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Effect of some Environmental Factors on Potato Bacterial Soft Rot

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ABSTRACT

Potato (*Solanum tuberosum* L.) is the third most important food crop of the world after wheat and rice. The aim of study to evaluate effect of some environmental (pH values, temperatures and relative humidity) on the growth and severity of bacterial soft rot. In vitro, the growth of three isolates of *Pectobacterium carotovorum p.v. carotovorum* were affected with different pH values and rate the growth tested isolates were the most increasing with 7.0 and 7.5 pH values. While rate the growth tested bacterial isolates increased with increasing temperatures and the best rate the growth was recorded on 30, 26 and 34°C. Under artificial inoculation conditions, severity of potato bacterial soft rot disease increased with increasing temperatures and was the most severed with 34, 30 and 26°c temperatures. Also, severity of potato bacterial soft rot disease increased with increasing relative humidity (RH) at 90 and 100% RH potato tubers were totally rotted.

Keywords: *Pectobacterium carotovorum p.v. carotovorum*, Potato, *Solanum tuberosum* L., Soft Rot, environmental factors, temperature, relative humidity

1. Introduction

Pectobacterium carotovorum p.v. *carotovorum* is a prominent phytopathogen responsible for bacterial soft rot disease affecting a wide spectrum of crops, particularly potato tubers, resulting in significant post-harvest losses worldwide (Toth *et al.,* 2011). This pathogen's virulence is largely attributed to its secretion of cell wall-degrading enzymes, chiefly pectinolytic enzymes like pectate lyase, which play a fundamental role in the maceration of host plant tissues and subsequent disease progression (Liao & Chuang, 2013). Soft rot is a serious problem for potato production and can cause significant economic losses during plant growth, harvest, transport and storage (Latour, *et al.,* 2008).

Among environmental factors influencing the physiology and pathogenicity of *P. carotovorum*, temperature and pH are particularly critical. Temperature not only regulates the bacterial growth rate but also modulates the enzymatic activity of pectinases, thereby impacting the extent and severity of infection. Similarly, fluctuations in pH can affect bacterial metabolic processes and enzymatic efficiency, both of which are essential determinants of the pathogen's ability to cause disease (Liao & Chuang, 2013). *Pectobacterium* spp. and *Dickeya* spp. population dynamic and symptoms development under the combined effect of the most important factors of storage environmental (Moh, *et al.,* 2012). Model soft rot of potato tubers with respect to temperature, relative humidity and duration of storage (Kushalappa and Zulfiqar, 2001).

This study aims to evaluate effect of some environmental factors on the growth of *Pectobacterium carotovorum p.v carotovorum*, in vitro and severity of potato soft rot disease under artificial inoculation conditions.

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2. Materials and methods

2.1. Sample Collection, Isolation and identification of *Pectobacterium carotovorum*

Infected potato tubers with bacterial soft rot disease were collected from different locations. Proceeds isolation and identification carried out in Bacterial Diseases Laboratory, Plant Pathology Department, Faculty Agriculture, Ain Shams University according to Lelliott & Dickey, (1984), Sambrook *et al.,* (1989), Pérombelon, (2002), Lagacé *et al.,* (2004), Czajkowski *et al.,* (2011), van der Wolf *et al.,* (2021), and Seleim & Bereika, (2023).

2.2. Effect of some environmental factors on bacterial soft rot

2.2.1. *In vitro*

Pure three isolates of Pectobacterium carotovorum p.v. carotovorum (Pcc1, Pcc2, and Pcc3) were grown on nutrient agar medium for 24 h at 30°C. Bacterial growth was suspended in sterile distilled water and adjusted to approximately $1x10^8$ colony forming units (CFU)/ml, with absorbance at A600nm as Optical Density (OD) = 0.1 measured using a spectrophotometer. Different pH values (6.0, 6.5, 7.0, 7.5, and 8.0) were prepared using nutrient broth medium in flasks containing approximately 100 ml. Approximately 1.0 ml of bacterial suspension was inoculated per flask and incubated for two days at 30°C. The inoculated flasks were placed on a shaker at 180 rpm to provide continuous aeration and mixing. Other inoculated flasks were incubated at different temperatures (18, 22, 26, 30, and 34°C) for three days under the same shaking conditions at 180 rpm. Bacterial growth in the nutrient broth was measured at 660 nm as Optical Density (OD) using a spectrophotometer (Huang *et al.,* 2020 and Zhang *et al.,* 2010).

2.2.2. *In vivo* (Under artificial inoculation conditions)

Potato healthy tubers "Spunta" cultivar approximately equal weight (65-75 gm) was used. The tubers were washed with running tap water and air dried, then sterilized by washing with 95% ethanol for 2 minutes followed by two successive rinses in sterile distilled water and allowed to dry at room temperature. The tubers were prepared for inoculation by using two methods as the following; a)Injection method with application of sterilized cork borer (0.5 mm in diameter and 1 cm in depth) to make a hole in the middle of each tuber, these tubers were inoculated with bacterial suspension (0.2 ml/hole) and the holes were closed again with the same cylinder which eliminated potato pieces before the inoculation and b) wounding method where sterilized tubers were wounded (1cm long and 0.5 ml depth) by using sterilized needle, with three wounds were done per tuber. These tubers were soaked with bacterial suspension for 5 minutes. Inoculated tubers were incubated in room chamber for three days. Inoculated tubers were maintained at different relative humidity (60, 70, 80, 90 and 100%) which were prepared by using two saturated salt solutions Kcl (86%) and Kno3(96%) according to Winston and Bate (1960) and Lahlali *et al*., (2008) and sterilized distilled water (100%) at 30 $°C$ for five days. While other Inoculated tubers were maintained at different temperatures (18, 22, 26, 30 and 34$°C$) with 100% relative humidity for three days, using sterilized plastic boxes as growth chamber. Twelve tubers (two tubers/box) were applied as replicates per treatment (Ranjan and Singh, 2020).

Severity of potato soft rot disease was determined as percentage of rotted tissue (tuber weight before inoculation-tuber weight after inoculation $x100$/tuber weight before inoculation) according to Schaad (1988) and socttler *et al.* (1989).

2.3. Statistical analysis

Statistical difference calculated using an ANOVA according to a pairwise multiple comparison (Duncan's method and Kruskal-Wallis tests). These analyses were performed using the Excel Add-in XISTAT software program (Addinsoft, 2021).

3. Results and Discussion

3.1. Effect of some environmental factors on *Pectobacterium carotovorum* subsp. *carotovorum* isolates, in vitro:

Different pH values were examined against the growth three isolates of *Pectobacterium carotovorum* subsp. *carotovorum* (figure,1). Rate growth tested bacterial isolates were affected with different pH values, where rate growth bacterial isolates were ranged from 1.04 to 1.24 with Pcc1

isolate, from 1.08 to 1.33 with Pcc2 isolate and from1.09 to 1.30 with Pcc3 isolate. Meanwhile, rate the growth tested isolates were the most increasing with 7.0 and 7.5 pH values, where rate the growth was 1.24 and 1.19 with Pcc1 isolate, were 1.33 and 1.28 with Pcc2 isolate and were 1.30 and 1.24 with Pcc3 isolate, respectively. However, pH values of 6.5 and 8.0 were moderately affected on the growth tested isolates, where rate the growth was 1.07 and 1.04 with Pcc1 isolate, were 1.17 and 1.08 with Pcc2 isolate and were 1.09 and 1.11 with Pcc3 isolate, respectively.

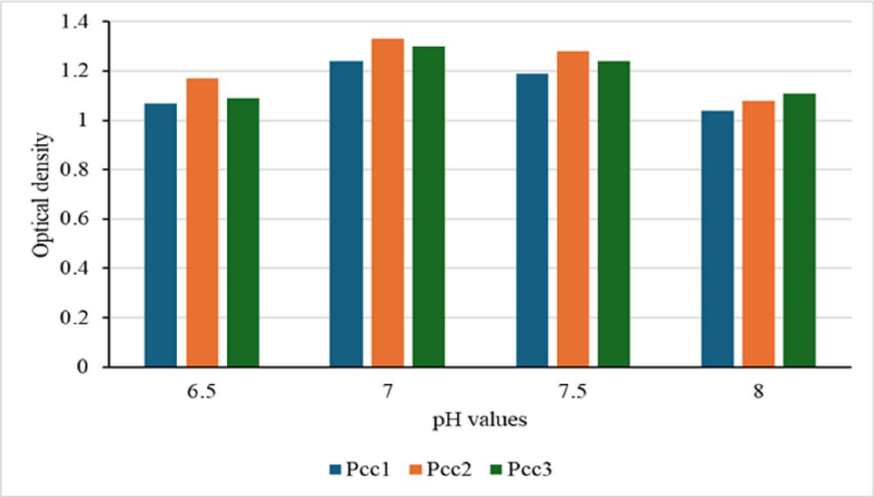

Fig. 1. Relationship between pH values and growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates as optical density after 72 hrs., in vitro

Different temperatures were examined against the growth of three isolates of *Pectobacterium carotovorum* subsp. *carotovorum* (figure,2). Rate growth tested bacterial isolates were increased with increasing temperatures, where rate growth bacterial isolates were increased from 1.08 to 2.27 with Pcc1 isolate, from 1.14 to 2.36 with Pcc2 isolate and from 1.11 to 2.30 with Pcc3 isolate by increasing temperatures from 18 to 30°c, respectively. Meanwhile, the best rate growth tested bacterial isolates were recorded on 30, 26 and 34°C, where rate the growth was 2.27, 2.30 and 2.30; was 2.20, 2.33 and 2.23 and was 1.93, 1.98 and 1.96 with Pcc1, Pcc2 and Pcc3 isolates, respectively. But rate growth tested isolates were moderately affected on 22 and 18°C , where rate the growth was 1.27 and 1.08 with Pcc1 isolate, 1.32 and 1.14 with Pcc2 isolate and 1.29 and 1.11 with Pcc3 isolate, respectively.

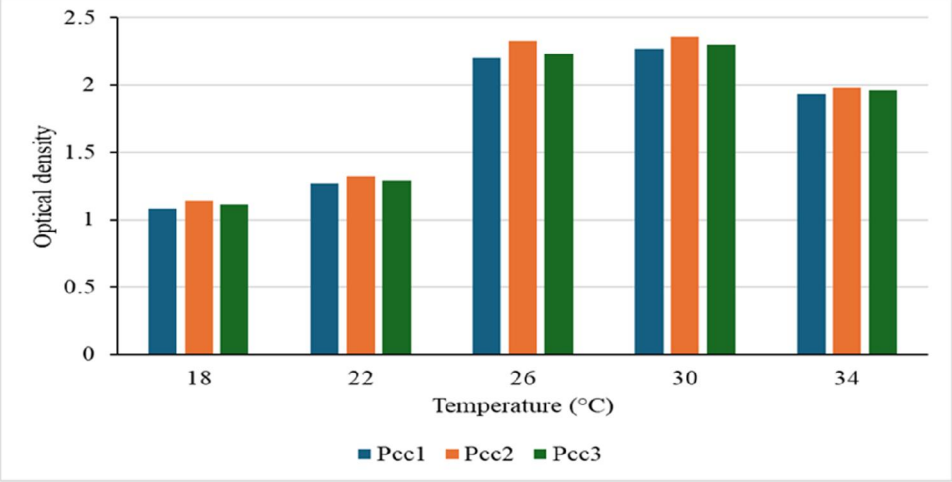

Fig. 2: Relationship between different temperatures and growth of *Pectobacterium carotovorum* subsp. *carotovorum* isolates as optical density after 72 hrs., in vitro

3.2. Effect of some environmental factors on severity of potato soft rot disease under artificial inoculation conditions:

Efficacy of temperatures were assessed on severity of soft rot disease of potato under artificial inoculation conditions (figure, 3). Disease severity increased with increasing temperatures, where the disease increased from 57.3 to 72.0 % by using injection method and from 41.5 to 58.3 % by using wounding method with increasing the temperatures from 18 to 34°c, respectively. Meanwhile, disease severity was the most severed with 34, 30 and 26°C temperatures, where the disease was 72.0, 71.9 and 70.4 % by using injection method and was 58.3, 57.7 and 56.2% by using wounding method, respectively. But disease severity was moderately severed on 22 and 18°C, where the disease was 62.4 and 57.3% by using injection method and was 48.1 and 41.5% by using wounding method, respectively.

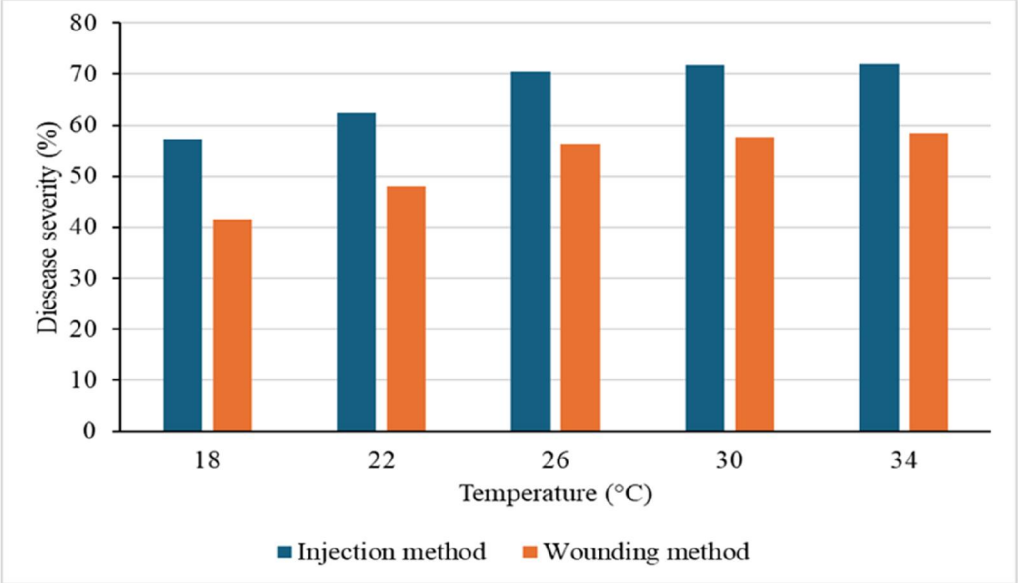

Fig. 3: Relationship between different temperatures and severity of potato soft rot disease under artificial inoculation conditions after three days

Efficacy of different relative humidity values were assessed on severity of soft rot disease of potato under artificial inoculation conditions (figure, 4). Disease severity increased with increasing relative humidity values, where the disease was increased from 88.9 to 100% by using injection method and from 77.9 to 94.5 % by using wounding method with increasing values of relative humidity from 60 to 100%, respectively. Meanwhile, relative humidity is considered important factors for development potato soft rot disease

Singh and Ranjan (2020) confirmed the role of *E. cartovora* subsp. *cartovora* in potato rotting at different storage treatment, temperatures and population levels of the pathogen, where the maximum rotting of potato tubers was recorded at 108 cfu cell/ ml, 35°C after 3 days of incubation, under artificial inoculation conditions. Raju *et al.* (2008) mentioned that rotting ability of *E. cartovora* subsp. *cartovora* enhanced by increasing temperature of 20 to 30°C and by increasing relative humidity levels, where maximum rotting was recorded at35 °C with 100% relative humidity in radish discs. Moh *et al.* (2012) showed a significant effect of temperature, relative humidity and initially applied bacterial concentration on population dynamic of the pathogens and soft rot development at the surface of wounded potato tubers, where temperature was the most important factor followed by the initially applied bacterial concentration and relative humidity.

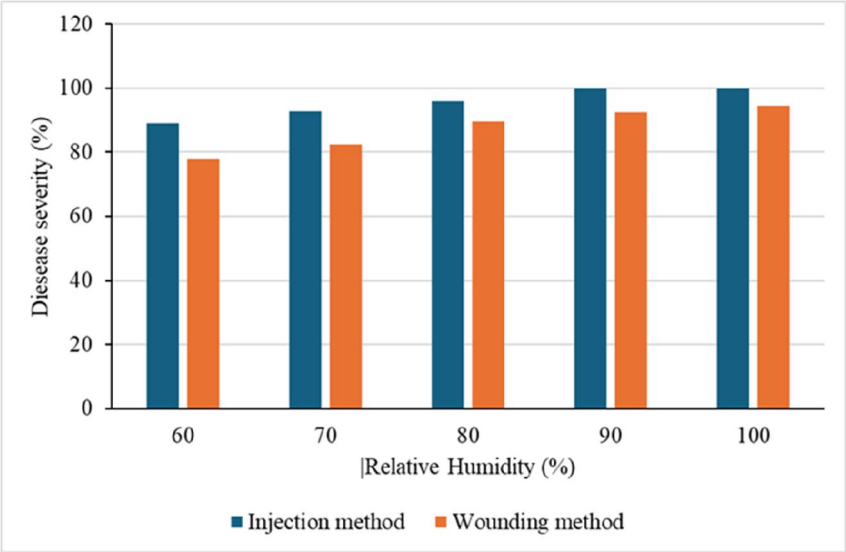

Fig. 4: Relationship between different relative humidity and severity of potato soft rot disease under artificial inoculation conditions after five days

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