zn	Cu
Abou El-Matameer	0.3	5.0	47	202	81	400	0.8	0.2	0.24
South El-Tahrir	0.3	4.0	63	425	90	800	1.1	0.3	0.27
Nubaria	0.3	6.0	38	325	35	400	0.7	0.1	0.12
Banger El-Sukar	0.2	8.0	250	775	190	1100	1.6	0.4	0.19

* The elements were estimated at ppm.

1.2. Pathogenicity:

Pathogenicity tests of the 13 isolates of *Ecc* on potato tubers (cv. Diamant) showed that all isolates were virulent to different degrees (Table 2). The *Ecc1, Ecc2, Ecc3,* and *Ecc6,* isolates were highly virulent (rot covered ≥ 75% of tuber diameter), seven isolates, *i.e. Ecc4, Ecc5, Ecc7, Ecc9, Ecc10, Ecc*11 and *Ecc13,* out of the 13 isolates were moderately virulent (rot covered < 75% > 25% of tuber diameter) while only two isolates were weakly virulent, *i.e.* rot covered 25% of tuber diameter or less (Table 2).

Table 2. Pathogenicity of thirteen isolates of *Erwinia carotovora* subsp. *carotovora* recovered from Behera governorate fields on Diamant potato tubers

Character	Bacterial isolates of *Erwinia carotovora* subsp. *carotovora*												
Rot developed (%)	*Ecc1*	*Ecc2*	*Ecc 3*	*Ecc 4*	*Ecc 5*	*Ecc 6*	*Ecc 7*	*Ecc 8*	*Ecc 9*	*Ecc 10*	*Ecc 11*	*Ecc 12*	*Ecc 13*
	76.75	77.25	75.00	51.75	65.00	84.75	65.00	24.75	54.25	57.50	65.00	23.45	54.25
Virulence	HV	HV	HV	MV	MV	HV	MV	WV	MV	MV	MV	WV	MV

* Diameter of the rotted area / Tuber diameter x 100 was determined.

** Virulence were determined on potato tubers cv. Diamant, HV= Highly virulent, *i.e.* rot covered ≥ 75% the tuber diameter, MV= Moderately virulent, *i.e.* rot < 75% - > 25% of tuber diameter, and WV=Weakly virulent, *i.e.* rot ≤ 25% of tuber diameter. Data are average of five replicate determined 5 days after inoculation at 28°C.

1.3. Host range:

Data in Table (3) showed that isolates *Ecc* (*Ecc* 1, *Ecc2* and *Ecc* 6) caused significant amount of soft rot on potato tubers and fruits of different vegetable hosts tested (Table 3). On the other hand, data showed that eggplant was the most susceptible to the infection with the three isolates of *Ecc* tested (*Ecc* 1, *Ecc2* and *Ecc6*) with average diameter of rotten area of 33.6 mm, followed by potato tubers (27.9 mm), while a proportionate weakly reaction was observed on pepper and squash as means of the rotted areas developed were 18.1 and 14.1 mm, respectively.

Table 3. Susceptibility of potato tubers and fruits of different vegetable crops to infection with *Erwinia carotovora* subsp. *carotovora* isolates recovered from Behera governorate fields

Host	Diameter of the rotted area (mm)*				
	Bacterial isolates				
	Control	*Ecc1*	*Ecc2*	*Ecc6*	Mean
Egg plant	0.00	32.5	25.8	42.5	33.6a
Potato	0.00	25.5	26.7	31.5	27.9b
Pepper	0.00	19.7	12.9	21.7	18.1c
Squash	0.00	13.3	9.8	21.7	18.1c
Mean	0.00c	22.7b	18.8 b	28.7a	14.1c
					----

* Data are average of five replicates.

- Diameter of the rotted area was determined 5 days after inoculation.
- Means followed by a different letter in the same column or raw are significantly different at p= 0.05.

1.4. Enzymes secretion and activity:

Results presented in Table (4) revealed that the tested isolates of *Ecc* (*Ecc6*, *Ecc10* and *Ecc12*) were able to produce polygalacturonase (PG) and pectate lyase (PL) in the culture media. However, the maximum enzyme activity was observed with *Ecc6* isolate (highly virulent), followed by *Ec10*, (moderately virulent) isolate. On the other hand, the least enzyme activity was observed in case of *Ecc12* (weakly virulent).

Table 4. The *in vitro* activity of polygalacturonase (PG) and pectatelyase (PL) of *Erwinia carotovora* subsp. *carotovora* isolates recovered from Behera governorate

Isolate	Virulence	PL activity * (absorbance at 550 nm)	PG activity (absorbance at 515 nm)
Ecc 6	HV **	0.12	0.325
Ecc10	MV	0.087	0.179
Ecc12	WV	0.027	0.041
Control		0.00	0.00

* Enzymes activity was assessed 3 hours after inoculation.
** HV = highly virulent; MV = moderately virulent and WV= weakly virulent.

2. Control studies:

2.1. Potato varietal resistance:

Data in Table (5) showed that tubers of cv. Nicola were the most tolerant cultivar tested. This was followed by cv. Cara, while cvs. Diamant and Sponta were the most susceptible to the infection with the tested isolates of *Ecc*. No significant differences were detected between Sponta and Diamant. Meantime, inoculated stems of the tested potato cultivars were affected in a similar manner (Table 6). The Diamant and Sponta cvs were the most sensitive to infection by *Ecc* while Nicola and Cara cvs were more tolerant to *Ecc* stem infection.

Table 5. Relative resistance of tubers of potato cultivars artificially inoculated with three isolates of *Erwinia carotovora* subsp. *carotovora* recovered from Behera governorate fields

Cultivar	Diameter of rotted area (mm)*				
	Bacterial isolate				
	Control	*Ecc6*	*Ecc1*	*Ecc2*	Mean
Diamant	0.0	30.0	22.5	15.0	22.5a
Sponta	0.0	22.3	20.5	17.3	20.1a
Cara	0.0	21.7	12.5	9.2	14.4b
Nicola	0.0	12.5	5.0	0.00	5.8c
Mean		1.6a	15.1b	10.4b	----

* Diameter of the rotted area was determined 5 days after inoculation.
- Data are average of five replicates.
- Means followed by a different letter in the same column or raw are significantly different.

Table 6. Relative resistance of aerial stems of potato cultivars artificially inoculated with *Erwinia carotovora* subsp. *carotovora* isolates

Cultivar	Control	*Ecc6*	*Ecc1*	*Ecc2*	Mean
Nicola	1	3	2	1	2.00
Sponta	1	5	4	3	4.00
Diamant	1	5	5	4	4.606
Cara	1	4	3	2	3.00

- Severity of wilting was rated according to the scale of five grades, *i.e.* 1= no symptoms, 2= one leaf wilted, 3= two or three leaves wilted, 4= four or more leaves wilted and 5= plant dead (He *et al.*, 1983).

2.2 Effect of soil extracts:

Data presented in Fig. (1) show that soil extracts from Abou El-Matameer area, which was severely affected with soft rot during the conducted survey, was conducive for *Ecc* growth (13.33×10^7 cfu/ml). On the other hand, soil extracts taken from El-Nubaria, South El-Tahrir and Banger El-Sukar showed pronounced high suppression effect on the bacterial growth compared to the control as populations developed were 8.67, 3.67 and 2.33×10^7 cfu/ml for the above three regions, respectively.

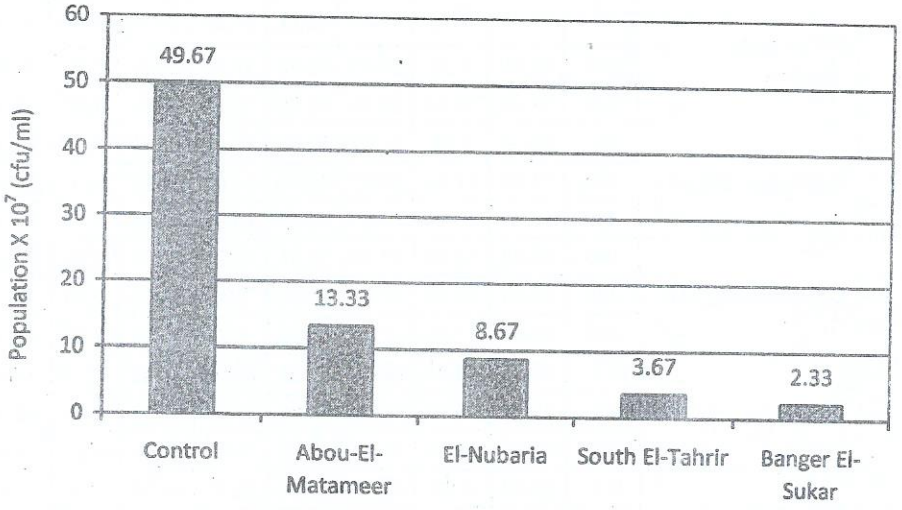

Fig. 1. Effect of soil extracts prepared from soil samples collected from different locations in Behera governorate on population growth of *Erwinia carotovora subsp. carotovora* (isolate *Ecc 6*) under laboratory conditions.

2.3 Effect of mineral salts:

Sulfate salts of calcium, magnesium, potassium, zinc, manganese and copper at the tested concentrations exhibited *in vitro* bactericidal effect and decreased population of *Ecc* in the amended medium (Table 7). The effect increased with time and was most pronounced four days after inoculation. The highest bactericidal effect (≥ 90% reduction) was obtained with 100 ppm magnesium sulfate, 200 ppm potassium sulfate, 100 ppm calcium sulfate 0.3 ppm zinc sulfate, and 0.3 ppm copper sulfate. Meantime, 0.3 ppm manganese sulfate decreased bacterial population by 89.68% (Table 7).

Table 7. The *in vitro* effect of certain salts on population growth of *Erwinia carotovora* subsp. *carotovora* (ECC6 isolate) in culture medium

Treatment	Salts concentration (ppm)	Incubation period							
		1 day		2 days		3 days		4 days	
		Count. (cfu/ml)	Reduction (%)	Count. (cfu/ml)	Reduction (%)	Count. (cfu/ml)	Reduction (%)	Count (cfu/ml)	Reduction (%)
Magnesium sulphate	50	88.67	2.91	41.67	58.88	17.33	83.23	15.00	83.99
	100	84.00	8.03	29.67	70.72	12.33	88.07	7.00	92.53
	200	59.33	35.04	22.33	77.96	9.67	90.64	7.00	92.53
Potassium sulphate	50	86.33	5.47	41.67	58.88	39.67	61.61	15.00	83.99
	100	71.67	21.53	29.67	70.72	14.67	80.96	12.33	86.84
	200	66.67	27.00	24.67	75.65	15.00	85.48	7.00	92.53
Calcium sulphate	100	69.33	24.09	27.00	73.35	15.00	85.48	7.00	92.53
	200	66.67	27.00	22.33	77.69	7.00	93.23	7.00	92.53
	400	44.33	51.46	15.00	85.20	7.00	93.23	7.00	92.53
Zinc sulphate	0.1	54.33	40.51	24.67	75.65	20.00	80.64	12.33	86.84
	0.2	39.33	56.94	22.00	78.29	17.33	83.23	7.00	92.53
	0.3	39.67	56.56	19.67	80.59	9.67	90.64	4.67	95.01
Manganese sulphate	0.1	84.00	8.02	67.33	24.67	39.33	61.94	24.67	73.66
	0.2	71.67	21.53	56.67	44.07	24.67	76.13	12.33	86.84
	0.3	64.33	29.56	49.33	51.23	12.33	88.07	9.67	89.68
Copper sulphate	0.1	34.67	62.04	20.00	80.26	12.33	88.07	9.67	89.68
	0.2	29.67	67.51	19.67	80.59	9.67	90.64	7.00	92.35
	0.3	29.67	67.51	17.33	82.90	7.00	93.23	4.67	95.01

2.4 Effect of antibiotics:

Results in (Table 8 and Fig. 2) show that streptomycin and ampicillin exhibited an inhibition effect on the bacterial growth of the tested isolates of *Ecc* at concentrations tested. This was most pronounced at 200 ppm. However, chloramphenicol did not exhibit a noticeable inhibition effect at all tested concentrations, and also, tetracycline showed low effect even at the high concentration tested (200 ppm).

Table 8. The *in vitro* inhibition effect of different antibiotics on the bacterial growth of *Erwinia carotovora* subsp. *carotovora* isolates recovered from Behera governorate fields

Isolate	Average of inhibition zone (mm)						
	Ampicillin (ppm)						
	0	25	50	75	100	125	200
Ecc 6	0.0	1.5	2.3	3.0	5.3	6.3	7.7
Ecc 1	0.0	7.3	11.7	12.7	12.7	13.3	14.3
Ecc 2	0.0	18.3	22.3	22.5	25.0	26.2	28.2
Mean	0.0	9.03	12.1	12.73	14.33	15.27	16.73
	Tetracycline (ppm)						
	0	25	50	75	100	125	200
Ecc 6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ecc 1	0.0	0.0	0.0	0.0	1.7	2.7	2.7
Ecc 2	0.0	0.0	0.0	1.7	3.3	6.7	10.3
Mean	0.0	0.0	0.0	0.57	1.67	3.13	4.33
	Streptomycin (ppm)						
	0	25	50	75	100	125	200
Ecc 6	0.0	2.0	4.3	6.0	10.3	13.3	15.3
Ecc 1	0.0	10.7	13.3	14.0	14.7	15.7	16.3
Ecc 2	0.0	19.3	22.5	23.0	25.2	26.3	30.3
Mean	0.0	10.67	13.37	14.3	16.73	18.43	20.63
	Chloramphenicol (ppm)						
	0	25	50	75	100	125	200
Ecc 6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ecc 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ecc 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0

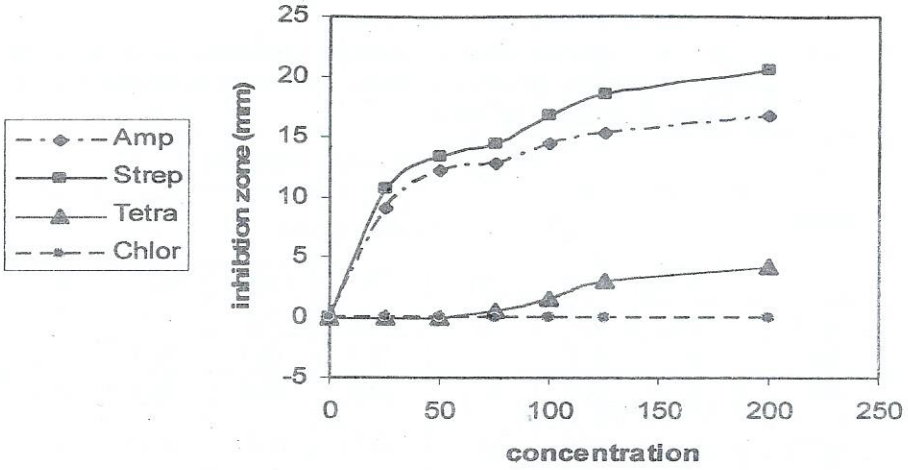

Fig. 2. The *in vitro* inhibition effect of different antibiotics on the bacterial growth of *Erwinia carotovora* subsp. *carotovora* isolates recovered from Behera governorate, 48 hours after inoculation. Amp= Ampicillin, Strep= Streptomycin, Tetra= Tetracycline, Chlor= Chloramphenicol.

Discussion

Isolates of *Erwinia carotovora* subsp. *carotovora* associated with soft rot of potato in Behera governorate were all monomorphic for the different morphological and physiological characteristics tested in the present study. All tested isolates were short rods, non spore forming, Gram negative, motile and exhibited positive reaction for catalase activity, gelatin liquefaction, growth on NaCl 6%, growth at 36°C, production of acid from arabinose, lactose, mannose, raffinose and sorbitol. However, the isolates showed negative reaction for hydrolysis of starch, sensitivity to erythromycin, and unable to produce acid from maltose, adonitol and dextrin. More variations between isolates were recorded for their virulence on potato tubers as 4 isolates out of the 13 tested were classified as highly virulent (rot covered ≥75% of potato tuber diameter) while 7 isolates were intermediate (rot <75- >25%) and 2 isolates were weakly virulent. Meantime, the isolates differed in virulence for the host range and even for the same isolate on the different host species studied. These findings were in harmony with reports published by several research workers in the world (Catara *et al.*, 2001; Hadas *et al.*, 2001; Toth *et al.*, 2003; Cetinkaya-Yildiz *et al.*, 2004; Fiori and Schiaffino, 2004; Agrios, 2005; Fiori *et al.*, 2005; Anajjar *et al.*, 2007 and Hibar *et al.*, 2007). The virulent isolates were found to be polygalacturonase (PG) and pectatelyase (PL) producers. The maximum enzyme activity was observed with the highly virulent isolate, followed by the moderately, while the least enzyme activity was noticed in case of the weakly virulent isolate of *Ecc.* This could be the major factors in the development of soft rot in potato.

Our results were in accordance with Nasuno and Starr, (1966), Hayashi *et al.*, (1997), Schober and Vermeulen (1999), El-Hendawy *et al.* (2002) and Basu *et al.* (2008). In addition, cv. Nicola was the most tolerant cultivar to the soft rot infection by *Ecc* recovered from Behera governorate. The Diamant and Sponta cvs however, were considerably susceptible to *Ecc* isolates while cv. Cara was moderately susceptible. Meantime, the results obtained from pathogenicity tests performed on aerial stems of the tested potato cultivars confirmed the obtained results on tubers. The present investigation revealed a relationship between soft rot bacterial incidence and soil extracts of the surveyed regions in Behera, *i.e.* Abou El-Matameer, El-Nubaria, South El-Tahrir region and Banger El-Sukar. Soil extract prepared from Abou El-Matameer region was conducive for *Ecc* growth (13.33×10^7 cfu/ml), while soil extracts of the other regions were suppressive for *Ecc* growth. This could explain the often observed high soft rot bacterial incidence in Abou El-Matameer compared to the other regions in the conducted survey. Meantime, the laboratory investigation in the present study showed that sulfate salts of calcium, magnesium, potassium, zinc, manganese and copper had an *in vitro* bactericidal effect on *Ecc*. A high bactericidal effect ($\geq$ 90% reduction) was obtained with 100 ppm magnesium sulfate, 200 ppm potassium sulfate, 100 ppm calcium sulfate, 0.3 ppm zinc sulfate, and 0.3 ppm copper sulfate, four days after inoculation. These findings are in agreement with Mills *et al.* (2006). The preservative salts may inhibit the proton motive force across the cell wall (Salmond *et al.*, 1984 and Eklund, 1985), the transport of nutrient (Brown and Booth, 1991), as well as the energy production pathway (decrease in ATP production), discussed by (Kabara and Eklund, 1991). The effectiveness of Ca^{2+} was higher than expected, which may be explained by its ability to destabilize membrane proteins and enzymes (Vaara, 1992). Such a destabilizing effect on the membrane proteins should affect solute transport and other membrane functions. On the basis of obtained data, such results may recommend the use of chelated Ca^{+2} as fertilizer or spray in potato fields to help in controlling soft rot diseases and set tubers. In addition, treating the tubers or soil with calcium sulphate before planting may reduce soft rot symptoms on potato tubers. Meantime, the antibiotics were of significant effect to control *Ecc* isolates affecting potato in Behera governorate. Streptomycin and ampicillin had a considerable inhibitory effect in the *in vitro* tests conducted on *Ecc* isolate particularly at 200 ppm. These findings were in agreement with those obtained by Abd El-Khair (1993) and Abd El-Khair and Haggag (2007).

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الصفات المميزة للبكتيريا اروينيا كاروتوفورا تحت النوع كاروتوفورا التي تصيب البطاطس في محافظة البحيرة- مصر وطرق مقاومتها

محمد أحمد الشيخ *، سيد احمد الغزاز، ايمان العرجاوى*، اسيا رشاد احمد*.**

* قسم أمراض النبات ، كلية الزراعة بدمنهور ، جامعة الإسكندرية.

** قسم أمراض النبات ، كلية الزراعة بالشاطبى ، جامعة الإسكندرية.

تم الحصول على ١٣ عزلة بكتيريا من درنات بطاطس مصابة بالعفن الطري من حقول مختلفة بمحافظة البحيرة وتم تعريف هذه العزلات على أنها بكتيريا اروينيا كاروتوفورا تحت النوع كاروتوفورا (*Erwinia carotovora* subsp. *carotovora*) وذلك باستخدام اختبارات فسيولوجية ومورفولوجية. وبإجراء المزيد من الدراسة كانت النتائج المتحصل عليها كالأتي:

الصنف نيكولا كان أكثر الأصناف مقاومة في عدوى كل من سوق ودرنات البطاطس بينما الصنف اسبونتا كان أكثر الأصناف قابلية للإصابة في عدوى الدرنات يليه في ذلك الصنف دايمونت. وجد أن الصنف دايمونت كان أكثر الأصناف قابلية للإصابة في عدوى سوق نباتات البطاطس أما الصنف كارا فأظهر قابلية متوسطة للإصابة في عدوى كل من السوق والدرنات.

سببت كل العزلات حدوث أعراض العفن الطري على كل من درنات البطاطس وثمار الباذنجان والفلفل والكوسة حيث وجد أن الباذنجان كان أكثر الأنواع النباتية قابلية للإصابة يليه البطاطس بينما كان الفلفل والكوسة اقل قابلية للإصابة.

تسبب مستخلص التربة من منطقة بنجر السكر في حدوث أعلى نسبة خفض في أعداد البكتيريا يليه منطقة جنوب التحرير بينما كان اقل نسبة خفض في مستخلص التربة من منطقة أبو المطامير. وأوضحت نتيجة التحليل الكيميائي لمستخلصات عينات التربة المجمعة من الأربع مناطق أن مستخلص التربة من منطقة بنجرالسكر تحتوي على أعلى تركيز من الكالسيوم والبوتاسيوم والماغنسيوم تتبعها في ذلك منطقة جنوب التحرير مقارنة بالمستخلصات من المناطق الأخرى.

أظهرت النتائج أن اقوى الاملاح تاثيراً على النمو البكتيرى كانت كبريتات النحاس وكبريتات الزنك وكبريتات الكالسيوم حيث كانت نسبة الخفض في أعداد البكتيريا تتراوح ما بين ٦٢,٠٤ - ٩٥,٠١ ٪ و ٤٠,٥١- ٩٥,٠١ ٪ و ٥١,٦٤- ٩٢,٠٣ ٪ على التوالي بينما كان أقل الاملاح تاثيراً على النمو البكتيري كبريتات المنجنيز حيث كانت نسبة الخفض ٨,٢ - ٨٩,٦٨ ٪ حيث أشارت النتائج أن هناك علاقة بين تركيز هذه الأملاح وزيادة نسبة الخفض في النمو البكتيرى.

أسفرت النتائج أن الاستربتومايسين كان أقوى المضادات الحيوية تاثيراً على النمو البكتيري يليه الامبسلين أما الكلورامفينيكول لم يظهر أي تاثير على أي من العزلات بينما كان التتراسيكلين ليس له تاثير على العزلتين *Ecc1* و *Ecc2* مع التركيزات المنخفضة وليس له تأثير على العزلة الثالثة مع كل التركيزات المستخدمة.

وجد أن العزلة *Ecc6* كانت الأعلى نشاطا في إنتاج الأنزيمات البكتينية وهي البكتات ليز والبولي جلاكتوبورنيز يليها *Ecc1* ثم العزلة *Ecc2* ووجد أن نشاط الأنزيمات المتحصل عليها من عدوى الدرنات بالعزلة *Ecc6* كانت أعلى من نشاط الأنزيمات الناتجة من نفس العزلة في راشح المزرعة كما وجد أن أعلى نشاط للأنزيمات المختبرة كان على درجة حرارة ٤٠ و ٣٠°م على التوالي.

مما سبق يمكن استنتاج والتوصية باستعمال عنصر الكالسيوم كسماد أو رشاً على نباتات البطاطس وذلك لزيادة صلابة الدرنات مما يقلل من الإصابة بأمراض العفن الطري لدرنات البطاطس كما أن إضافة الجبس الزراعي كبريتات الكالسيوم للتربة قبل الزراعة يؤدي إلى تقليل الإصابة بالعفن الطري.