

Potent Plant Extracts: Combatting Potato Tuber Soft Rot Disease Caused by *Pectobacterium carotovorum* subsp. *carotovorum*

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Abstract. The objective of this research was to assess the efficacy of three plant extracts—Cumin, pomegranate, and Black pepper against *Pectobacterium carotovorum* subsp. *carotovorum*, a bacterium responsible for causing soft rot disease in potato tubers. This ailment poses a significant threat to potato production, impacting the quality of potatoes throughout storage, transit, and shipment. To conduct the investigation, twenty isolates of the pathogenic bacterium were acquired from naturally infected potato tubers and identified as *P. carotovorum* subsp. *carotovorum* through various microbiological characteristics. Through in vitro experiments, the plant extracts were examined for their antibacterial properties against the bacterium. The findings revealed that all three plants extract exhibited inhibition of bacterial growth. Notably, pomegranate demonstrated the most potent inhibitory effect among the three extracts. In light of these in vitro results, it is recommended to employ all extracts at a concentration of 50 mg to effectively control soft rot disease in potato tubers during storage. The concentration of 50 mg was identified as the most effective in impeding the growth of the pathogenic bacterium. In summary, this study demonstrated the potential of all tested plant extracts to mitigate potato tuber soft rot disease, addressing a significant challenge in potato production.

Keywords: Soft rot disease, Potato, plant extract, antibacterial.

1. Introduction

Potato production is greatly impacted by two main factors: environmental factors related to climate change (Rabia et al., 2018) and bacterial diseases that result in significant losses in both quality and quantity of production (Sulaiman et al., 2020). The decline in potato production can happen under various circumstances, including in the field, during transit, storage, and marketing. The two contributing factors, unfavorable climate and infected seed tubers, result in the susceptibility of the production to significant losses (Doolotkeldieva et al., 2016). The damage to potato crops caused by microbial diseases has a detrimental impact on both potato production and the potato-based food industry in Saudi Arabia. Potato crops are vulnerable to five diseases caused by bacterial species. Common scab is caused by *Streptomyces scabies* (Charkowski et al., 2020), ring rot by *Clavibacter michiganensis* subsp. *sepedonicus* (Charkowski et al., 2020), and bacterial wilt or brown rot by *Ralstonia solanacearum* (Prior et al., 2016). Blackleg and soft rot of the plant stem are caused by the genus *Dickeya* spp. (Parkinson et al., 2014), and soft rot of tubers is caused by *Pectobacterium* spp. (Adeolu et al., 2016), a member of the beta-Proteobacteria in the Pectobacteriaceae family within the order Enterobacterales. Of these bacteria, *P. carotovorum* subsp. *carotovorum* (Pcc) is the primary

causative agent of potato tuber soft rot (Naas et al., 2018). This disease results in substantial yield reduction and economic losses, up to 60% during field transit and storage (Mantsebo et al., 2014).

The infection of potato tubers with soft rot begins when the causative pathogen, *P. carotovorum* subsp. *carotovorum* (Pcc), attaches to the skin of the developing tubers during the growing season. After harvest, the potato is typically disease-free unless it is wounded during shipment (allowing Pcc to penetrate the tuber) or exposed to moisture (enabling Pcc to rapidly reproduce and infect the exterior tissues of the tuber). The resultant diseased tubers exhibit small, creamy to tan, water-soaked surface spots that spread internally (Rosenzweig et al., 2016). This decay can spread quickly, causing the tissue to become soft, viscous, and water-soaked, and eventually fleshy, forming blister-like lesions that often release a watery substance (Czajkowski et al., 2015).

The challenges posed by bacterial soft rot have driven many researchers in plant pathology to search for new and effective ways to manage this disease, such as using plant extracts as a potential alternative to antibiotics (Hossain et al., 2019).

The primary objective of this study was to explore multiple aspects through experimental approaches. These included the isolation of the key causative agent of potato tuber soft rot disease, identified as *Pectobacterium P. carotovorum* subsp. *carotovorum*. Additionally, the study involved the examination of pathogenicity in healthy slices and the *in vitro* assessment of the antibacterial activity of three distinct plant extracts against the identified pathogen.

2. Materials and Methods

2.1. Isolation of the bacterial causal pathogen

The purpose of this study was to investigate the primary causative agent responsible for soft rot disease in potato tubers. Infected potato tubers were gathered from various locations in Jeddah during the 2022 season. The isolation of the pathogen involved a series of steps: first, the infected tubers underwent washing, followed by treatment with a 1% sodium hypochlorite solution. Subsequently, the tubers were cut into small pieces, and the diseased tissue was homogenized in sterilized water. The resulting suspension was then streaked onto nutrient sucrose agar plates (NSA) and incubated at a temperature of 27-29°C for 48 hours. Single colonies were meticulously selected and maintained as pure cultures on sterilized slants, which were stored in the refrigerator for further analysis.

2.2. Pathogenicity tests

2.2.1 Preparation of bacterial suspension

The bacterial suspension of each isolate was prepared by growing the pure culture in 100 ml sterilized nutrient sucrose broth (NSB) and incubating it at 27±2°C for 48 hours on a rotary shaker at 150 rpm. The resulting bacterial suspension was then centrifuged at 7000 rpm for 3 minutes, after which the pellet was re-suspended in sterilized water. The bacterial suspension was adjusted to a concentration of 1x10⁸ cfu/ml using a spectrophotometer (Spectronic 20D from Milton Roy Company) at 620 nm, following the method described by McGuire and Kelman (1984).

2.2.2. Test for soft rotting

To evaluate the pathogenicity of the isolated bacterial strains, a pectinolytic activity test was conducted on healthy potato slices. The healthy potato tubers were washed thoroughly with tap water and dried, then sliced into 1 cm thick pieces that were surface sterilized with 95% ethanol. These sterilized slices

were placed on petri dishes containing moist sterilized filter paper. The bacterial suspension (1×10^8 cfu/ml) was streaked onto the surface of the potato slices, and the dishes were incubated at 27-29°C for 72 hours. The development of soft rot symptoms was monitored daily.

2.2.3. Disease severity assessment

Disease severity of the soft rot disease (weight loss) caused by the tested isolates estimated mathematically using the equation of Yaganza, et al., (2004), as follow: Disease Severity Index % (DSI) = $\frac{Tw1 - Tw2}{Tw1} \times 100$

Where:- Tw1 = Total weight of slices with rotting tissue.

Tw2 = Total weight of slices without rotting tissue.

2.3. Identification of the pathogenic bacteria

The bacteria isolated, responsible for inducing soft rot in potato slices and tubers, were identified as pathogenic through a comprehensive assessment of their morphological, cultural, and physiological characteristics. This identification process adhered to the criteria outlined in authoritative references such as Bergey's Manual of Determinative Bacteriology, Bergey's Manual of Systematic Bacteriology, and the guidelines established by Lelliott and Stead (1987).

2.4. Control of potato soft rot causal pathogen by plant extracts (In vitro study)

The purpose of the experiment was to compare the antibacterial activity of different plant extracts against the bacterial soft rot pathogen *P. carotovorum* subsp. *carotovorum* in vitro using the Kirby-Bauer disc-diffusion method on plates supplemented with nutrient sucrose agar. Sterile discs were saturated with varying concentrations of the tested plant extract (0, 10, 20, 30, 40 and 50 µg/ml) and the antibacterial activity was evaluated.

2.4.1. Preparation of bacterial suspension

The bacterial suspension of *P. carotovorum* subsp. *carotovorum* isolate No. 3, which was responsible for the highest disease severity, was prepared as mentioned above.

2.4.2. Kirby-Bauer disc diffusion method

The Kirby-Bauer disc diffusion method was used to assess the antibacterial activity efficacy of three plant extracts. In this method, a suspension of a 48-hour old culture of the tested bacteria was spread over a sterile petri plate and two 5mm diameter discs loaded with different concentrations of plant extract were placed on the plate. The plate was then incubated for 24 hours at $27 \pm 2^\circ\text{C}$, and the inhibition zone against bacterial growth was observed. The experiment was carried out four times for each concentration and repeated twice for accuracy.

2.5. Control of potato soft rot causal pathogen by best concentration of three plant extracts (In vivo study): Storage conditions against *P. carotovorum* subsp. *carotovorum*

The experiment aimed to evaluate the efficacy of three plant extracts in suppressing the soft rot disease caused by *P. carotovorum* subsp. *carotovorum*. The experiment consisted of two parts: treated tubers and control tubers. Treated potato tubers were immersed in 50 µg/ml of plant extract for 30 min. and left to dry completely, while control tubers were submerged in sterilized distilled water. The treated potato tubers were then inoculated with 200 µl of a suspension of *P. carotovorum* subsp. *carotovorum* No.3, while the control tubers were inoculated with 200 µl

of sterilized distilled water. Both the treated and control tubers were kept in clean sterilized plastic containers with sterile moist cotton and incubated at 27-29°C for 96 hours to monitor the evolution of the soft rot disease. The disease assessment was recorded following the method described by Saleh et al. (1996).

Statistical analysis

The data collected underwent statistical analysis employing Analysis of Variance (ANOVA) through the Statistical Analysis System (SAS Institute Inc., 1996). Following this, mean comparisons were conducted using Tukey's test, as outlined by Gomez and Gomez (1984).

3. Results

3.1. Pathogenicity test on potato slices

Twenty isolates were procured from potato tubers exhibiting natural disease symptoms. These isolates were subsequently assessed for their pathogenicity on healthy potato slices. Repeated pathogenicity tests revealed that only 15 of these isolates exhibited pathogenic characteristics, inducing typical soft rot symptoms with varying severity levels, ranging from strong to weak. Control slices, on the other hand, displayed no symptoms. The detailed results of the pathogenicity tests on the slices are outlined in Table 1.

Table 1: Disease severity index of selected bacterial soft rot isolates.

Isolates No	Disease severity %	Isolates No	Disease severity %
1	25 f	11	0 i
2	15 h	12	25 f
3	20 g	13	56 c
4	22 g	14	70 a
5	20 g	15	e
6	35 e	16	i
7	42 d	17	oi
8	34 e	18	66 b
9	45 d	19	67a
10	0 i	20	0 i

Values in the column followed by different letters indicate significant differences among treatments according to a least significant differences test ($P = 0.05$).

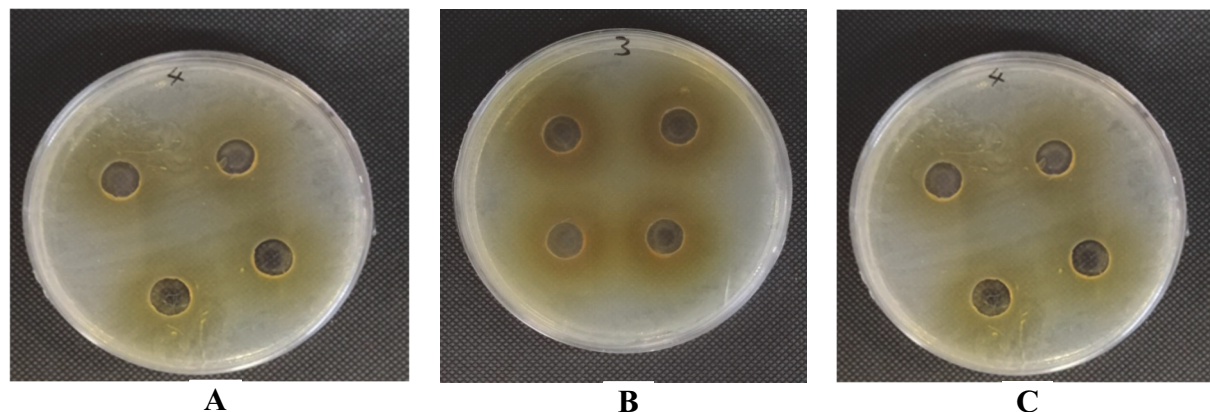
3.2. Identification of pathogens

The identification process for the pathogenic bacteria involved a thorough examination of morphological and physiological characteristics, encompassing factors such as cell shape, motility, Gram staining reaction, and various physiological tests. Drawing upon these characteristics it was deduced that all the examined isolates could be classified as *Pectobacterium carotovorum* subsp. *carotovorum* (Jones) Dye.

3.3. Control of potato soft rot causal pathogen by different plant extract (In vitro study)

Results showed that the tested pomegranate extract revealed more bactericidal activity on soft rot causal pathogen *P. carotovorum* subsp. *carotovorum* isolate no. 3 than other extract. The data demonstrated that thyme extract was on the first ranking with bacterial growth reduction

percentage estimated (1.4 mm), followed by clove extract with growth reduction estimated (0.92). The result of this experiment was recorded in Figs. (1).



(Fig. 1). Effect of certain plant extracts on inhibition of pathogen growth, a, Cumin, b, pomegranate, and c, Black pepper.

3.4. Evaluation of, cumin, pomegranate, and black pepper extract on soft rot diseases under storage conditions

Table 2 presented data that showed the ability of various plant extracts to reduce the severity of the potato tuber disease. The data revealed that all of the tested plant extracts were able to reduce the severity of the disease.

Of all the extracts, pomegranate extract showed the highest reduction in disease severity. This indicates that pomegranate extract was particularly effective in reducing the impact of the disease on potato tubers. This is a promising result as it suggests that pomegranate extract may be a potential alternative for controlling the disease.

Following pomegranate extract, the cumin and black pepper extracts also showed a reduction in disease severity. These results indicate that both cumin and black pepper extracts may also have potential for controlling the disease, although to a lesser extent compared to pomegranate extract. It is important to note that further studies are needed to validate these results and to determine the optimal concentration and application methods for these plant extracts to effectively control the disease. However, the results presented in Table 2 suggest that these plant extracts may offer a natural alternative for controlling potato tuber diseases.

(Table 2). Evaluation of certain oils on soft rot diseases under storage conditions

Oils	Diseases index	Disease reduction
Pomegranate	7.00 c	89.2
Black pepper	15.46 b	76.2
Cumin	5.66 c	91.3
Control healthy	0.0 d	-
Control infected	65.0 a	-

*Means with the same letter vertically are not significantly different according to Duncan’s multiple range test at level of ($P \leq 0.05$).

Discussion

In an effort to address this issue, a study was conducted to evaluate the pathogenicity and virulence of different bacterial isolates in relation to soft rot in potato tubers. The results showed that only 15 isolates were identified as pathogenic, capable of causing soft rot symptoms, and demonstrated varied levels of virulence. These results aligned with those found by previous researchers in the field, including Ismail and Moustafa (2012), Ashmawy et al. (2015), Czajkowski et al. (2015), Shmas et al. (2016), and Azaiez et al. (2018).

The identity of these ten isolates was confirmed through a range of techniques, including Gram staining, and hanging drop and physiological study. These methods were found to be effective in identifying *Pectobacterium* spp., which was consistent with the results of previous studies such as those by Ngadze et al. (2012) and Benada et al. (2018).

Additionally, the study evaluated the effect of different plant extracts, including black pepper, pomegranate, and cumin, on the suppression of soft rot in potato tubers during storage conditions. The results showed that these plant extracts exhibited antibacterial activity against *P. carotovorum* subsp. *carotovorum*, with pomegranate extract causing complete suppression of the disease. These results were consistent with those found by Abo-Elyousr and Bagy (2019).

Overall, this study highlights the importance of addressing the issue of soft rot in potato production, as it negatively impacts the quality and quantity of this crucial crop in KSA. The results provide valuable insights into the pathogenicity and virulence of different bacterial isolates, as well as the potential for using oils as a means of suppressing the disease. The study's findings on the antibacterial activity of various plant extracts against *P. carotovorum* subsp. *carotovorum* indicated distinct effects on reducing bacterial growth for each extract.

The study aligns with the findings presented by Abo-Elyousr and Bagy (2019), where the researchers specifically examined the impact of pomegranate, black pepper, and cumin at a concentration of 50 µg/ml in suppressing potato tuber soft rot during storage conditions.

In summary, plant extracts have demonstrated effectiveness in controlling soft rot disease. These natural remedies exhibit antibacterial properties capable of inhibiting the growth and dissemination of soft rot-causing pathogens. Moreover, they offer an eco-friendly alternative and are often more cost-effective compared to synthetic chemical counterparts. Nevertheless, it is crucial to recognize that not all plant extracts exhibit the same level of efficacy, and some may have adverse effects on plants or the environment. Therefore, additional research is imperative to comprehensively understand the potential advantages and limitations of employing plant extracts for soft rot disease control.

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المستخلصات النباتية القوية: مكافحة مرض التعفن الطري على درنات البطاطس الذي تسببه بكتيريا *Pectobacterium carotovorum subsp. carotovorum*

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المستخلص. الهدف من هذا البحث هو تقييم فعالية ثلاثة مستخلصات نباتية - الكمون والرمان والفلفل الأسود ضد بكتيريا *Pectobacterium carotovorum subsp. carotovorum*، وهي بكتيريا مسؤولة عن التسبب في مرض العفن الطري في درنات البطاطس. يشكل هذا المرض تهديداً كبيراً لإنتاج البطاطس، مما يؤثر على جودة البطاطس أثناء التخزين والنقل والشحن. لإجراء البحث، تم الحصول على عشرين عذلة من البكتيريا المسببة للأمراض من درنات البطاطس المصابة طبيعياً وتم تعريفها على أنها *P. carotovorum subsp. carotovorum* من خلال الخصائص الميكروبيولوجية المختلفة. ومن خلال التجارب المعملية، تم إجراء تجربة لدراسة المستخلصات النباتية لمعرفة خصائصها المضادة للبكتيريا. وأظهرت النتائج أن مستخلصات النباتات الثلاثة أظهرت تثبيط نمو البكتيريا. ومن الجدير بالذكر أن الرمان أظهر التأثير المثبط الأكثر فعالية بين المستخلصات الثلاثة. في ضوء هذه النتائج المخبرية يوصى باستخدام جميع المستخلصات بتركيز ٥٠ ملغم للسيطرة بفعالية على مرض العفن الطري في درنات البطاطس أثناء التخزين. تم تحديد تركيز ٥٠ ملغ باعتباره الأكثر فعالية في تثبيط نمو البكتيريا المسببة للأمراض. أظهرت هذه الدراسة قدرة جميع المستخلصات النباتية التي تم اختبارها على التخفيف من مرض العفن الطري على درنات البطاطس، مما يعالج تحدياً كبيراً في إنتاج البطاطس..

الكلمات المفتاحية: مرض التعفن الطري، البطاطس، مستخلصات نباتية، مضاد النمو.

