Zagazig J. Agric. Res., Vol. 43 No. (3) 2016

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

FACTORS AFFECTING DEVELOPMENT OF POTATO TUBERS SOFT ROT DURING STORAGE

Ahmed M. Khairy[1*], M-R.A. Tohamy[1], M.A. Zayed[1] and H.M. El-Mosalmy[2]

1. Plant Pathol. Dept., Fac. Agric., Zagazic Univ., Egypt

2. Agric. Bot. Dept, Fac. Agric., Zagazig Univ., Egypt

ABSTRACT

Five soft rot bacteria with different degree(s) of virulence were selected out of 25 isolates previously recovered from marketed potato and storehouses in Sharkia Governorate. Pathogenicity test of these isolates to produce soft rot symptoms on Diamant potato tubers was verified. Bacteriological properities of the identified isolates revealed identity with *Erwinia carotovora* subsp. *carotovora* (Jone 1901) Bergey *et al*. (1923). The five selected pathogenic isolates namely FS5, FM3, ES8, EM1 and EM2 were arranged in descending order according to their pathogenic potential. Medium to low growth temperature range from 5-20°C, acidity (pH4.5), usual ventilation during storage and late physiological maturity were unfavorable for greater rot development. Higher growth temperature (30-35°C), anaerobic condition, alkalinity (pH 8.5) and early maturity parameters (90-100 days) increased disease development during storage. Tubers susceptibility to rot revealed that potato Spunta cultivar was the highest one followed by Diamant and King Edward. Liability to Erwinia soft rot differed according to the hosts. It was enormously recognized on garlic, onion bulb, tomato, eggplant and pepper compared limited soft rot on cucumber, squash and some fruitiness.

Key words: *Erwinia carotovora*, potato soft rot, bacteriological properities, ventilation and acidity.

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) crop is usually considered to be the fourth most important staple food source after rice, maize and wheat. Approximately 22% of the crop is lost due to viral, bacterial, fungal and pest attacks, in incurring an annual loss of over 65 million tones due to bacterial soft rot alone that accounts for 30-50% of this huge loss (Czajkowski *et al*., 2011). *Erwinia carotovora* subsp. *carotovora* (Ecc) has been described as a pathogen causing soft rot of different vegetables and several ornamental bulb crops. In literature, attention has focused mostly on ecology, epidemiology, pathogenicity and resistance of the diseases (Pérombelon and Kelman, 1980; Pérombelon, 1992 and 2002).

A comprehensive assessment of pathogenesis, which encompasses the interactions between host, pathogen and the environment leading to latent and active infections, by focusing exclusively on soft rot erwinia pathogenesis or disease development in potato tubers and stems is the subject of several review (Pérombelon, 2002). Disease development, with emphasis placed on the transition from latent to active infection, is examined in two steps: (i) the role of environmental conditions and host reaction (Fawzi, 1980), and (ii) the implications of recent molecular studies on erwinia pathogenicity (Yishay *et al*., 2008).

Erwinia carotovora subsp. *carotovora* was confirmed and identified through biochemical tests and pathogenicity (Tohamy and Sárvari, 1982b; Tohamy, 1984; Akbar *et al.,* 2015) on wide variety of crop species, chiefly potato. Black leg of potato plants and soft rot of tubers either in stored or in the cultvated crop were

***Corresponding author: Tel. :** +201114740138
E-mail address: khkhairy79@yahoo.com

intensively studied during storage condition (Tohamy and Sárvari, 1982a; Tohamy and Sárvari, 1982b; Reeves *et al*., 1999; Bortel and Sauthoff, 2000; Mustafa and Alawami, 2012; Prajapat *et al*., 2013). Development of soft rot is shown to be affected by storage temperature, wetness, percentage of RH and limited aeration during storage (Tohamy, 1984 as well as Kushalappa and Zulfiquar, 2001). The disease is being progressive and affecting tuber quality in Egypt during storage regardless of the variety Makhlouf and Abdeen (2014). Control of post harvest diseases especially in potato soft rot is being difficult and relies on integration of cultural methods, ranging from pre-plant seed-piece handling to post-harvest processing (Farrar *et al*., 2009).

This study aimed to diagnose and identify the cause of the soft rot disease in rotten potato tuber samples collected from different localities at El-Sharkia Governorate and to study the effect of certain growth factors on pathogen growth and rot development during storage was also undertaken.

MATERIALS AND METHODS

Isolation, Identification and Pathogenicity Tests

Samples of potato tubers showing typical soft rot symptoms were collected from storehouses and vegetable markets in (Faqous and El-Salhia localities) El-Sharkia Governorate during 2012-2013 seasons. Samples were transferred directly to the laboratory for diagnosis.

Samples of potato tubers exhibiting soft rot, were washed with tap water, surface sterilized with 95 percent alcohol and flaming. Sterilized tubers were aseptically cut at the region of infection adhering to the healthy tissues and small portion from the infected tissue was crashed and suspended in two ml sterile water in sterilized Petri dishe. The suspension was left for 15 min., then streaked on nutrient ager (NA) medium. After incubation for 24 hr., at 28ºC, separated *Erwinia* – like colonies were picked up and transferred to nutrient agar slants for further investigations. Method of identification was carried out according to (Kiraly *et al.,* 1970 as well as Cuppels and Kelman, 1974).

Bacterial suspensions (10^8 cfu/ml) were prepared from the selected 25 isolates, grown on NA medium for 48 hr. Uniform tubers of Diamant cv., kindly obtained from Daltex Agriculture Company were washed and surface sterilized with 95% ethanol and flaming. Four uniform healthy tuber replicates were used for each tested isolate. Inoculation was carried out with the standardized bacterial suspension (s): A hole of (two cm depth) was made in each tuber with flamed cork – borer (one cm in diam.) and was filled with 0.5ml of the prepared bacterial suspension and kept for 10 min; then closed with the respective removed cylinder. The inoculated tubers and control were tightly sealed by sterile vaspar (vaseline / paraffin. 1:1 *V:V*), and were kept in sterilized polyethylene bags for five days at 30ºC. Control tubers were prepared by the same way using sterile distilled water instead of the bacterial suspension. At the end of incubation period, the volume of rotted tissues was recorded in cm^3 according to Fawzi (1980). The mean volum of rotted tissue produced by each isolate was taken as indicator for the rotting ability of each isolate.

Effect of Incubation Temperatures of *Erwinia carotovora* subsp. *carotovora* on Potato Tuber Soft Rot Development

The most virulent five bacterial isolates (FM3, FS5, EM1, EM2, and ES8) were grown on nutrient broth medium under different incubation temperatures (5, 15, 25, 30 and 35ºC) for 48 hr. A healthy tubers of potato Spunta cv. were surface sterilized, inoculated separately with the adjusted bacterial suspension (10^8 cfu/ml) grown at the previously mentioned incubation temperature and kept at 30ºC for 4 days as shown in pathogenicity test. Control treatment was carried out with sterile distilled water instead of bacterial suspension. Three replicates were used for each treatment. Percentage of rotted tissue weight was calculated according to (Saettler *et al.,* 1989) as follows:

$$\text{Rotted tisues weight (\%) per tuber} = \frac{\text{Weight of rotted tissue}}{\text{Weight of healthy tuber before inoculation}} \times 100$$

Effect of Ambient Temperatures on Soft Rot Development Caused by *Erwinia carotovora* subsp. *carotovora*

Bacterial suspensions (10^8 cfu/ml) of the most virulent five tested bacterial isolates were used to inoculate potato tubers of Spunta cv. Tubers inoculated as mentioned before were kept at 5, 15, 25, 30 and 35ºC for five days. Water was used instead of bacterial suspension in control treatment. Three replicates were used for each treatment. The percentage of rotted tissue weight was calculated for each treatment as previously mentioned in effect of incubation temperature.

Effect of Gaseous Atmosphere on Potato Tuber Rot Development

Healthy tubers of potato Spunta cultivar were surface sterilized and inoculated with a bacterial suspension (10^8 cfu/ml) of the most virulent five bacterial isolates. Inoculated tubers were divided into two groups. One group was kept in plastic net bags as aerobic condition and the other group was kept in polyethylene bags as anaerobic condition. All treatments were stored at 30 ºC for five days. Water instead of bacterial suspension was used in control treatment and three replicates were used for each treatment. Percentage of rotted tissue weight was measured and used to calculate the percentage reduction of disease incidence as mentioned before in effect of ambient temperatures.

Effect of Growth Medium pH of *Erwinia carotovora* subsp. *carotovora* on Soft Rot Development

The most virulent five bacterial isolates were grown in Erlenmeyer flasks containing nutrient broth previously adjusted to 4.5, 6.5, 8.5, and 10 pH values which were prepared according to Kiraly *et al.* (1970), and incubated for 48 hr., at 30°C. Whole tubers of potato Spunta cv. were separately inoculated with the bacterial growth suspension developed at different pH values (0.5 ml/tuber). Inoculation and incubation at 30ºC was carried out as previously mentioned. In control treatment water was used instead of bacterial suspension .Three replicates were used for each treatment. Percentage of rotten tissue weight measured and calculated as previously mentioned in effect of ambient temperatures.

Potato Cultivar Test

Surface sterilized healthy tubers of Diamant, King Edward and Spunta were used. Inoculation was carried out using the most five virulent bacterial isolate suspensions (10^8 cfu/ml). Incubation at 30ºC for five days was carried out as previously mentioned. Water instead of bacterial suspension was used in control treatment. Three replicates were used for each treatment. Percentage of rotten tissue weight was measured and calculated as previously mentioned in effect of ambient temperatures.

Effect of Different Cultivar Maturity on Percentage of Soft Rot Caused by *Erwinia carotovora* subsp. *carotovora*

Healthy tubers of Lady Rosetta, Caruso and Mundial cultivars each of them have two different growth stage (early mature cv. 90-100 days and late mature cv. 120 days) were used. Bacterial suspension of the most five virulent isolates was prepared as previously mentioned. Inoculation and incubation was carried as previously mentioned. Three replicates were used for each treatment. Percentage of rotted tissue weight was measured and calculated as previously mentioned.

Host Range

The usual common varieties of potato tubers (*Solanum tuberosum,* Spunta cv.), healthy fruits of pepper (*Capsicum annuum*, California wonder 300 cv.), squash (*Cucurbita pepo,* Eskandarany cv.), eggplant (*Solanum melongena*, Black beauty cv.), tomato (*Solanum lycopersicum,* Alicante cv.), cucumber (*Cucumis sativus* ,Pot Luck cv.), onion bulb (*Allium cepa,* Red onion cv.), Garlic head (*Allium sativum,* Garlic cheives cv.) as well as fruits of Apple (*Malus domestica,* green apple cv.), Orange (*Citrus sinensis*, navel orange cv.), Lemon (*Citrus limon*, Citrus aurantifolia cv.) and Olive (*Olea europaea,* Picholine cv.) were used. Inoculum with the five virulent isolates was used at 10^8 cfu/ml. Inoculation was carried out by pricking the tubers, fruits, bulbs and head garlic with sterilized needle previously dipped in bacterial suspension. All inoculated tubers, fruits, bulbs and garlic heads were kept in polyethylene bags as anaerobic condition, and stored at 30ºC for five days. Control treatment similarly inoculated

with sterile water instead of bacterial growth. Three replicates were used for each treatment. Disease measurement was expressed as the percentage of rotted tissue weight and calculated as previously mentioned.

Statistical Analysis

Data were arranged in an Excel datasheet (Microsoft office 2010). The averages were estimated. Data then were analyzed according to Snedecor and Cochran (1980) using computer COSTAT-C program to statistically analyze the variance of differences using ANOVA test including Fisher test and LSD analysis.

RESULTS AND DISCUSSION

Isolation, Identification and Pathogenicity Test

The selected twenty five bacterial isolates were found to be pathogenic and produced typical soft rot symptoms in Diamant potato tubers, with varied virulence. The most aggressive isolates, were arranged in descending order, and were coded as FS5, FM3, ES8, EM1 and EM2, with rotting potentials expressed as rot volume that recorded 19.8, 14.3, 11.5, 10.6 and 9.8 cm^3, respectively. Smears of the tested isolates showed short rod bacteria without sporulation and Gram negative. They were able to grow at 36ºC. Other morphological and physiological properties were conforming with those of *Erwinia carotovora* subsp. *carotovora* (Jones, 1901) Bergey *et al*. (1923) according to (Tohamy and Sarvari, 1982b; Boone *et al.,* 2001; Brenner *et al*., 2005). Difference between carotovora and atroseptica were detected on their growth at 36ºC, utilization of ethanol from alcohol agar medium as well as fermentation of α-methyl glucoside according to (Tohamy, 1984).

Effect of Cardinal Temperature of *Erwinia carotovora* subsp. *carotovora* on Tuber Rot Development

Table 1 shows the influence of cardinal temperature on rot development produced by *Erwinia carotovora* subsp. *carotovora*. The highest percentage of rot produced was recorded with isolates grown at 35ºC being 48.4% for the mean rot of the five isolates under investigation. The highest rot percentage was recorded by FS5, FM3 that showed 73.47% and 65.50%, respectively. The moderate rot was recorded for ES8, EM1and EM2 being 45.74%, 32.53% and 24.76., indicating a case of strain variation in rotting ability of different carotovora isolates. Such variation was recorded by Pérombelon and Kelman (1980) and Tohamy and Sarvari (1982b).

Incubation of carotovora strains at a lower temperature, at 30ºC, resulted in smaller rot percentage being 18.53% compared to that produced following incubation at 35ºC, that showed 48.40%. Moreover, negligible rot percentage may be recognized for isolates incubated at 5ºC, being 0.094%, that may be attributed to the lower pathogen density produced at such incubation temperatures. The same effect is shown to be extended to the rot produced by isolates incubated at 15°C.

In this regard the cardinal temperature of different species of the pathogen affiliated to the genus Erwinia were previously reported (Malcolmson, 1959; Hyman *et al.,* 1998). The differentiation between different species and subspecies according to the maximum growth temperature was indicated by Tohamy (1984).

Effect of Ambient Temperature on Tuber Rot Development

Table 2 shows the effect of ambient temperature on rot development produced by the five *E. carotovora* subsp. *carotovora* isolates under investigation. The highest mean percentage of rot produced was recognized for tubers incubated at 35°C, being 19.54% compared to 11.79% for those kept at 30°C. Moreover, a pronounced decrease in the mean rot percentage was recognized for tubers kept at lower temperature ranging between 5°C to 25°C without any significant differences.

The increased rot percentage of tubers kept at high temperatures namely 35°C and 30°C, may be attributed to the favourable effect of temperature on initial and extended proliferation of the pathogen *in situ*. However, the high temperature of incubation, regardless of humidity,

Table 1. Effect of cardinal temperatures of *Erwinia carotovora* subsp. *carotovora* on the percentage of tuber rot weights of Spunta cv. in grams

Pathogenic isolate	Percentage of rot produced after incubation of cultures at ºC					Mean
	5ºC	15ºC	25ºC	30ºC	35ºC	
FM3	0.08	0. 56	12.48	19.52	65.50	19.63
FS5	0.14	0.69	17.64	26.33	73.47	23.65
EM1	0.09	0.50	2.76	15.57	32.53	10.29
EM2	0.10	0.48	2.52	15.05	24.76	8.58
ES8	0.06	0.29	5.52	16.19	45.74	13.56
Mean	0.094	0.50	8.18	18.53	48.40	15.14

LSD 0.05 for Temperature (T) =10.29 Isolate (I) = 6.84 TxI = 12.50

Table 2. Effect of ambient temperature on percentage of soft rot caused by *Erwinia carotovora* subsp. *carotovora*. infected Spunta potato tubers

Pathogenic isolate	Percentage of rot at different temperature ºC					Mean
	5ºC	15ºC	25ºC	30ºC	35ºC	
FM3	0.33	0.38	0.86	13.19	21.25	7.20
FS5	0.71	0. 44	1.43	22.48	30.31	11.07
EM1	0.25	0.31	0.54	8.29	12.47	4.37
EM2	0.11	0. 25	0.25	6.21	12. 00	3.79
ES8	0.26	0.32	0.63	8.81	14.16	4.84
Mean	0.33	0.33	0.74	11.79	19.54	6.55

LSD 0.05 for Temperature (T) = 6.526 Isolate (I) = 4.716 TxI = 10.776

may enhance periderm formation and making the cut tissues at the site of inoculation less liable to invasion. Suberization and periderm formation were intensively studied in literature (Appel, 1906; Priestley and Woffenden, 1923; Artshwager, 1927) Moreover, the lytic enzymes, responsible for rot induction are known to be favoured by higher temperatures (Hasegawa *et al*., 2005) and calcium treatment (Schober and Vermeulen, 1999).

The variation of rotting ability of isolates under investigation is quite clear and ranging between high rot percentage, for the isolate FS5 (11.07%) and low for the isolates EM2 (3.79%), and conforming with results presented in Table 1 on the effect of cardinal temperatures.

Effect of Gaseous Atmosphere on Percentage of Potato Tuber Rot Tissue Caused by *Erwinia carotovora* subsp. *carotovora* Isolates

Results in Table 3 reveal decreased rot percentage under aerobic conditions compared to those kept under anaerobic restricted ventilation. The percentage reduction of rot ranged between 94.5 and 96.2 under aerobic compared to 33.88% and 81.4% under anaerobic conditions. Oxygen depletion associated with a build-up of carbon dioxide around tubers are important factors that promote soft rot development (Perombelon and Kelman, 1980). It is worthy to mention that *Erwinia carotovora* strains are facultative anaerobes. If tubers in soil or during storage are covered with a thin film of water, depletion of oxygen and build-up of carbon dioxide reduce the activity of oxidase enzymes such as polyphenoloxidase and peroxidase responsible for transforming phenolic compound to quinone. Quinone is a toxic material for erwinia growth. Accordingly oxygen depletion reduces tubers natural resistance to pectolytic bacteria. The build-up of carbon dioxide also causes lenticels to enlarge and facilitate entry of pectolytic bacteria into the tuber (Kushalappa and Zulfiquar, 2001 as well as Toth *et al*., 2003). Also, endogenous pectate lyase play an important role through induction of defence response against erwinia soft rot in potato tubers (Wegener, 2002).

Effect of Growth Medium pH of *Erwinia carotovora* subsp. *carotovora* on Soft Rot Development

Data in Table 4 show that acid reaction of the medium possibly inhibited the growth of Ecc isolates, hence decreased soft rot percentage during storage. In this respect, the lowest rot percentage was recorded at pH 4.5 compared to the highest significant rot at pH 10. It is also documented in our laboratory studies, unpublished data, that pH value significantly influenced Erwinia growth. No bacterial growth was occurred either below pH 4.5 or above pH 10.5, these is being justified by those reported by Elian *et al*. (2005). Value of pH5 characterizes many fruits juice and is probably an important factor in their general resistance to bacteria decay, but higher pH value furthers affect the post-harvest development decays of various fungi. In fact, fungi cause most of the decays in harvested fruits, whereas bacteria are important mainly in vegetables. Unlike the fruits, various other plant organs such as roots, tubers, stems or leaves, where the pH level is generally higher and ranges between 4.5 and 7.0 might often be attacked by bacteria (Lund, 1983; Bartz and Eckert, 1987; Tohamy, 1992). Exceptions to that rule are some "fruit-vegetables", such as tomatoes, bell peppers and cucumber, in which bacterial decay is quite common. The pH of soft rot lesions on tomato fruits is higher (pH $\geq$ 5.0) than that of surrounding healthy tissue (pH = 4.3 - 4.5), suggesting that the bacteria are capable of buffering their environment in a range suitable for their growth and the activity of their pectolytic enzyme (Bartz and Eckert, 1987).

Potato Cultivars Test

Data in Table 5 shows the reaction of certain potato cvs. against Ecc isolates under investigation. Results indicate that the tested potato cultivars reacted differently and varied significantly in their degree of susceptibility. Spunta cv. was the highest in susceptibility that showed 53.31% followed by Diamant cv. 23.33% while king Edward was the most tolerant cv. as shown by the limited degree of rot tissues (10.65%). Difference in susceptibility to bacterial soft rot among potato cvs. may be attributed as mentioned by Fric and Williams (1976) to one or all of the following (i) differences in physiological properties of cultivar tubers. (ii) changes in the properties associated with disease development. (iii) differences in genetic make-up of tested cultivars. Also, Wegener (2002) observed that potato lines expressing the pectate lyase showed a better resistance to Erwinia soft rot than the non-transgenic counterpart. Tuber slices methods suggested by Tohamy and Sarvari (1982 a) and Nabhan *et al.* (2006) helps the research workers to study the susceptibility degree of newly introduced potato cvs., to bacterial soft rot on tubers and investigates the associated physiological changes in potato tissues.

Table 3. Effect of gaseous atmosphere on percentage reduction of potato tuber rot caused by *Erwinia carotovora* subsp. *carotovora*

Pathogenic isolate	Rot percentage under different condition			
	Aerobic	Rot decrease (%)	Anaerobic	Rot decrease (%)
FM3	4.5	94.5	32.5	66.93
FS5	5.0	94.7	66.3	33.88
EM1	3.7	95.3	21.1	79.01
EM2	2.8	96.2	17.6	81.4
ES8	4.2	95.8	23.4	75.6
Mean	4.04	95.3	32.18	67.36
LSD	3.07	--------	25.21	--------

Table 4. Effect of growth medium pH of *Erwinia carotovora* subsp. *carotovora* isolates on percentage of rot produced in spunta potato tubers

Pathogenic isolate	Rot percentage at different pH values				Mean
	4.5	6.5	8.5	10	
FM3	10.61	15.75	28.83	44.83	25.00
FS5	9.21	17.59	34.59	64.54	31.48
EM1	5.54	12.05	12.85	17.35	11.94
EM2	1.02	6. 68	11.76	12.58	8.01
ES8	6.45	13.26	16.66	33.63	17.5
Mean	6.56	13.66	20.93	34.58	19.18

LSD 0.05 for pH = 11.49 Isolate (I) = 7.13 pH x I = 15.21

Table 5. Reaction of some potato cultivars to *Erwinia carotovora* subsp. *carotovora* isolates expressed as percentage of rot tissue produced

Pathogenic isolate	Cultivar			Mean
	Diamant	King Edward	Spunta	
FM3	27.42	18.64	61.39	35.81
FS5	40.08	19.01	69.77	42.28
EM1	16.05	6.31	27.16	16.50
EM2	5.38	0.56	16. 92	7.62
ES8	27.75	8.73	54.94	30.47
Mean	23.33	10.65	53.31	29.10

LSD 0.05 for Cultivar (C) = 16.14 Isolate (I) = 7.64 CxI = 18.45

Effect of Different Cultivar Maturity of some Potato Cultivars Inoculated by *Erwinia carotovora* subsp. *carotovora* on the Percentage of Soft Rot

Table 6 indicate that potato cultivars reacted differently according to their physiological maturity stage, against *E. carotovora.* Significant differences between tested isolate was also observed herein. FS5 isolate was the most significant one followed by FM3, ES8, EM1 and EM2. No. significant differences between early and late growth stage of Caruso and Lady rosseta cultivars. On the other hand, significant differences were detected between the two tested stages in Mondial cultivar where less rotted tissues weight in late mature stage. Late mature growth stage (120 days) of Caruso cv. exhibited the highest susceptible degree (23.42%) followed by Mondial cv. (15.86%) then Lady rosseta (10.92%). When these cvs., harvested at early mature stage (90-100 days). The percentage of soft rotted tissues increased for the three tested cultivars at early mature stages. Increase resistance in 120 days of late mature stage might be attributed to the decrease moisture contents in tuber tissues compare to the early mature stage (90- 100 days) and to the increase of epidermis layers. It is worthly to mention that, if tubers remain too along in the soil after vine death, however, they can become over mature. In such cases, starch convert back to sugar and specific gravity declines causes more rot in tissues (Pavlista, 1995).

Host Range

Data in Tables 7 and 8 show that the bacterial isolates FM3, ES8 and FS5 of Ecc caused the highest disease severity on garlic, onion bulb, tomato, eggplant and pepper fruits. However, the lowest percentage of disease severity was observed on cucumber and squash. Disease severity differed between hosts according to the interaction between the pathogenic isolate and the host tissues (Table 7). The same effect of FS5, FM3 and ES8 was observed in case of orange and olive comparing with apple and lemon. These results agree with these recorded by Altin and Bora (2001), Toth *et al*. (2003) and Yishay *et al.* (2008).

Table 6. Effect of different physiological potato maturity on the percentage of soft rot produced by *Erwinia carotovora* subsp. *carotovora* isolates

Pathogenic isolate	Percentage of rot produced by cultivars with different maturity stage						Mean
	Lady rosseta (late)		Caruso (early)		Mondial (early)		
	100 day Early	120 day Late	100 day Early	120 day Late	100 day Early	120 day Late	
FM3	24.07	14.01	31.22	24.10	38.47	17.43	24.88
FS5	28.3	16.14	36.18	44.42	38.63	25.81	31.58
EM1	16	9.56	18.03	14.12	26.11	14.79	16.435
EM2	10.40	4.28	9.15	12.24	24.35	13.76	12.36
ES8	21.69	10.62	24.91	22.26	29.77	7.53	19.46
Mean	20.09	10.92	23.89	23.42	31.46	15.86	20.94
LSD 0.05 for	Mature stage = 13.446		Isolate (I) = 12.04		CXI = 15.71		

Table 7. Host range of *Erwinia carotovora* subsp. *carotovora* tested isolates determined as percentage rot weight in tested vegetables

Pathogenic isolate	Hosts							Mean
	Tomato	Pepper	Eggplant	Squash	Cucumber	Onion	Garlic	
FM3	55.89	60.30	62.40	57.21	52.28	90.50	97.16	67.96
FS5	77.50	70.30	70.57	59.37	53.14	90.84	98.08	50.76
EM1	49.85	52.86	52.73	49.37	47.88	78.94	93.74	60.76
EM2	48.65	34.32	43.89	48.26	43.54	73.50	89.16	54.47
ES8	50.72	53.83	60.54	55.57	50.92	87.31	95.51	64.91
Mean	63.72	54.32	57.99	53.95	49.55	84.21	94.73	59.77

LSD 0.05 for Host (H) = 54.32 Isolate (I) = 53. 11 H X I = 52.95

Table 8. Host range of *Erwinia carotovora* subsp. *carotovora* tested isolates determined as percentage rot weight in tested fruits

Pathogenic isolate	Hosts				Mean
	Apple	Orange	Lemon	Olive	
FM3	15.76	17.93	10.37	22.87	16.73
FS5	17.38	24.39	15.97	28.43	21.54
EM1	8.68	13.69	5.62	7.69	8.92
EM2	6.86	11.61	5.42	8.95	8.21
ES8	9.48	14.82	7.98	14.85	11.87
Mean	11.63	16.48	9.07	16.55	13.43

LSD 0.05 for Host (H) = 9.066 Isolate (I) = 9.355 HxI = 15.21

REFERENCES

Akbar, A., A. Musharaf, N. Azra, S.Z. Khan and A. Zahoor (2015). Characterization of the causal organism of soft rot of tomatoes and other vegetables and evaluation of its most aggressive isolates. Am. J. Plant Sci., 6 (4): 511-517.

Altin, N. and T. Bora (2001). Biological control studies by fluorescent pseudomonas against *Erwinia carotovora* subspp. *carotovora* caused soft rot on potato. Proc. Ninth Turkish Phytopathology Congress, 3–8 September, Tekirdag, Turkey, 104–110.

Appel, O. (1906). Zur kenntris des wund vershlusses beiden kartoffeln. Ber. Dt. Bot. Ges. 24:118-122.

Artshwager, E. (1927). Wound periderm formation in the potato as affected by temperature and humidity. J. Agric. Res., 35: 995-1000.

Bartz, J.A. and J.W. Eckert (1987). Bacterial diseases of vegetable crops after harvest. A review. Plant Pathol., 50:351-376.

Bergey, D.H., F.C. Harrison, R.S. Breed, B.W. Hammer and F.M. Huntoon (1923). Bergey's Manual of Determinative Bacteriology, 1st Ed. The Williams and Wilkins Co, Baltimore, 442.

Boone, D.R., R.W. Castenholz and G.M. Grrity (2001). Bergy's Manual of systematic Bacteriology, volume 1, 2nd Ed. the Archae and the Deeply Branching and Photrophic Bacteria. New York, USA: Springer Publishing.

Bortel, H. and W. Sauthoff (2000). *Erwinia chrysanthemi*, EPPO, 2 (53): 212-216.

Brenner, D.J., N.R. Krieg, J.T. Staley and G.M. Garrity (2005). Bergey's Manual of Systematic Bacteriology, Vol. 2, parts A, B and C, Springer-Verlag, New York, NY.

Cuppels, D. and A. Kelman (1974). Evaluation of selective media for isolation of soft rot bacteria from soil and plant tissue. Phytopathol., 64: 468-475.

Czajkowski, R., M.C.M. Perombelon, J.A. Van Veen and J.M. Van der Wolf (2011). Control of black leg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. Plant Pathol., 60 (6): 999-1013.

Elian, S.Y., J.T. Russell and A. Joseph (2005). Inhibitory effects of organic and inorganic salts on the growth of *Erwinia carotovora* , a soft rot causative agent in potato tubers: Physico. Chem. Basis Potato Res., 80: 105-111.

Farrar, J.J., J.J. Nunez and R.M. Davis (2009). Losses due to lenticel rot are an increasing concern for Kern County potato growers. California Agric., 63 (3):127-130.

Fawzi, F.G. (1980). Studies on soft rot of some economical plants in Egypt, ph.D. thesis, Fac. Agric. Ain. Shamas Univ., Egypt.

Fric, F. and P.H. Williams (1976). Physiological Plant Pathology, Springer-Verlag, Berlin, 4: 617-631.

Hasegawa, H., A. Chatterjee, Y. Cui and A.K. Chatterjee (2005). Elevated temperature enhances virulence of *Erwinia carotovora* subsp. *carotovora* Strain EC153 to plants and stimulates production of the quorum sensing Signal, N-acyl homoserine lactone, and extracellular proteins. Am. Soc. for Microbiol., 71 (8): 4655–4663.

Hyman, L.J., I.K. Toth and M.C.M. Perombelon (1998). Methods for the detection and quantatification of *Erwinia carotvora* subsp *atroseptica* on Potatoes. Laboratory Manual. Invergowrie. Scottish Crop Res. Inst., 60-65.

Kiraly, Z., Z. Klement, F. Solymosy and J. Voros (1970). Methods in Plant Pathology. Akademiai kiado, Badapest, printed in Haugary.

Kushalappa, A.C. and M. Zulfiquar (2001). Effect of wet incubation time and temperature on infection and storage time and temperature on soft rot lesion expansion in potatoes inoculated with *Erwinia carotovora* subsp.*carotovora,* J. Potato Res., 44 (3): 233-242.

Lund, B.M. (1983). Bacterial Spoilage Post-Harvest Pathology of Fruits and Vegetables. London: Acad. Press, 218–257.

Makhlouf, A.H. and R. Abdeen (2014). Investigation on the effect of chemical and biological control of bacterial soft rot disease of potato in storage. J. Biol., Agric. and Healthcare, 4(10): 31-45.

Malcolmson, J.F. (1959). A study of Erwinia isolates obtained from soft rot and blackleg of potatoes. Trans. Brit. Mycol. Soc., 42: 261-269.

Mustafa, H.S.A. and A.M.Y. Alawami (2012). Susceptibility of newly introduced potato cultivars infection with bacterial soft rot and the associated physiological changes. J. Agric. Sci. and Technol., 2 (8): 976-982.

Nabhan, S., S. Al-Chaabi and M. Abu-Ghorrah (2006). Evaluation of pathogenicity of different Erwinia isolates causing potato soft rot and black leg, and assessment of susceptibility of some potato cultivars under laboratory conditions, Arab. J. Pl. Prot. 24: 20-27.

Pavlista, A.D. (1995). Potato production stages: Scheduling key practices. Histrolical Material from University of Nebraska-Lincoln Extension. Paper 1584.

Pérombelon, M.C.M. (1992). Potato black leg: epidemiology, host-pathogen interaction and

control. Netherlands J. Plant Pathol., 98 (2): 135–146.

Pérombelon, M.C.M. (2002). Potato diseases caused by soft rot erwinias: an overview of pathogenesis. Plant Pathol., 51: 1–12.

Pérombelon, M.C.M and A. Kelman (1980). Ecology of the soft rot erwinias. Ann. Rev. Phytopathol., 18: 361–387.

Prajapat, R., A. Marwal and P.N. Jha (2013). *Erwinia carotovora* associated with Potato: A critical appraisal with respect to Indian perspective. Int. J. Curr. Microbiol. Appl. Sci., 2 (10): 83-89.

Priestley, J.H. and L.M. Woffenden (1923). The healing of wounds in potato tubers and their propagation by cut sets. Ann. Appl. Biol., 10: 96-115.

Reeves , A.F., O. M. Olanya, J. H. Hunter and J. M. Wells (1999). Evaluation of potato varieties and selections for resistance to bacterial soft rot. Am. J. Potato Res., 76 (4): 183-189.

Saettler, A.W., N.W. Schaad and D.A. Roth (1989). Detection of Bacteria in Seed and Other Planting Material. APS Press, St. Paul, Minnesota, USA.,122.

Schober, B.M. and T. Vermeulen (1999). Enzymatic maceration of witloof chicory by the soft rot bacteria *Erwinia carotovora* subsp. *carotovora*: the effect of nitrogen and calcium treatments of plant on pectic enzyme production and disease development, Eur. J. Plant Pathol., 105 (4): 341- 349.

Snedecor, G.W. and W.G. Cochran (1980). Statistical methods. Oxford and J. PJ. Publishing Com 7th Ed.

Tohamy, M.R.A. (1984). On the Soft Rot Storage Diseases of Potato in Hungary with Special Respect to Those Caused by *Erwinia*. Ph.D. Thesis, Hung. Acad. of Sci., Budapest; Agric. Sci. Univ., Keszthely.

Tohamy, M.R.A. (1992). Serological differentiation between different isolates of *Erwinia carotovora* subsp. *carotovora* (Jones) Dye and *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye. Zagazig J. Agric. Res., 19 (3): 1249-1258.

Tohamy, M.R.A. and I. Sárvari (1982a). A new technique for the rapid detection of susceptibility of different potato varieties to infection with soft rot bacteria. KGST Potato Breeding Committee, Keszthely, Hungary, Aug., 16-20.

Tohamy, M.R.A. and I. Sárvari (1982b). Isolation and identification of potato soft rot causative organisms from Hungarian and Egyptian materials. Proc. of the 2nd Egyptian-Hungarian Conf. of Plant Prot., 258-268. a Conf. held at Alex. Univ., Egypt, Sept., 21-24.

Toth, I.K., K.S. Bell, M.C. Holeva and P.R.J. Birch (2003). Soft rot erwiniae: from genes to genomes. Molecular Plant Pathol., 4 : 17–30.

Wegener, C.B. (2002). Induction of defence responses against Erwinia soft rot by an endogenous pectate lyase in potatoes. Physiol. Mol. Plant Pathol., 60:91–100.

Yishay, M.L., S. Burdman, A. Valverde, T. Luzzatto, R. Ophir and I. Yedidia (2008). Differential pathogenicity and genetic diversity among *Pectobacterium carotovorum* spp *carotovorum* isolated from monocot and dicot hosts support early genomic divergence within this taxon. Environ. Microbiol., 10: 2746–2759.

العـوامـــل المـــؤثـــرة علـــى تكشـــف العفـــن الطـري فـي درنـــات البطـاطـس أثنـاء التخـزين

أحمد محمد خيرى[١] - محمد رضا أحمد تهامى[١] - محمد أمين عبدالمنعم زايد[١] - حسن محمد حسن المسلمى[٢]

١- قسم أمراض النبات - كلية الزراعة - جامعة الزقازيق – مصر

٢- قسم النبات الزراعي- كلية الزراعة - جامعة الزقازيق – مصر

أختيرت خمس عزلات مختلفة فيما بينهما فى درجة شراستهم المرضية من بين ٢٥ عزله تم عزلها من بعض أسواق وثلاجات التخزين بمحافظة الشرقية، كان لها القدرة على إنتاج العفن الطري في درنات بطاطس الصنف دايمونت، أوضحت نتائج التعريف باستخدام الطرق المورفولوجية والفسيولوجية أن هذه العزلات تتبع بكتيريا ايروينيا كاروتوفورا تحت نوع كاروتوفورا ورتبت تنازليا طبقا لقدرتها المرضية، ثبطت درجات الحرارة المنخفضة (٥-٢٠°م) وحموضة البيئة (pH 4.5) نمو المسبب المرضى وبالتالي إنتاج العفن كما ثبطت درجات الحرارة المنخفضة والظروف الهوائية أثناء التخزين وأصناف البطاطس متأخرة النضج تكوين العفن الطري على العكس من ذلك زاد العفن تحت ظروف درجة الحرارة المرتفعة والقلوية والظروف اللاهوائية والأصناف مبكرة النضج التي سببت زيادة العفن، أوضحت نتائج اختبار الأصناف أن صنف سبونتا كان الأكثر قابلية للإصابة فى حين اختلفت قابلية العوائل للإصابة حيث رتبت تنازليا كالآتي : الثوم، البصل، الطماطم، الباذنجان، الفلفل فى حين أن كل من الخيار والكوسة كان أقلهم إصابة، كما كانت ثمار الزيتون والبرتقال أكثر قابلية للإصابة من ثمار التفاح والليمون.

المحكمون :

١- أ.د. نبيل صبحي فرج أستاذ أمراض النبات المتفرغ بمعهد بحوث أمراض النبات – مركز البحوث الزراعية.

٢- أ.د. أحمد زكـي علـى أستاذ أمراض النبات المتفرغ – كلية الزراعة – جامعة الزقازيق.