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Effect of different irrigation rates on the occurrence and development of potato bacterial lenticels rot in Egypt

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ABSTRACT

"Lenticel rot" is a type of soft rot that develops within potatoes' natural openings due to increased respiration rates. When potatoes are exposed to waterlogged soil and high temperatures, their lenticels expand, providing an access route for pectolytic bacteria. This study is the first to shed light on lenticel rot in Egypt. The study's goal is to investigate how inappropriate watering rates affect the development of lenticel infections. The causal bacteria were identified as *Pectobacterium carotovorum* subsp. *carotovorum* using both BIOLOG analysis and DNA sequencing, following the pathogenicity test. In a pot experiment (with 4 kg of soil in each pot), the highest watering rate (1000 mL per pot), combined with soil infestation, resulted in the greatest disease incidence. The ratio of infected (IR) tubers increased from 0.0±0.0 in the treatment without infestation to 65.4±10.7 in the infested treatment ($P < 0.001$) under the high watering rate. No significant difference in IRs was observed between the different watering rates in non-infested treatments. Additionally, there was no significant difference in IRs between non-infested and infested treatments following regular and moderate watering rates (500 mL and 750 mL, respectively). High irrigation rates altered soil chemistry, decreasing organic matter (OM) and nitrogen (N) availability while increasing phosphorus (P) and potassium (K). In conclusion, the irrigation rate has a significant impact on disease development compared to the sole presence of the pathogen in the soil. This implies that the irrigation rate influences disease development only when the pathogen is actively present.

Keywords: Pectolytic bacteria, *Pectobacterium carotovorum*, soil chemistry, BIOLOG and DNA-sequencing.

INTRODUCTION

The potato is the fourth most important crop worldwide (FAO, 2019). It is one of Egypt's most important cash crops. In 2022, Egypt's potato exports accounted for 6.2% of worldwide table potato exports. Notably, in 2023, Egypt achieved a remarkable milestone by exporting a record 440,000 tonnes of potatoes to European Union (EU) nations, underscoring its economic relevance and the strong global demand for Egyptian potatoes (1). Additionally, during the 2023-24 season, Egypt's potato exports to the EU, Russia, and Arab nations totalled approximately 650,000 tonnes, solidifying Egypt's position as the world's fifth-largest exporter of table potatoes (2).

Climate change significantly influences disease-causing organisms through temperature, rainfall, and humidity. Consequently, climate change is expected to influence the emergence of infectious diseases (Thomas, 2020). Potatoes are grown mostly during the cold season. However, climate change may lead to warmer seasons, which may cause new, unexpected potato diseases, especially under uncontrolled irrigation conditions. "Warmer world would be a sicker world" (Alhammadi and Al-Shrouf, 2013; Harvell *et al*., 2002).

Bacterial soft rot in potatoes is one of the most economically significant diseases worldwide, affecting both quantity and quality (Charkowski *et al*., 2020). Pectolytic bacteria, responsible for soft rot, invade potato tubers through wounds and natural openings, such as lenticels and stomata, and vulnerable tissues such as stem ends and eyes. The infection takes place primarily in the field and spreads during packing (Inglis *et al*., 2011). Lenticel rot is a unique form of bacterial soft rot in which the bacteria enter through the lenticels. Lenticels, small pores on the tuber surface, facilitate the exchange of oxygen and carbon dioxide during tuber cellular respiration. Under high moisture conditions, a water film forms on the tuber surface, causing lenticel enlargement and creating anaerobic conditions (Chakrabarti *et al*., 2022). Elevated temperatures enhance tuber respiration, reduce oxygen levels, and accelerate lenticel enlargement. These conditions create favourable circumstances for pathogen entry (Bethke, 2023). These diseases may cause severe economic losses in both the processed and raw yields of potatoes. (Farrar *et al*., 2009 and Inglis *et al*., 2011). Additionally,

shipments subjected to wet conditions during harvesting are exposed to lenticel soft rot (Smith and Ramsey, 1947).

Lenticel rot symptoms include tan to dark brown circular, water-soaked patches or tiny lesions surrounding lenticels on the tuber surface. Infected tissue normally does not go deeper than 4 mm into the tuber. Neighbouring lesions can combine to form bigger, oddly-shaped, sunken lesions. These signs are known as bacterial lenticel spots (Bethke, 2023). Under damp conditions, the lesions enlarge and quickly become puffy. The bacteria responsible for lenticel rot and soft rot, which can cause early mortality, initially colonize the outer surface of tubers and spread via surface irrigation water (Farrar *et al*., 2009). These bacteria were grouped into two genera, *Pectobacterium* and *Dickeya*. To encompass different species and subspecies, named *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliensis*, *P. wasabiae*, and *D. dianthicola.* Additionally, other bacterial species, including *Bacillus, Clostridium, and Pseudomonas,* may also produce lenticel spot symptoms. (Inglis *et al*., 2011). According to De Boer, (2008), the best management methodology for lenticel rot is controlling irrigation practices.

The disease triangle comprises virulent Pectobacteriaceae strains, susceptible potato varieties, and favourable environmental conditions (high temperatures and humidity) that induce hypoxia (Maciag *et al*., 2024). To date, no potato variety has demonstrated resistance to soft rot (lenticel rot) caused by virulent Pectobacteriaceae strains (Lebecka *et al*., 2004). In this study, different irrigation rates are compared to the development of lenticel rot in Egypt, both with and without the presence of the agent that causes it.

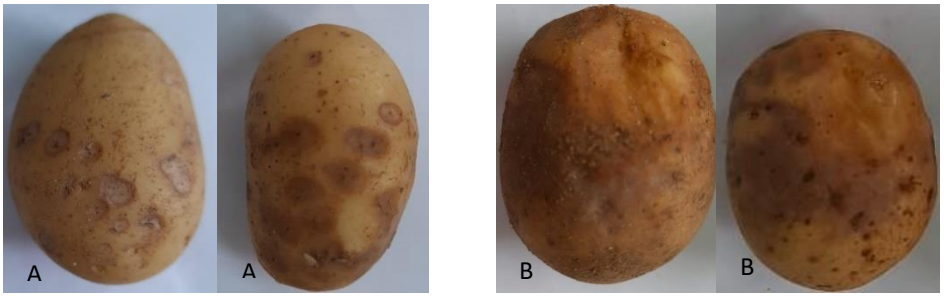
MATERIAL AND METHODS

Sample collection:

Samples were obtained from Mariam Farm in Nubaria, Behera governorate, exhibiting characteristic lenticel infection symptoms at the end of March 2021. Notably, the affected tubers had rotten, discoloured lesions surrounding the lenticels. Over time, these rotting lesions continued to enlarge until the tuber eventually collapsed **(Fig. 1)**.

Pathogen isolation:

The pathogen was isolated as follows: infected tubers were washed, and their surfaces were disinfected. Next, portions of the rotten areas were macerated in sterilized phosphate buffer (0.05 M). The resulting suspension was plated onto Logan media (Logan, 1963 and Schaad *et al*., 2001) and incubated for 24 hours at 28 °C. Colonies displaying typical characteristics were selected for subsequent pathogenicity and identification methods.



A) Conventional farm (var. Princess) B) Organic farm (var. Toscana)

Fig. 1. Typical lenticel rot symptoms on naturally infected potato tubers

Pathogenicity test:

The isolated bacteria were checked for their pathogenic potential using surface sterilized potato tubers of the Spunta cultivar. Holes were made with a cork borer (5 mm diam.) at a depth of 2 cm and inoculated with 150 µl of a bacterial suspension (10^6 CFU/ml) in sterile 0.01 M phosphate buffer (PB). Control tubers were prepared using sterile PB instead. The removed potato cylinder was placed in each hole and sealed with sterile vaspar. All treatments were then placed in moist conditions at 28 °C for 4–7 days. Tubers were regularly monitored to observe the development of soft rot.

Identification of the pathogenic isolates:

Two bacterial isolates out of the total five tested were subjected to the following identification methods:

a. Identification by conventional PCR:

Oligonucleotide primers EXPCCR (5-GCCGTAATTGCCTACCTGCTTAAG-3') and EXPCCF (5'GAACTTCGCACCGCCGACCTTCTA-3') were used in a standard PCR assay (Kang *et al*., 2003). The PCR reactions were performed in a 50-µl PCR mixture (Cosmo PCR RED MMIX, WF-10203001-M Willowfort, UK). PCR amplification was carried out as follows: one cycle of 4 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 60 °C,

and 2 min at 72 °C; and a final extension for 7 min at 72 °C. PCR products were separated on a 1.5% agarose gel in tris-acetate-EDTA (TAE) buffer and visualised by staining with RedSafeTM Nucleic Acid Staining Solution. A Molecular 1Kb DNA Ladder was employed (Gene Direx, Life Direx, Bio-Helix).

b. Identification by BIOLOG system:

BIOLOG Revolutionary "GEN III: Microbial Identification System" (MicroLogTM Manual System Version 5.2.1, Serial No. 150733/USA). GEN III Database version 2.8.0 for G+ and G-bacteria (Hayward, CA 94545 USA) was employed using the protocol (A) following the published protocol.

c. **Identification by DNA-sequencing:**

DNA extraction from bacterial colonies was carried out with the DNA Easy Pure Genomic DNA Kit Trans (code #EE101-01). The V6 to V8 region of the 16S rRNA gene was amplified from the extracted DNA using primers 968 f and 1401 r, as reported by Hiddink *et al*., (2005). The PCR products were purified using Qiagen's QIAquick gel extraction kit (cat. no. 28704).

For sequencing, 20 ng of each purified PCR product was added to a 20 µl PCR reaction containing 8 µl of BigDye terminator, 1 µl primer (3.2 pmol) (either forward or reverse), and up to 20 µl of nuclease-free water. The thermal profile for amplification was as follows: initial denaturation at 96 °C for 1 min., followed by 25 cycles of denaturation at 96 °C for 10 sec., annealing at 50 °C for 5 sec., and extension at 60 °C for 4 min. The resulting fluorescent PCR reaction mixtures were purified using BigDye® XTerminator™ Kit (Applied Biosystems™, Bedford, MA01730. USA) following the recommended protocol.

Each purified PCR product was combined with 10 µl of Hi-Di™ Formamide (PN 4440753), a highly deionized formamide, and the mixture was heat-denatured and immediately placed on ice before injection into the genetic analyzer instrument. The genetic analyzer used was the Applied Biosystems 3500 genetic analyzer HITACHI (8-capillary) 8 ch Ruo 622-0010, Tokyo, Japan, and POP-7™ polymers were employed for sequencing.

The resulting partial 16S rRNA gene sequences were compared to sequences in the GenBank DNA database using the BLASTN method, which is accessible at http://blast.ncbi.nlm.nih.gov/Blast.cgi (nucleotide blast). Bioedit software was used to refine and edit forward and reverse DNA sequences. Chimaeras were detected with DECIPHER version 2.25.2. Finally, the sequences were uploaded to the GenBank database via the submission portal at https://submit.ncbi.nlm.nih.gov/subs. Mega11 software was used to generate the Neighbour-joining phylogenetic tree. The Muscle algorithm was used for alignment. Evolutionary analyses were performed, following the approach demonstrated by Tamura *et al*., (2021). The Neighbour-Joining method proposed by Saitou and Nei, (1987) was employed to infer the evolutionary history. The resulting optimal tree is displayed, with evolutionary distances calculated using the Jukes-Cantor method (Jukes and Cantor, 1969) expressed in terms of base substitutions.

Effect of different watering rates in presence and absence of the pathogen at ambient temperature on Potato lenticel rot development and tuber yield:

A pot experiment was carried out twice throughout the 2022 and 2023 seasons at the Agricultural Research Centre's Giza experimental research station. The experiment began in late January and lasted until early May each year. **Figure 2** depicts the lowest and highest temperatures recorded throughout the two trials.

The experiment used sandy soil with the following characteristics: pH 8.81, electrical conductivity (EC) at a ratio of 2.5:1 of 0.25 dSm^{-1}, organic matter (OM) content of 0.29%, nitrogen (N) concentration of 7.72 ppm, phosphorus (P) concentration of 17.56 ppm, and potassium (K) concentration of 155 ppm. Physical and chemical studies were carried out at the Mansura University Faculty of Agriculture's Central Analysis Lab.

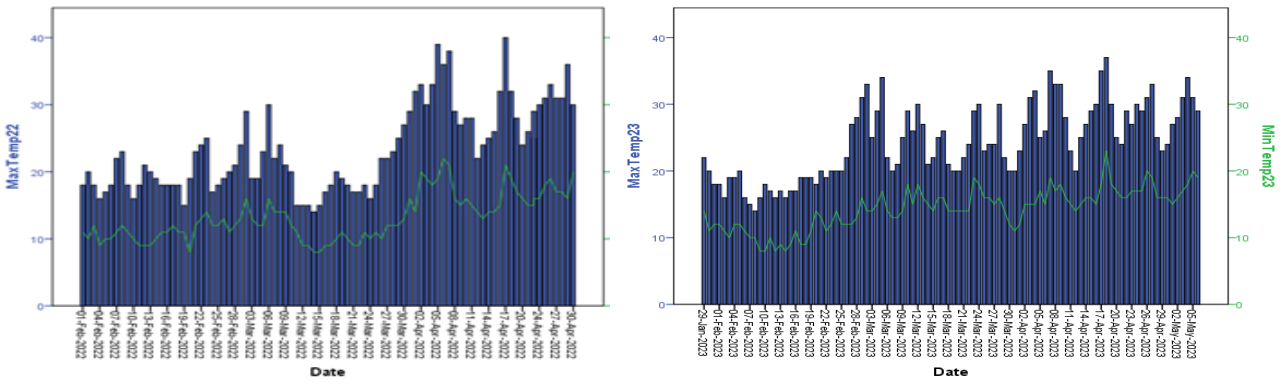


Fig. 2. The minimum and maximum temperature during the two experiments (according to https://www.accuweather.com)

Sterilized pots with a diameter of 30 cm were filled with 4 kg of sandy soil collected from a potato field in the Nubaria district, Behera governorate. Spunta variety seed tubers were planted using standard eyepieces, and adequate, equal watering was provided until germination. The experiment was divided into two groups, as summarized in **Table 1**. The bacterial suspension was prepared by incubating fresh inoculum (grown on nutrient agar) for 48 hours at 28 °C. The suspension contained a mixture of the two identified and registered pathogenic isolates, P1 and P6. The bacterial density was adjusted spectrophotometrically to approximately 1 x 10^9 CFU ml^{-1} (OD600). Forty ml of this suspension were mixed with the soil in each pot, achieving a final inoculum density of 10^7 CFU g^{-1} soil. Three watering levels were employed every 3-4 days, regular (500 ml/pot), moderate (750 ml/pot), and high (1000 ml/pot). At the end of the experiment, tubers from each pot were harvested, counted, weighed, and stored under controlled humid conditions for another two weeks.

Table 1. Layout of the experiment

Watering rate** / Treatment	500 ml/pot (regular)	750 ml/pot (moderate)	1000 ml/pot (high)
Control (non-infected)	5 pots	5 pots	5 pots
Inoculated*	5 pots	5 pots	5 pots

*A mixture of two strains (P1 & P6) were used, achieving a final inoculum density of 10^7 CFU g^{-1} soil.
**The watering was done regularly every 3-4 days with the predetermined amounts of water.

Effect of various watering levels in soil chemical characteristics:

Hygroscopic moisture content (HW%), organic matter (OM%) and organic carbon (OC%) were evaluated using Gravity Convection Oven Redline by Binder 53 (Model: RE53, Serial No.: RL12-15750, Germany) as described by Walkley and Black, (1934). Electric conductivity was measured using a Jenco EC -meter (Model: 3173, Serial no.: 00465, Salinity 0.0～80.0 ppt ±1 % FS, U.S.A) after Dellavalle, (1992). Soil pH was measured by Jenway pH -meter (Model: 3505, Serial no.: 03282, U.K) (Jackson, 1967). Total and available N was measured using Behr automatic Steam Distillation Unit for Kjeldahl Nitrogen, Model: S 2, Serial no.: 406 1587, Germany) as described by Jackson, (1967). Available P was measured using UV/VIS Spectrophotometer / PG instruments, Model: T80, United Kingdom (Olsen *et al*., 1954). Available K was measured by Flame photometer, Model: PFP7, United Kingdom) following the methods explained by Hesse, (1971).

Statistical analysis:

Post-hoc analysis was conducted using Dunnett t-tests in SPSS 23. In this analysis, the control group served as a reference, and other groups were compared against it. We specifically compared infection rates (IR) and tuber weight across different watering rates for both infected and non-infected treatments. In case of significant interaction between watering rate and infection treatment, we split the data and evaluated the impact of infection treatment under each watering rate separately. Additionally, we assessed the impact of watering rates for both infected and non-infected treatments separately.

RESULTS

Pathogenicity test:

Five different isolates developed on Logan medium plates, were selected and tested for pathogenicity. Two isolates namely P1 and P6, exhibited typical soft rot symptoms on potato tubers after artificial inoculation, as depicted in **Fig (3)**

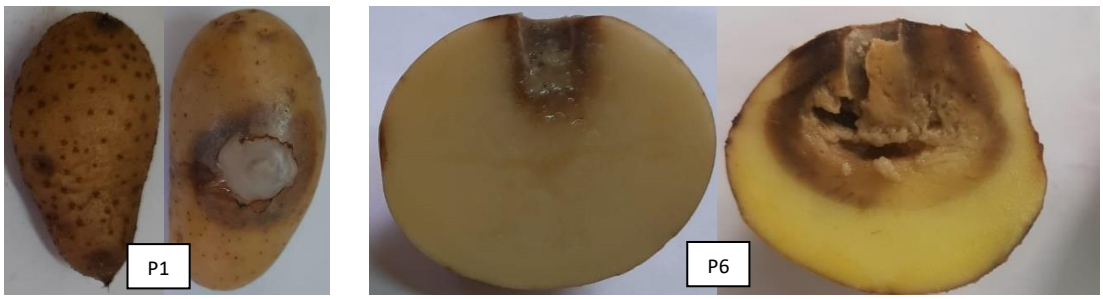


Fig. 3. Symptoms of soft rot observed after artificial inoculation of potato tubers by the two isolated, identified, and registered isolates, P1 and P6, seven days post- inoculation (10^6 CFU/ml) in a sterile 0.01 M phosphate buffer (PB).

Identification of pathogenic isolates:

a. Identification by Conventional PCR:

Figure (4) illustrates the identification of isolates P1 and P6 using specific primers EXPCCR and EXPCCF, as reported by Kang *et al*., 2003. A 1-kilobase marker was also employed. The typical band observed corresponds to a size of approximately 400 base pairs.

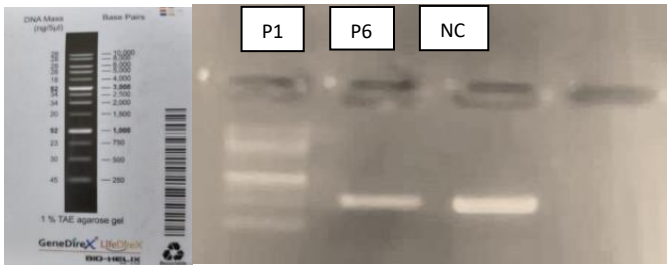


Fig. 4. The identification of isolates P1 and P6 using specific primers EXPCCR and EXPCCF, as reported by Kang *et al*. in 2003. A 1-kb marker was used. The typical band observed corresponds to a size of approximately 400 bp.

b. Identification by BIOLOG System:

Isolate P1 showed a 66.9% similarity with *Pectobacterium carotovorum* ss *carotovorum* B followed by an 18.2% similarity with *Pectobacterium carotovorum* ss *carotovorum* A **(Supp. 1)**.

c. Identification by DNA-sequencing:

The two isolates showed 99% similarity with *Pectobacterium carotovorum* subsp. *carotovorum.* The first one (P1) was identified as *Pectobacterium carotovorum* subsp. *carotovorum* strain P1 (Acc.No. OR142440). The second one was identified as *Pectobacterium carotovorum* subsp. *carotovorum* strain P6 (Acc. No. OR142620). **Figure 5** shows the phylogenetic tree of *Pectobacterium carotovorum* subsp*. carotovorum* strain P1 (Acc.No. OR142440) and *Pectobacterium carotovorum* subsp. *carotovorum* strain P6 (Acc. No. OR142620)

The proportion of sites containing at least one unambiguous base is present in indicated next to the internal nodes. This study included 21 nucleotide sequences and ambiguous positions were removed pairwise, resulting in a final dataset of 459 positions.

Effect of different watering rates in the presence and the absence of the pathogen at ambient temperature on Potato lenticel rot development and tuber yield:

A) Potato lenticel rot development:

Lenticel rot symptoms are depicted in **Figure 6**. These symptoms manifest as circular, water-soaked spots or small lesions on the surface of tubers, with colours ranging from tan to dark brown. These spots are typically found around the lenticels, which are small pore-like structures on the tuber's surface. The depth of the infection is generally shallow, usually not extending beyond 4 mm into the tuber.

In general, significant differences were observed among various watering rates (F=5.3, *P* = 0.001), as well as between infected and non-infected treatments (F=15.0, *P* <0.001). Additionally, a significant interaction between treatment and watering rate was noted (F=5.4, *P* =0.001). When the data was further analysed by splitting it, no significant variance in the IR between different watering rates in absence of the pathogen (**Fig 7A and Table 2**). There was no significant difference in IRs observed between regular and moderate watering rates when the pathogen was present, being 0.0±0.0% and 20.0±12.2% for different watering rates respectively. Notably, IRs increased: infected treatment under regular watering showed an average of 0.0±0.0%, while infected treatment under high irrigation exhibited a significantly higher IR of 65.4±10.7% (*P* <0.001). Moreover, under high watering conditions, the ratio of infected tubers showed similar increase between non-infected and infected treatments.

Spontaneous disintegration of tissues after 14 days of storage was recorded as follows: In the absence of pathogen inoculation, the proportion of rotten tubers increased sporadically. Specifically, when a high watering regime was employed, the tubers IR rose from 0.0±0.0% to 6.6±6.6%. This suggests that excessive watering led to the spontaneous breakdown of tissues in the tubers. When the pathogen was introduced, the situation changed. Under a regular watering rate, the IR of stored tubers increased from 0.0±0.0% to 13.2±8.1%. Similarly, under moderate watering rates, the IR escalated from 20.0±12.2% to 30.0±20.0%. The most significant increase occurred under high watering rates, where the IR surged from 65.4±10.7% to 93.4±6.6%. These results pertain to the second season (2023). Interestingly, the disease incidence during the first season was similar but not explicitly shown in the data.

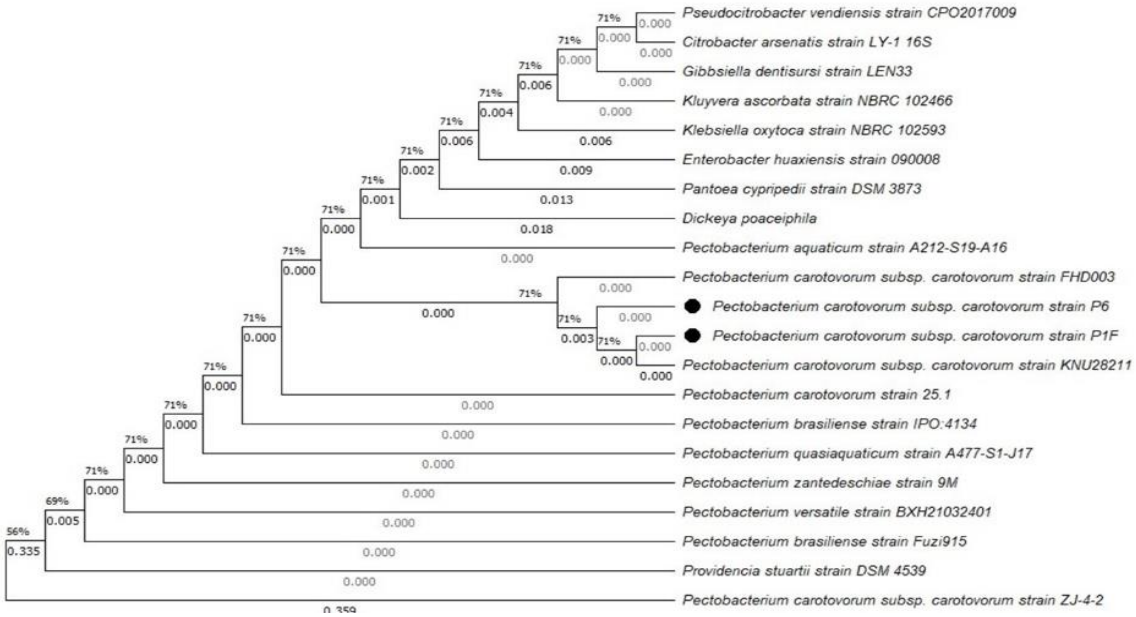


Fig. 5. The phylogenetic tree of *Pectobacterium carotovorum* subsp*. carotovorum* strain P1 (Acc.No. OR142440) and *Pectobacterium carotovorum* subsp. *carotovorum* strain P6 (Acc. No. OR142620).

The evolutionary history was inferred using the Neighbor-Joining method proposed by Saitou and Nei, (1987). The optimal tree is displayed, with evolutionary distances calculated using the Jukes-Cantor method (Jukes and Cantor, 1969) expressed in terms of base substitutions per site. Additionally, the proportion of sites containing at least one unambiguous base in each descendant clade is indicated next to the internal nodes. This analysis involved 21 nucleotide sequences, and ambiguous positions were removed pairwise, resulting in a final dataset of 459 positions.

Fig. 6. Lenticel rot symptoms (circular, water-soaked spots or small lesions on the surface of tubers, with colours ranging from tan to dark brown around the enlarged lenticels) for potatoes grown during the ARC experiment under high watering rates for infested treatment, Spunta potato variety, 90 days (Experiment 2)

Table 2. Lenticel rot infection ratio per pot under different treatments

		Ratio of infection (mean ± SE) ***		
Watering rate** / **Infection treatment**	**Sampling date**	**500 ml/pot [a]**	**750 ml/pot [b]**	**1000 ml/pot**
Control (non-infected)	End of the experiment	0.0±0.0	0.0±0.0	0.0±0.0
	14 days post harvest	0.0±0.0	6.6±6.6	6.6±6.6
Infected*	End of the experiment	0.0±0.0	20.0±12.2	65.4±10.7
	14 days post harvest	13.2±8.1	30.0±20.0	93.4±6.6

*A mixture of 2 strains (OR142440-OR142441, OR142620)

**The irrigation was made regularly every 3-4 days with the mentioned amount of water.

***Mean ± SE (Mean of 5 pots)

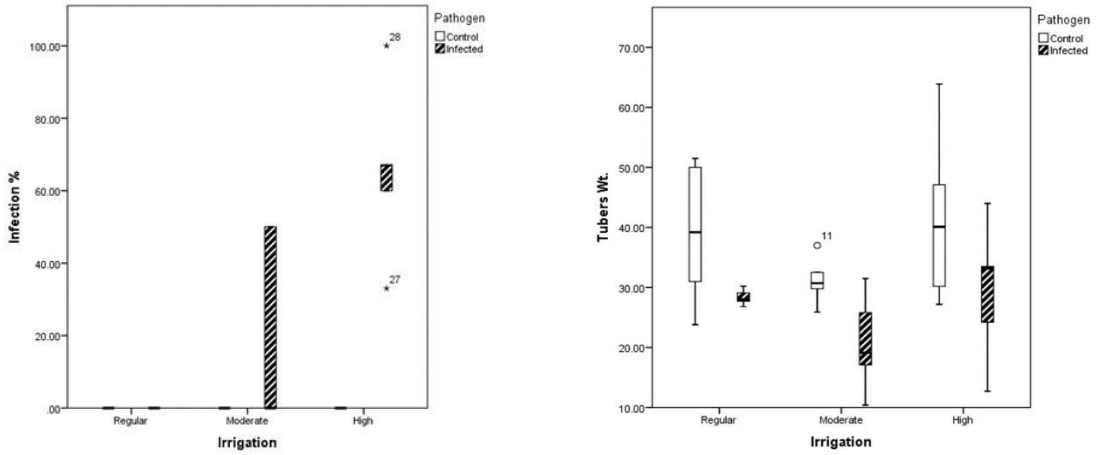


Fig. 7. Effect of *Pectobacterium carotovorum subsp. carotovorum* strain P1 (Acc.No. OR142440) and *Pectobacterium carotovorum subsp. carotovorum* strain P6 (Acc. No. OR142620) and watering rates on lenticel rot incidence and tubers weight (total wt. in gm).

A: The infected treatment under high irrigation exhibited a significantly higher IR of 65.4±10.7 (P <0.001) as compared to all other treatments.

B: A significant decrease in tubers wt. was recorded between control and infected treatments (F=6.6, P = 0.034) under the moderate watering rate.

Data represent 5 replicates per treatment (Experiment 2)

B) Potato Yield:

In general a trend of significant difference between different watering rates (F=2.7, P = 0.089) was recognized and a significant difference between infected and non-infected treatments as well (F= 9.5, P = 0.005).

Under the regular watering rate, a trend of a significant decrease in potato tubers weight was recognized for infected treatments (F=4.0, P = 0.081) and the control treatment. Moreover, a significant decrease in tubers wt. was recorded for control and infected treatments (F=6.6, P = 0.034) under the moderate watering rate (**Fig 7B and Table 3**).

Table 3. Weight of potato tubers in grams per pot under different treatments

Watering rate** / Treatment	Weight of potato tubers in grams at the end of the experiment (mean ± SE) ***		
	500 ml/pot [a]	750 ml/pot [b]	1000 ml/pot
Control (non-infected)	39.1±5.3	31.2±1.8	41.7±6.6
Infected*	28.4±0.6	20.8±3.6	29.5±5.2
% of decrease	**27.5**	**33.3**	**29.2**

*A mixture of 2 strains (OR142440-OR142441, OR142620)

**The irrigation was made regularly every 3-4 days with the mentioned amount of water.

***Mean ± SE (Mean of 5 pots)

[a] Trend of a significant decrease in tubers' weight. (P = 0.081)

[b] Significant decrease in tubers wt. (P = 0.034)

Effect of various watering levels on soil chemical characteristics:

Chemical analysis was conducted only for soil following regular and high watering rates (Table 4). The high watering rate is characterized by a higher Hygroscopic moisture content (HW%) of 4.11%, compared to 2.35% for the regular watering rate. Additionally, Electric conductivity (EC) was slightly higher for soil following the high watering rate (0.29 dSm^{-1}) compared to (0.25 dSm^{-1}) for soil following the regular watering rate.

The high watering rate resulted in a lower pH (8.61), as well as lower levels of total N (1.52%), organic carbon (0.12%), organic matter (0.21%), and available N (5.09 ppm), compared to 8.81, 2.1%, 0.17%, 0.29%, and 7.22 ppm, respectively, for the regular watering rate. Conversely, higher levels of available P (21.21 ppm) and K (190 ppm) were recorded for the high watering rate, compared to 17.56 ppm and 155 ppm, respectively, for soil following the regular watering rate.

Table 4. Chemical characteristics for the sandy soil following two different watering rates

	Hygroscopic moisture content (HW%)	EC (2.5: 1) dSm^{-1}	pH	Total N %	Organic carbon OC (%)	Organic matter OM (%)	Available macro elements (ppm)		
							N	P	K
Regular watering rate	2.35	0.25	8.81	2.10	0.17	0.29	7.72	17.56	155.00
High watering rate	4.11	0.29	8.61	1.52	0.12	0.21	5.09	21.21	190.00

Analysis was made at the end of the experiment for non-infested soils
Regular watering rate (500 ml/4 kg) High watering rate (1000 ml/4 kg)
Watering was made every 3-4 days

DISCUSSION

The current study demonstrated that *Pectobacterium carotovorum* subsp. *carotovorum* causes lenticel rot disease, verified using both BIOLOG and 16S sequencing approaches. *P. carotovorum* subsp. *carotovorum* is one of the species known to cause lenticel rot (Farrar *et al*., 2009; Inglis *et al*., 2011; and Bethke, 2023). However, this research is the first to shed light on lenticel rot in Egypt. Plants face various stressors, including pathogens, which necessitate precise environmental conditions for inducing plant diseases. This phenomenon is known as the "disease triangle," closely tied to the specific relationship between plants and pathogens. Disease occurs only when a virulent pathogen interacts with a susceptible plant cultivar under specific environmental circumstances (Francl, 2001). Lenticel rot, caused mainly by soft rot Pectobacteriaceae (SRPs), serves as an excellent example of this phenomenon. Waterlogging, a significant abiotic stress, leads to hypoxia—a condition characterized by a reduction in oxygen availability by 1-5%. In response, plants have evolved an ethylene-based system for hypoxia sensing, which involves physiological adjustments to environmental conditions. Potatoes, in particular, exhibit heightened sensitivity to hypoxia. SRPs take advantage of this vulnerability by inducing the production of virulence factors using cyclic diguanylate (c-di-GMP). As a consequence, potato tubers reduce their defenses to conserve energy, rendering them susceptible to the detrimental effects of reactive oxygen species and acidification, ultimately contributing to soft rot disease (Maciag *et al*., 2024). Hypoxia reduces plant aerobic respiration, slowing metabolism, development, and tolerance to additional stressors like necrotrophic diseases (Chung *et al*., 2020). Plant pathogenic bacteria can use root local hypoxia caused by microbial metabolism in the soil to compete for oxygen with their host, leading to symptom development (Gravot *et al*., 2016; Siedt *et al*., 2023).

Our study reveals that lenticel rot severity increases when both the pathogen and humidity are present. Higher watering rates exacerbate the disease when the pathogen is active. However, there is no significant difference in infection rates (IRs) between infested and non-infested treatments under regular or moderate watering rates. In conclusion, field flooding due to soil saturation significantly facilitates the development of lenticel infection at warm temperatures, as well as in humid conditions around the tuber during storage and transit. It is established that these diseases, caused by *Pectobacterium* and *Dickeya*, are facultative anaerobes that can survive in the absence of oxygen. Such diseases tend to occur at temperatures exceeding 16°C (Bethke, 2023). Researchers have long claimed that lenticel rot would not occur if the temperature is below 72°F (22.2°C) and the relative humidity was below 94.8% (Smith and Ramsey, 1947). Reduced oxygen levels and higher carbon dioxide accumulation around tubers encourage lenticel rot development. In some cases, nearby lesions can merge to create larger, irregularly shaped depressions. When exposed to moisture, these lesions can rapidly expand and take on a swollen appearance due to gas production by bacteria within the lenticels. However, if environmental conditions remain dry, lenticel spot lesions tend to remain limited in size, retaining their sunken, dry, and hard characteristics. Therefore, to manage bacterial infections in potatoes, it is recommended to maintain soil moisture levels above 65%. Avoid irrigation if soil moisture exceeds 70%, and strictly avoid it when soil moisture surpasses 90% (Inglis *et al*., 2011). Proper crop handling after harvest and careful tuber sorting, storage, and transit (Gudmestad, 2008) should be considered. In our study, disease progress increased after 2 weeks of storage. This may be explained by a sufficient number of virulent soft rot bacterial cells which increase the lenticel rot symptoms (Bethke, 2023). According to Inglis *et al*., (2011), lenticel spot symptoms often appear 4-10 days following harvest and packing.

According to Farrar *et al*., (2009), global warming may exacerbate the damage caused by lenticel rot. The impact of global warming has also facilitated the spread of infectious diseases across various geographical regions. In our study, the infected samples were collected at the end the March 2021 when the temperature was 11°C (minimum) to 21°C (maximum). In our experiments, potato tubers were planted in sandy soil at the

end of January and harvested them in early May. Throughout the growing season, temperatures consistently exceeded 22°C. Specifically, during the experiment, temperatures ranged from 8°C (minimum) to 22°C (maximum) in February and increased further to 11°C (minimum) to 37°C (maximum) in April. Remarkably, the temperature during the harvest season exceeded 30°C. However, it is advisable to harvest potatoes when the temperature falls within the range of 10°C to 18°C (Inglis et al., 2011). Consequently, farmers must adapt to climate changes by adjusting their planting seasons to avoid warmer temperatures, which could elevate the risk of such diseases.

The disease, however, has been an issue in Kern County (California, USA) when potatoes were planted in January and February for a June harvest in sandy soils. Because potatoes are cultivated in sandy soil and develop shallow roots, farmers tend to irrigate frequently, especially in hot weather. This additional watering can generate an environment conducive to the spread of *Pectobacterium (Erwinia)* soft-rot diseases (Farrar *et al*., 2009).

High irrigation rates disrupt soil chemistry by reducing organic matter (OM), organic carbon, and nitrogen (N) availability while increasing phosphorus (P) and potassium (K) levels. Elevated moisture levels can accelerate the microbial breakdown of organic materials, converting them into CO_2 and further depleting organic carbon. Ammonium conversion to nitrate decreases with higher watering rates. Nitrate (NO_3) serves as the preferred nitrogen source for many plants, including potatoes. Reduced nitrification rates may lead to insufficient nitrate availability for potato uptake, impacting tuber growth and overall crop health (Farzadfar *et al*., 2021). Additionally, pectolytic bacteria enhance nitrate respiration, producing lytic enzymes and virulence factors (Maciag *et al*., 2024). These microbial activities contribute to the decline in available nitrogen and organic matter. Waterlogging negatively impacts soil by causing compaction, reducing oxygen levels, accumulating toxic microbial metabolites, and diminishing the redox potential, leading to decreased soil pH (Manik *et al*., 2019). Oxygen deficit due to waterlogging led to nitrate conversion to nitrogen gas, depleting soil nitrogen. This anaerobic condition may impact soil microbes responsible for enhancing P and K availability to plants, explaining the elevated soil P and K levels observed with high watering rates (Aslam and Aslam, 2023). Most beneficial soil microorganisms cannot tolerate hypoxia (Maciag *et al*., 2024), further supporting the link between increased watering rates and higher soil P and K levels. Moreover, waterlogging is reported to cause a 33% yield decrease, a trend expected to worsen due to climate change and soil structure decline (Tian *et al*., 2021). To mitigate the adverse effects of excessive irrigation using flood (furrow) methods, adopting drip irrigation is strongly advisable. Drip irrigation is a water-efficient technique that provides precise and targeted watering (Singh *et al*., 2013).

CONCLUSION

This pioneering study sheds light on lenticel rot in Egyptian potato cultivation. We found that irrigation rates significantly impact disease development beyond the pathogen's presence in the soil. Effective management involves careful irrigation practices, avoiding excess soil moisture, and adopting drip irrigation is highly recommended. Additionally, preventing soil infestation and promoting disease-resistant potato varieties as well as certified, pathogen-free seeds are crucial. Further investigations into temperature effects will guide optimal cultivation practices in Egypt, considering climate change implications.

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تأثير معدلات الري المختلفة على حدوث وتطور تعفن العديسات البكتيري في البطاطس في مصر

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تعفن العديسات هو نوع مميز من التعفن الطري يحدث داخل الفتحات الطبيعية (العديسات) في البطاطس نتيجة لارتفاع معدل التنفس. عندما تتعرض البطاطس للتربة المشبعة بالماء ودرجات حرارة مرتفعة، تتسع العديسات الخاصة بها، مما يسهل نقطة دخول لبكتيريا التحلل. يظهر المرض على شكل بقع دائرية بين اللونين البني والبني الداكن، وغالبًا ما يكون محاطًا بمناطق مشبعة بالماء بالقرب من العديسات في الدرن. مع تقدم المرض، تنتشر المناطق المتأثرة، مما يؤدي في النهاية إلى انهيار الدرنه بالكامل. تهدف الدراسة إلى استكشاف كيف يمكن أن تؤثر معدلات الري غير المنضبطه على تطور العدوى العديسات. في مصر، تم اكتشاف المرض لأول مرة في نوعين من البطاطس زُرعت في مزرعتين مجاورتين في محافظة البحيرة. كانت هذه الأصناف هي "برنسيس" (التي نمت في حقول رملية تزرع بشكل تقليدي) و"توسكانا" (التي نمت في حقول رملية عضويًه) خلال ربيع عام 2021. في وقت الحصاد، لوحظت أنسجة تعفن العديسات في العديد من الدرنات. كان موقع العدوى مميزًا بتلون بني كبير. في التجارب التشخيصية، تم تحديد البكتيريا المسببة باسم *Pectobacterium carotovorum* subsp. *carotovorum*باستخدام الفحص البيوكيميائي BIOLOG وتسلسل الحمض النووي، بعد اختبار المرضية .في تجربة الزراعه في حاويات (4 كجم)، أثرت نظم الري المختلفة (500 مل، 750 مل، و 1000 مل لكل حاويه) على تطور المرض. أظهرت أعلى معدل ري (1000 مل لكل حاويه)، بالاشتراك مع العدوي البكتيريه للتربة بالممرض أعلى معدل إصابة بالمرض أثناء حصاد البطاطس. زادت نسبة الدرن المصاب من 0.0±0.0 في المعاملة بدون العدوي إلى 10.7±65.4 في المعاملة المعديه .(P <0.001) لم يكن هناك فرق ملحوظ في نسب الإصابة بالمرض بين معدلات الري الأخري. معدلات الري العالية أثرت على كيمياء التربة، حيث انخفضت المادة العضوية (OM) وتوافر النيتروجين (N) مع زيادة الفوسفور (P) والبوتاسيوم .(K) **الخلاصه،** يؤثر معدل الري بشكل كبير على تطور المرض مقارنةً بالوجود المنفرد للمسبب في التربة. وهذا يعني أن معدل الري يؤثر على تطور المرض فقط عندما يكون المسبب حاضرًا بنشاط.

الكلمات المفتاحية: البكتيريا البيكتوليتية ، بيكتوباكتيريوم كاروتوفوروم ، الكيمياء التربوية ، بيولوج ، تسلسل الحمض النووي (الدي أن أي)

Appendix **(Supp. 1)**.

Program	MicroLog M/5.2.01 35
Project	ML5
File Name	
User	admin
Instrument	Manual Entry
Instrument S/N	0
Incubation Hours	22.00
Plate Number	1
Plate Type	GEN III
Protocol	A
Sample ID	
Date & Time of Read	Nov 06 2022 6:02 PM
Biolog ID DB	GEN_III_v2.8.0.I5G

Result	Species ID: Pectobacterium carotovorum
Comment	
Notice	

Rank	PROB	SIM	DIST	Organism Type	Species
1	---	0.669	1.691	GN-Ent	Pectobacterium carotovorum ss carotovorum B
2	---	0.182	2.288	GN-Ent	Pectobacterium carotovorum ss carotovorum A
3	---	0.104	2.628	GN-Ent	Pectobacterium aroidearum A
4	---	0.081	2.782	GN-Ent	Pectobacterium atrosepticum A

Key: <x: positive, x: negative, <x-: mismatched positive, x+: mismatched negative, {x: borderline, -x: less than A1 well

Well Color Values

Plate	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	< 250	< 250	< 250	< 250	0	< 250	< 250	< 250	< 250
B	< 250	< 250	< 250	< 250	< 250	< 250	0	0	0	< 250	< 250	< 250
C	< 250	< 250	< 250	< 250	0	0	0	< 250	0	< 250	< 250	0
D	0	< 250	0	< 250	< 250	< 250	< 250	< 250	0	< 250	< 250	0
E	0	0	0	0	< 250	< 250	0	0	< 250	< 250	< 250	< 250
F	< 250	< 250	< 250	< 250	0	0	< 250	0	< 250	< 250	< 250	< 250
G	0	< 250	0	0 +	< 250	0	0	< 250	< 250	0	< 250	0
H	0	0	0	0	0	0	0	< 250	< 250 -	0	< 250	0

Report Date Nov 06 2022 6:08 PM

Supp. (1) Identification of P1 by BIOLOG System

BIOLOG Revolutionary "GEN III: Microbial Identification System" (MicroLogTM Manual System Version 5.2.1, Serial No. 150733/USA).