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Management of soft rot disease, caused by *Erwinia carotovora* subsp. *carotovora* in potato tubers

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ABSTRACT

Six isolates of *Erwinia carotovora* were isolated from naturally infected samples in different localities in Egypt. All isolates gave the same results in the pathological, morphological, physiological and biochemical tests and identified as *E. carotovora* subsp. *carotovora*. The isolates showed variation in their virulence. The isolate ET was selected because this isolate was the nearest isolate to *E. carotovora* and also it was the most severe isolate on potato tubers. The effects of oxalic acid and biological control agents, *Penicillium simplicissmum* GP17-2, *Pseudomonas putida* T15, *Pseudomonas vranovensi* A30, *Pseudomonas resinovorans* A5 and *Pseudomonas brassicacearum* N32 on disease progress were evaluated. Oxalic acid at concentrations of 5, 10 and 20 mM and biological control agents significantly reduced soft rot development in wounded entire potato tubers cv. Kara as compared with the control. Disease development was significantly decreased by the tested treatments with increasing soaking time. Data obtained in this study showed that the tested concentrations of oxalic acid proved to be superior to biological control agents in reduction of soft rot disease except for GP 17-2 and T15 isolates.

Key words: Soft rot disease, *Penicillium simplicissmum*, *Pseudomonas putida*, oxalic acid, potato, induced resistance.

INTRODUCTION

Potato is an essential crop worldwide. Among potato disease, bacterial soft rot is considered a common disease all over the world. Infection of potato plants by late blight could increase the possibility of secondary infection by *Erwinia carotovora* subsp. *carotovora* (Lui *et al*., 2005). *E. carotovora* subsp. *carotovora*, the most common soft rot pathogen, is a gram negative, necrotrophic, facultative anaerobe of the family *Enterobacteriaceae*, motile via flagella (Pérombelon and Kelman, 1980; Pérombelon, 2002). The symptoms of bacterial soft rot include fast softening of the tuber tissues which could be reduced to a watery mass within a week under humid conditions and optimum temperatures. The pathogen invades the roots through stomata, lenticels, wounds and the stolen of mother plant then multiplies quickly causing maceration of tuber tissues by secretion of different extracellular degrading enzymes (Elphinstone, 1987; Pérombelon and Salmond, 1995). Induced resistance in plants can be stimulated either by localized infection with pathogens or by treatment with certain chemicals such as oxalic acid, salicylic acid (SA) and acetylsalicylic acid (ASA) and several beneficial microorganisms such as plant growth promoting fungi and plant growth promoting rhizobacteria (Kabeil *et al.*, 2008; Elsharkawy *et al*., 2012, 2013, 2018). Rhizosphere microorganisms provide the best alternative strategy to protect potato plants from pathogen infection in order to reduce pesticides application (Kabeil *et*

al., 2008). SA plays a crucial signaling role in triggering plant defense response against different fungal, bacterial and viral pathogens through systemic acquired resistance (SAR) and activating SAR gene expression in most studied systems (Malamy and Klessing 1992; Kessmann *et al*., 1994; Sticher *et al*., 1997). The potential of acetylsalicylic acid (ASA) to stimulate plant defense response against infection with potato soft rot disease caused by *E. carotovora* subsp. *carotovora* was reported by Lopez *et al*. (2001). They showed that soaking potato tubers in ASA solution at concentration of 0.0125% (w/v) significantly decreased the incidence of soft rot disease. They also found that the wounding of potato tubers was the most effective inoculation method and no phytotoxicity was recorded of ASA treatment.

The present study was conducted to reveal the effects of rhizosphere microorganisms, *Penicillium simplicissmum* GP17-2, *Pseudomonas putida* T15, *Pseudomonas vranovensi* A30, *Pseudomonas resinovorans* A5 and *Pseudomonas brassicacearum* N32 and different concentrations of oxalic acid on potato soft rot caused by *E. carotovora* subsp. *carotovora* under storage conditions.

MATERIALS AND METHODS

Isolation of *Erwinia carotovora*

Infected materials were washed and surface sterilized. Samples were macerated in sterile water in test tube and streaked over nutrient agar media (pH 7.0) then incubated for 2 days at 29ºC. The growing isolate was purified using single colony protocol and incubated at 29ºC for 2 days.

Pathogenicity tests

To evaluate the rooting ability of the isolates, standard tubers (70 g in weight) of Kara cultivar were washed and sterilized by flaming. A 0.5 ml suspension of bacterial isolates (10^7cfu/ml) were inoculated on potato tubers by making a hole in each tuber and closing the hole with the removing part after inoculation. The tubers were incubated in sterilized polyethylene bags at 29^{o}C for 5 days. Sterilized distilled water was used to inoculate control tubers instead of the bacterial suspension. The diameters of rotted area were measured in centimeters as described by Hollis and Goss (1950).

Identification of the isolates

Identification the bacterial isolates was done based on cultural, morphological characteristics (Don *et al*., 2005; Krieg and Holt, 1984; Garrity *et al*., 2005). Physiological and biochemical characteristics were evaluated (Graham, 1964; Leilliott *et al*., 1966; De Boer and Kelman 1978; Klement *et al*., 1990)**.**

Effect of biological control agents on inducing resistance against potato soft rot

Inocula preparation of biological control agents

Bacterial isolates were allowed to grow on KB medium and shaken at 160 rpm at 30°C for 24h, then cells were harvested by centrifugation at 12000 rpm for 10 min, washed with SDW and resuspended in 10 Mm $MgCl_2$. The harvested suspensions were adjusted to 4×10^8 CFU/g dry weight soil as a final density.

The fungus GP17-2 was cultured on PDA medium for 7 days. Twenty mycelial disks (5 mm) of each culture were taken from the growing margin of a colony and transferred to a flask containing potato dextrose broth (200 ml). The fungal culture was then maintained at 25°C for 12 days without shaking. The culture filtrate was separated by filtering through two layers of Whatman No. 2 filter paper, and then through a 0.22 μm Millipore filter (Millipore products division, Bedford, USA).

In this experiment, the effect of treating potato tubers with biological control agents and oxalic acid on soft rot disease severity was studied. Potato tubers of the potato cultivar kara was surface sterilized in 1% sodium hypochlorite for 4 minutes followed by washing for 5 times in sterilized distilled water. Tubers were dried at 30°C. The tubers were submerged in different concentrations of oxalic acid 5, 10 and 20 mM and suspensions of biological control agents for the periods of 0.5, 1 and 2 hours. They were evaluated for their susceptibility to rotting after 0, 3, 6- and 9-days storage periods. Tubers were inoculated with two drops of bacterial suspension ($5x10^8$ CFU/ml) as mentioned before. Treated tubers were kept in clean sterilized plastic bags with sterilized moist cotton and incubated at 29°C for 9 days. Tubers were cut into halves to see rotting symptoms. The disease severity was evaluated using the following formula:

DSI = (A - B / A) x 100

Where, A = Tuber weight with rotting, B = Tuber weight without rotting.

Statistical analysis

The statistical significance was assessed by one-way analysis of variance (ANOVA) using XLSTAT-Pro and compared with Duncan's multiple range test (DMRT). Values were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Isolation, purification and pathogenicity of *Erwinia carotovora* isolates

Six isolates were isolated from naturally infected potato, tomato, cucumber and apple samples. The isolates showed the typical symptoms of rot disease on potato tubers. The tested characters were; cell shape, gram reaction, catalase activity, gelatin liquefaction, starch hydrolysis, glucose, maltose, and growth at 37ºC. The isolates were varied in the degree of their virulence and pathogenicity. Table (1) showed that the six tested isolates were pathogenic and produced symptoms of soft rot on potato tubers. The isolate ET gave the highest disease index followed by isolates EA and EP1 and then isolates EP2 and ET1. Whereas, the isolate EC caused the least disease index. These results are in agreement with those reported by many workers (Zayed and maayouf, 1989; Choi *et al*., 1990; Togashi and Nami, 1991; Arsenijevic *et al*., 1996 and Clark *et al*., 1998).

Identification of the causal pathogen

Morphological and physiological characteristics

Some isolates revealed the same morphological, physiological and biochemical characters as *Erwinia carotovora* according to Bergey's Manuals of Systematic Bacteriology (2005). The tested characters were cell shape, gram reaction, catalase activity, gelatin liquefaction, starch hydrolysis, growth on NaCl (5%) and growth at 37ºC. Identification of isolated pathogenic bacteria was carried out using the morphological and physiological characteristics. Results indicated that isolates ET and ET1 were rod-shape, motile, non-sporing, gram negative, gelatin liquefaction positive, starch hydrolysis negative, urease negative, catalase test positive and grow at 37°C. Also, these tested isolates produced acid and gas from sucrose, glucose and maltose (Table 2). On the basis of the obtained data and those reported by Staley *et al*. (2005), it could be stated that ET and ET1 isolates are identified as *Erwinia carotovora* subsp. *corotovora*.

Effect of biological control agents and oxalic acid in inducing resistance against potato soft rot

Data in Tables (3, 4, 5 and 6) indicated that soaking tubers of kara cultivar

in all tested concentrations (5, 10 and 15 mM) of oxalic acid and also biological control agents have significantly reduced soft rot development in wounded entire potato tubers as compared with control. The reduction of soft rot severity increased with increasing time of soaking tubers. Tubers of the tested cultivar soaked for 2h in both tested isolates GP17-2 and T15 and oxalic acid at the highest concentration (20mM) exhibited the highest reduction on disease severity followed by soaking tubers for 1h and then soaking for 30 min as compared with the control (non-treated). Data also revealed that treated potato tubers with all tested conc of oxalic acid and stored for 9 days showed the highest decrease in disease severity (Table 6). Moreover, soaking potato tubers of the tested cultivar in oxalic acid in all tested concentrations caused higher decrease in soft rot severity than treatment with biological control agents except for GP17-2. Increasing storage periods after submerging in oxalic acid were significantly reduced soft rot developments of tested potato tubers. These results are in agreement with the results of Ward *et al*. (1991), Ukness *et al*. (1992) and Wafaa (1996) who reported that increasing intervals time of inoculation with pathogen after aspirin application enhanced resistance induction against bacterial soft rot. Potato soft rot disease caused by *E. carotovora* subsp. *carotovora* was reduced in potato tubers in *P. fluorescence*, *B. subtilis*, and *B. thuringiensis* treatments (Algeblawi and Adam, 2013).

In conclusion, data obtained in this study showed that the utilization of rhizosphere microorganisms as well as oxalic acid were effective and reduced the severity of soft rot disease in potato tubers. It is worthy to note that GP17-2 and T15 reported the best effects against *E. carotovora* subsp. *carotovora* than the other rhizosphere microorganisms.

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REFERENCES

Algeblawi, A.F.A. (2013). Biological control of *Erwinia carotovora* subsp. *carotovora* by *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus thuringiensis*. Int. J. Chem. Environ. Biolog. Sci. (IJCEBS), 1(5): 771-774.

Arsenijevic, M.; V. Tukulja and P. Mitrovic (1996). Bacterial soft rot of cabbage head. Zastita Bilja, 47:301-311.

Don, J.; Noel, R.K. and James, T.S. (2005). Bergey's Manual of Systematic Bacteriology 2nd ed. Vol. 2. George, M.U.S.A., 325-340.

Clark, C.A.; W.M. Hay; P.J. Bond; C. Chen; K.Y. Goh; X. Liang; X. Liu and P. Lotrakul (1998) First report of *E. carotovora* subsp. *carotovora* causing bacterial root rot of sweet potato (*Ipomea batatas*) in Louisiana. Plant Disease, 82:129.

Choi, J.E.K.; S. Hang and J.C. Kang (1990). Bacterial soft rot of iris caused by *Erwinia carotovora* subsp. *carotovora*. Korean J. Plant Pathol., 2:289-291.

De Boer, S.H. and A. Kelman (1978). Influence of oxygen concentration and storage factors on susceptibility of potato tubers to bacterial soft rot (*Erwinia carotovora*). Potato Res., 21:65-80.

Dye, D. W. (1969). A taxonomic study of the genus Erwinia. II. The ''carotovora'' group. N. Z. J. Sci., 12, 81–97.

Elphinstone, J. G. (1987). Soft rot and blackleg of potato Erwinia spp.

Technical Information Bulletin, International Potato Center, No. 21:18pp.

Elsharkawy, M.M.; M. Shimizu; H. Takahashi and M. Hyakumachi (2012). Induction of systemic resistance against *Cucumber mosaic virus* by *Penicillium simplicissimum* GP17-2 in *Arabidopsis* and tobacco. Plant Pathol., 61:964-976.

Elsharkawy, M.M.; M. Shimizu, H.; Takahashi, K.; Ozaki, M. and Hyakumachi (2013). Induction of systemic resistance against *Cucumber mosaic virus* in *Arabidopsis thaliana* by *Trichoderma asperellum* SKT-1. Plant Pathol. J., 29:193-200.

Elsharkawy, M.M.; Suga, H. and Shimizu, M. (2018). Systemic resistance induced by *Phoma* sp. GS8-3 and nanosilica against *Cucumber mosaic virus.* Environ. Sci. Pollut. Res., (in press). doi:10.1007/s11356-018-3321-3

Garrity, G.M.; D.J. Barenner; N.R. Krieg and G.T. Staley (2005). Bergey's Manual of systematic Bacteriology. Second Edition, vol. 2, pp.721-730, the Protobacteria, Part B. the Gamaproteobacteria. Library of Congress, USA.

Graham, D. (1964). Taxonomy of the soft rot coliform bacteria. Ann. Rev. Phytopathol., 2:13-42.

Hollis, J.P. and R.W. Goss (1950). Factors influencing invasion of potato by *Erwinia carotovora*. Phytopathology, 40: 860- 868.

Kabeil, S.S.; S.M. Lashin; M.H. El-Masry; M.A. El-Saadani; M.M. Abd-Elgawad and A.M. Aboul-Einean (2008) American-Eurasian J. Agric Environ. Sci., 4(1):44–54.

Kessmann, J.; T. Staub, C.; Hofmann, T.; Maetzke, J.; Herzog, E.; Ward, S. Uknes and J. Ryals (1994). Induction of systemic acquired resistance in plants by chemicals. Annual Rev. of Phytopathology, 32: 439-459.

Klement, Z.; K. Rudolph and D.C. Sands. (1990). Methods in phytopathology. Akademiai Kiado, Budapest. 133 pp.

Krieg, N.R. and J.G. Holt. (1984). Beregy's Manual of systematic Bacteriology, Vol.1.Williams and Wilkins, Baltimore.

Leilliott, R.A.; E. Billing and A.C. Haywarrd (1966). A determinative scheme for the flurescent plant pathogenetic pseudomonads. J. Appl. Bacteriol., 29: 470-489.

Lopez, M.M.; M.J. Lopez-Lopez; R. Marti; J.J. Zamora Lopez-Sanchez, and R. Beltra (2001). Effect of acetylsalicylic acid on soft rot produced by *Erwinia carotovora* subsp. *carotovora* in potato tubers under greenhouse conditions. Potato Res., 44: 197-206.

Lui, L.H.; A. Vikram; Y. Abu-Nada; A.C.; Kushalappa, G.S.V. and Raghavan, K. Al-Mughrabi (2005) Volatile metabolic profiling for discrimination of potato tubers inoculated with dry and soft rot pathogens. Amer. J. Potato Res., 82:1–8

Malamy, J. and D.F. Klessing (1992). Salicylic acid and Plant disease resistance. Plant J., 2: 643-654.

Pérombelon, M.C.M. and A. Kelman (1980). Ecology of the soft rot Erwinias. Annu. Rev. Phytopathol., 18:361-387.

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 G.P.C. Salmond (1995). Bacterial soft rots. In Pathogenesis and host specificity in

plant diseases. Ed. Singh US, Singh RP, Kohmoto K. Pergamon, Oxford. Vol.1 pp 1-20.

Staley, J.T.; D.R. Boone; G.M. Garrity; P. Devos; M.G. Fellow; F.A. Rainey; K.H. Schlifer; D.J. Brenner, et al. (2005). Bergey's Manual of Systematic Bacteriology, Vol. 2, Williams and Wilking Company Baltimore Med., USA, 469 pp.

Sticher, L.; B. Mauch-Mani and P. Metraux (1997). Systemic acquired resistance. Annual Review of Phytopathology, 35: 235-270.

Togashi, J. and T. Nami (1991). Bacterial soft rot of Chinese chive (*Allium tuberosum* Rottler) Annals of the phytopathological Society of Jaban 57:720-723.

Ukness, S.; B. Mauch-mani; M. Moyer; S. Potter; S. Williams; S. Dincher; D chandler; E. Slusarenko and Ward and J. Ryals (1992). Acquired resistance in Arabidopsis. Plant Cell, 4:645- 656.

Wafaa, M. El-Sayed (1996). Induction resistance to bacterial soft rot disease of potato tubers by application of acetyl salicylic acid (Aspirin). Annals Agric. Sci., Ain Shams Univ., Cairo, 41 (2): 993-1006.

Ward, E.R.; S.J. Unkess; S.C Williams; S.S.Dincher; D.L.W Wirderhold; D.C. Alexander; P. Ahl-Goy; J.P. Metroux and J.A. Ryals (1991). Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell, 3:1085-1094.

Zayed, A. and M. Maayouf (1989). Frist record of soft rot on imported potato varieties in great Libyan Jamahiriya. J. Plant Protection, 7(2): 172- 173.

Table (1): The isolated bacteria, their host plants and the degree of pathogenicity.

Isolates	Host plant	Degree of pathogenicity	ID	Isolates	Host plant	Degree of pathogenicity	ID
(1)	Potato	+++	EP1	(4)	tomato	++	ET1
(2)	Potato	++	EP2	(5)	apple	+++	EA
(3)	tomato	+++	ET	(6)	cucumber	+	EC

+ + + = highly virulent + + = moderately virulent + = weakly virulent

Table (2): Morphological, physiological and biochemical activities of the isolates.

Characteristics	Isolates					
	ET	ET1	EA	EP1	EP2	EC
Cell shape (Rods, single)	+	+	+	-	-	-
Gram reaction	-	-	-	-	-	+
Catalase activity	+	+	-	+	-	-
Gelatin liquefaction	+	+	-	-	+	+
Hydrolysis of starch	-	-	+	+	+	-
Soft rot symptoms	+	+	+	-	-	-
glucose	+	+	-	+	-	-
Maltose	+	+	+	-	-	-
Growth at 37 ^{o}C	+	+	+	-	-	+

Table (3): Effect of submerging potato tubers in different inducers (for 30min before pathogen inoculation) against bacterial soft rot of potato tubers.

Treatment	Days after inoculation					
	3		6		9	
	No. of infected tubers	Infection % to control	No. of infected tubers	Infection % to control	No. of infected tubers	Infection % to control
Healthy	0	0	0	0	0	0
Control	6	30	11	55	20	100
GP17-2	0	0	0	0	1	5
T15	0	0	0	0	1	5
A5	1	5	1	5	2	10
A30	2	10	4	20	6	30
N32	2	10	3	15	5	25
Oxalic acid (5Mm)	1	5	1	5	2	10
Oxalic acid (10Mm)	0	0	0	0	1	5
Oxalic acid (20Mm)	0	0	0	0	1	5

Table (4): Effect of submerging potato tubers in different inducers (for 1hour before pathogen inoculation) against bacterial soft rot of potato tubers.

Treatment	Days after inoculation					
	3		6		9	
	No. of infected tubers	Infection % to control	No. of infected tubers	Infection % to control	No. of infected tubers	Infection % to control
Healthy	0	0	0	0	0	0
Control	5	25	12	60	20	100
GP17-2	0	0	0	0	1	5
T15	0	0	0	0	1	5
A5	0	0	1	5	2	10
A30	2	10	3	15	5	25
N32	2	10	3	15	4	20
Oxalic acid (5Mm)	0	0	0	0	1	5
Oxalic acid (10Mm)	0	0	0	0	1	5
Oxalic acid (20Mm)	0	0	0	0	1	5

Table (5): Effect of submerging potato tubers in different inducers (for 2hours before pathogen inoculation) against bacterial soft rot of potato tubers.

Treatment	Days after inoculation					
	3		6		9	
	No. of infected tubers	Infection % to control	No. of infected tubers	Infection % to control	No. of infected tubers	Infection % to control
Healthy	0	0	0	0	0	0
Control	6	30	12	60	20	100
GP17-2	0	0	0	0	0	0
T15	0	0	0	0	0	0
A5	0	0	0	0	1	5
A30	1	5	2	10	3	15
N32	0	0	1	5	2	10
Oxalic acid (5Mm)	0	0	0	0	1	5
Oxalic acid (10Mm)	0	0	0	0	0	0
Oxalic acid (20Mm)	0	0	0	0	0	0

Table (6): Disease severity of potato soft rot after treatment with different inducers at 9 days after inoculation.

Treatment	Treatment time		
	30min	1 hour	2 hours
Control	89.9a	95.5a	92.47a
GP17-2	2.14f	1.44	1.36g
T15	3.14e	2.25d	1.60fg
A5	6.62d	4.01c	3.59d
A30	28.81b	22.93b	19.54b
N32	23.73c	21.93b	14.43c
Oxalic acid (5Mm)	3.83e	2.21d	2.21e
Oxalic acid (10Mm)	2.46f	2.07de	2.05ef
Oxalic acid (20Mm)	2.04f	1.91e	1.84f

مكافحه مرض العفن الطرى المتسبب عن بكتريا *Erwinia carotovora subsp. Carotovora* في درنات البطاطس

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المستخلص

تم عزل ٦ عزلات من بكتريا *Erwinia carotovora* من عينات مصابه طبيعيا من مناطق مختلفه في مصر. أعطت كل العزلات نفس النتائج في الأختبارات المورفولوجيه والمرضيه والفسيولوجيه والكيميائيه وتم تعريفها على أنها بكتريا *Erwinia carotovora*.

أظهرت العزلات إختلافات في قدرتها المرضيه. تم إختيار عزله ET في هذة الدراسه لأنها كانت أقرب عزله لبكتريا Erwinia carotovora وكانت أيضا أكثر العزلات من حيث الشدة المرضيه على درنات البطاطس. تم تقييم تأثير المعاملات بحمض الأوكساليك والعزلات النافعه *Penicillium simplicissmum* GP17-2, *Pseudomonas putida* T15, *Pseudomonas vranovensi* A30, *Pseudomonas resinovorans* A5 *and Pseudomonas brassicacearum* N32 على تطور المرض. وجد أن المعامله بحمض الأوكساليك بتركيزات ٥، ١٠، ٢٠ مل مولر والعزلات النافعه قد قللت من تطور مرض العفن الطرى فى صنف البطاطس كارا بالمقارنه بالكنترول. وجد أن تطور المرض قد قل بزياده وقت غمس درنات البطاطس في معاملات المقاومه. أظهرت النتائج التى تم الحصول عليها فى هذه الدراسه أن التركيزات المستخدمه من حمض الأوكساليك كانت أكثر تأثيراً من المعامله بالعزلات النافعه فيما عدا التى عادلت حمض الأوكساليك GP 17-2 وT15.