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 simplicissum* 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 simplicissum*, *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
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* 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 simplicissimum* GP17-2, *Pseudomonas putida* T15, *Pseudomonas vranovensis* 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 29°C. The growing isolate was purified using single colony protocol and incubated at 29°C 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 (10^7 cfu/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°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; Leilliot 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 MgCl₂. 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≤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 37°C. 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 Manual 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 37°C. 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. *carotovora*.

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. fluorescens, 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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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 °C                  | +       | +       | +      | -       | -       | +      |
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Table (3): Effect of submerging potato tubers in different inducers (for 30min before pathogen inoculation) against bacterial soft rot of potato tubers.

| Treatment          | 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          | 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          | 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                      | 1                      | 5                      |
| Oxalic acid (20Mm) | 0                      | 0                       | 0                      | 0                      | 1                      | 5                      |
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Table (6): Disease severity of potato soft rot after treatment with different inducers at 9 days after inoculation.

| Treatment                  | Treatment time |
|----------------------------|----------------|
|                            | 30 min         | 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          |

Table: 6

Disease severity of potato soft rot after treatment with different inducers at 9 days after inoculation.

| Treatment                  | Treatment time |
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| T15                        | 3.14e          | 2.25d          | 1.60fg         |
| A5                         | 6.62d          | 4.01c          | 3.59d          |
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| Oxalic acid (20Mm)         | 2.04f          | 1.91e          | 1.84f          |