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Evaluation of certain biogenic compounds and chemical pesticides for controlling bacterial soft rot disease in onion (*Allium cepa* L.) in Karbala Province

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Abstract

This study aimed to investigate the occurrence of bacterial soft rot disease in onion crops in Karbala Province, assess its pathogenicity, determine the susceptibility of the most common onion cultivars, and evaluate the effect of selected biogenic agents and chemical pesticides on the pathogen for control purposes. Pathogenicity tests of *Pectobacterium carotovorum* subsp. *carotovorum* revealed that isolate Pcc7 was the most virulent, inducing the largest necrotic area (62.62 mm) within 72 hours, followed by isolates Pcc5 and Pcc2, with lesion sizes of 58.22 mm and 40.1 mm, respectively.The evaluation of five onion cultivars for susceptibility to soft rot showed that the Local Red cultivar was significantly more susceptible to *P. carotovorum* subsp. *carotovorum*, producing the largest lesion size of 21.2 mm, followed by Texas Early Grano with 9.6 mm. The results also indicated that both melatonin and glutathione exhibited potent inhibitory effects on *P. carotovorum* subsp. *carotovorum* (Pcc7) in vitro, achieving 100% inhibition at a concentration of 1.0 mg/L. In terms of chemical control, three pesticides—Beltanol, Ganger, and Oxyride—demonstrated high efficacy in inhibiting bacterial growth. Both Beltanol and Ganger achieved complete Inhibition (100%) at the highest tested concentration (1.0 mL/L).

Keywords: Keywords: *Pectobacterium carotovorum* subsp. *Carotovorum*, Onion , Soft rot

Introduction

Onion (*Allium cepa* L.) is one of the major crops cultivated in many countries due to its high nutritional value and health benefits. Taxonomically, it belongs to the family Liliaceae. Onion is considered among the oldest cultivated plants worldwide and is grown under a wide range of climatic conditions [1]. It holds great economic importance and is widely used in human diets [2].

Onion is susceptible to numerous diseases, including viral, fungal, nematode, and bacterial infections [3, 4]. Bacterial diseases and bulb rots represent a significant threat to onion production worldwide. The most prominent bacterial diseases include leaf blight, soft rot, center rot, streaking, and various forms of bulb rot [5]. Among these, bacterial soft rot, caused by the pathogenic bacterium *Pectobacterium*

carotovorum subsp. *Carotovorum*, is considered one of the most destructive. Infection usually begins in the field and develops rapidly during transportation and storage, leading to significant post-harvest losses [6]. Economically important bacterial species responsible for soft rot include *P. carotovorum* subsp. *carotovorum, Erwinia chrysanthemi,* and *P. carotovorum* subsp. *atroseptica* [7, 8].

Controlling plant diseases is a key factor in preserving the quality and quantity of food, feed, and fiber crops. Farmers often rely on chemical pesticides for disease management. However, concerns about environmental pollution from excessive pesticide use have led researchers to explore alternative strategies, including biological resistance [9]. Biological control agents are typically bacterial or fungal strains isolated from soil [10].

Materials and Methods

Sample Collection

Onion bulb samples showing typical symptoms of soft rot or water-soaked decay were collected during the 2024–2025 season from various fields, storage facilities, and local markets in Karbala Province. The samples were placed in labeled plastic bags indicating the collection location, date, and cultivar. They were transferred to the Plant Pathology Laboratory, Department of Plant Protection, College of Agriculture, University of Kerbala for diagnosis and further studies.

Table (1): Regions from which onion samples infected with soft rot disease were collected and the codes of the isolates

Isolate Code	Number of Samples	Collection Area	Type of Collection Site	Governorate
Pcc1	20	Karbala Center	storage, market	Karbala
Pcc2	15	Al-Husseiniya	storage, market	
Pcc3	17	Al-Nasr District	market	
Pcc4	19	Al-Khayrat	field, market	
Pcc5	8	Al-Amel District	market	
Pcc6	13	Al-Hur	field, storage, market	
Pcc7	20	Tuwairij	market	
Pcc8	14	Al-Jadwal Al-Gharbi	field, market	
Pcc9	18	Umm Rawaia	field, storage, market	
Pcc10	12	Al-Rashida	field, storage, market	
Pcc11	16	Ain Al-Tamur	field, market	
Pcc12	14	Al-Rujayba	field, storage, market	

Isolation and Purification of the Pathogen

The method of Doololbeldieva et al. (2016) was followed with slight modifications. Symptomatic onion bulbs were washed with running tap water to remove soil particles, then surface sterilized using 2% sodium hypochlorite (NaOCl) for two minutes, and

rinsed several times with sterile distilled water. The outer scales were removed, and the internal tissue was macerated in a sterile mortar with a small amount of sterile normal saline solution (0.85% NaCl).

A portion of the suspension was streaked onto sterilized Nutrient Agar (NA) medium using a sterile inoculating loop. The plates were incubated at 28 ± 2°C for 24 hours. Colonies were then re-streaked onto fresh NA medium using the dilution method to obtain single colonies for purification. The incubation conditions remained the same.

Pathogenicity Test of Bacterial Isolates

Uniform and healthy onion bulbs were selected for testing For the five varieties: Local Red ,White Grano, Giza (Egyptian) ,Texas Grano and Local White. They were washed thoroughly, surface-sterilized with 10% sodium hypochlorite for two minutes, and rinsed multiple times with sterile distilled water. A 5 mm hole was made in the center of each bulb using a sterile cork borer.

The bulbs were placed in sterilized plastic containers lined with moist sterile filter paper. Each hole was inoculated with 100 µL of a 24-hour-old bacterial suspension (10^6 CFU/mL). Three bulbs were used for each isolate, with one bulb per container. The containers were incubated at 28 ± 2°C for six days. The virulence of each isolate was determined based on the diameter of the rotted tissue.

Susceptibility of Onion Cultivars to *P. carotovorum* subsp. *carotovorum* (Isolate Pcc7)

Five onion cultivars commonly grown in Iraq were tested: Texas Grano, White Grano, Giza (Egyptian), Local Red, and Local White. Ten bulbs per cultivar were surface-sterilized with 10% sodium hypochlorite for two minutes and rinsed thoroughly with sterile distilled water. A 5 mm diameter and depth hole was made in each bulb using a sterile cork borer, 30 minutes prior to inoculation.

Each hole was inoculated with 1 mL of the bacterial suspension of isolate Pcc7. The bulbs were placed in 12 × 8 cm sterilized plastic containers lined with moist filter paper and sealed to maintain humidity. The containers were incubated in darkness at 28 ± 2°C for six days. A completely randomized design (CRD) was used with 10 replicates per cultivar, along with a control treatment using sterile distilled water. The diameter of the rotted area was measured and the data were statistically analyzed.

Control of *P. carotovorum* subsp. *carotovorum* (Pcc7)

Evaluation of Chemical Pesticides

Thirteen sterile flasks, each containing 150 mL of autoclaved NA medium, were prepared. After cooling the medium to 45°C, the chemical pesticides Beltanol, Ganger, B-Oxyride, and Nordox were added separately at the recommended concentrations (1.0, 0.75, and 0.5 mL/L).

The media were poured into sterile Petri dishes and inoculated with a 24-hour-old suspension of Pcc7. The plates were incubated at 28 ± 2°C for 48 hours. The experiment was conducted using a completely randomized design (CRD) with three

replicates per treatment and an untreated control. The number of bacterial colonies was counted, and the inhibition percentage was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{(\text{Colony count in control} - \text{Colony count in treatment})}{(\text{Colony count in control})} \times 100$$

Evaluation of Melatonin and Glutathione

Seven sterile flasks containing 150 mL of NA medium were prepared. After cooling, melatonin and glutathione were added separately at concentrations of 0.5, 0.75, and 1.0 mg/L. The media were poured into sterile Petri dishes and inoculated with a 24-hour-old suspension of Pcc7.

The plates were incubated at 28 ± 2°C for 48 hours. The experiment was conducted using a completely randomized design (CRD) with three replicates per treatment and a control treatment without any additives. Colony counts were recorded, and inhibition percentages were calculated as previously described.

Results and Discussion

Isolation and Purification of the Pathogen

Twelve bacterial isolates (Pcc1 to Pcc12) were obtained from onion samples collected from fields, markets, and storage facilities across various areas in Karbala Province. After 24 hours of incubation at 28 ± 2°C, the bacterial colonies appeared cream to shiny white in color, circular, with smooth and regular edges, a convex surface, and diameters ranging from 0.5 to 1 mm (Figure 1). These morphological characteristics are consistent with descriptions of *Pectobacterium carotovorum* in both local and international studies regarding colony shape and growth on culture media [11, 12,13].

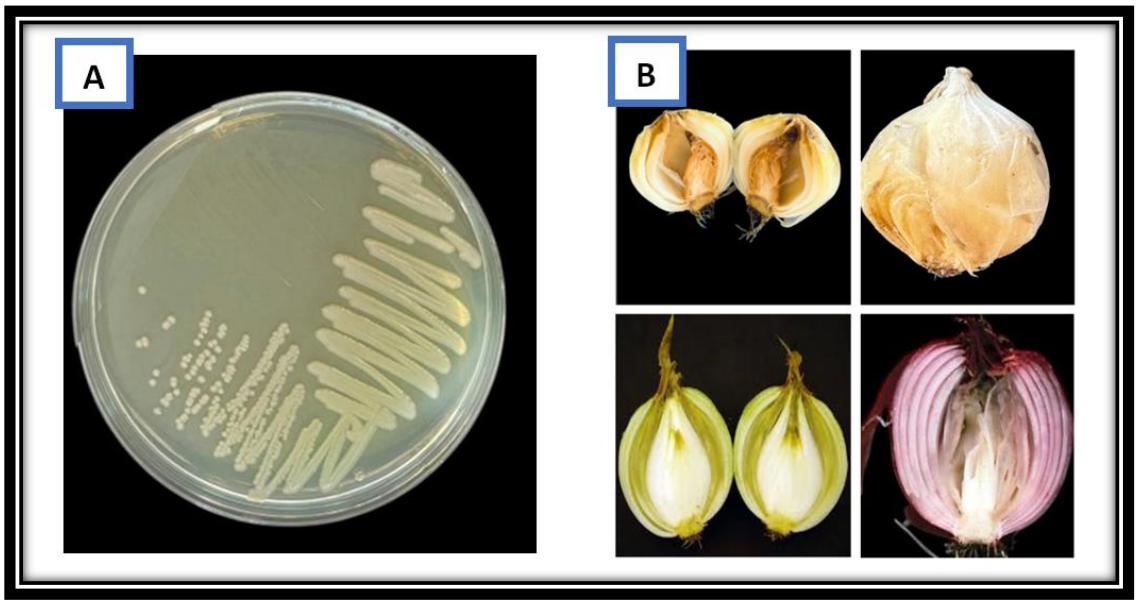

Figure 1. Isolation and purification of the bacteria causing soft rot disease on onion, showing: (A) Bacterial growth on culture medium (B) Infected onion samples

Pathogenicity Test of Bacterial Isolates

Pathogenicity testing of the bacterial isolates on onion bulbs (Figures 2 and 3) confirmed the ability of all isolates to induce typical soft rot symptoms such as tissue maceration and foul odor. The severity of the symptoms varied among the isolates, with lesion sizes ranging from 1.4 mm to 62.62 mm after 72 hours.

Isolate Pcc7 was the most virulent, causing the largest rot area (62.62 mm), followed by isolates Pcc5 (58.22 mm) and Pcc2 (40.1 mm). Isolate Pcc6 was the least virulent, with only 1.43 mm of tissue maceration. Based on these findings, Pcc7 was selected for molecular identification and further analysis.

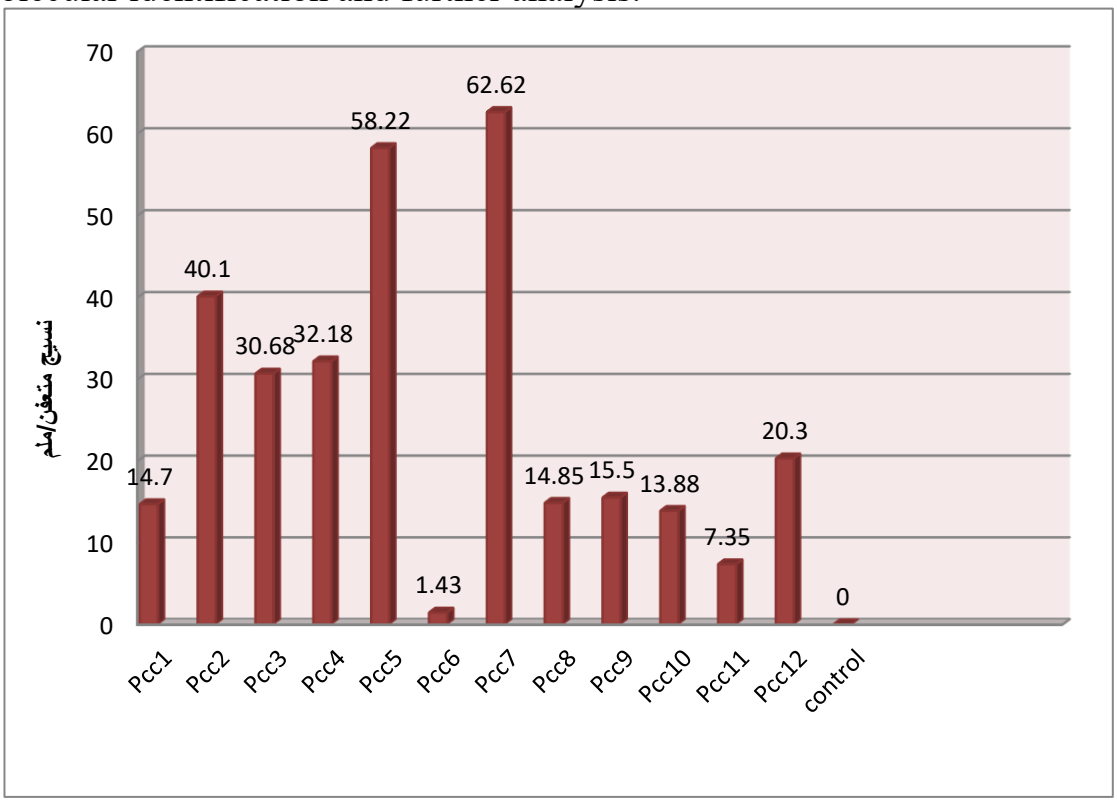

Figure (2): Pathogenicity test of bacterial isolates causing soft rot disease on onions

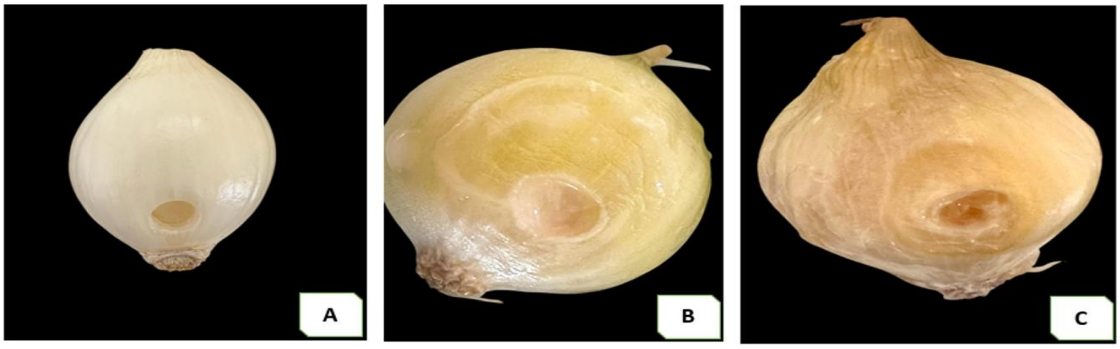

Figure 3. Pathogenicity of bacterial isolate Pcc7 on onions, showing:
(A) Control treatment (sterile distilled water only) (B) Pathogenicity after 3 days (C) Disease progression after 6 days

Susceptibility of Onion Cultivars to *P. carotovorum* subsp. *carotovorum* (Pcc7)

Results (Figure 4) showed that the Local Red cultivar was significantly more susceptible to soft rot than the other cultivars tested. It developed the largest lesion size, averaging 21.2 mm, followed by Texas Early Grano (9.6 mm), White Grano (6.9 mm), and Giza (Egyptian) cultivar (2.8 mm). The Local White cultivar exhibited the lowest susceptibility, with only 3.0 mm of tissue maceration. All cultivar responses differed significantly from one another.

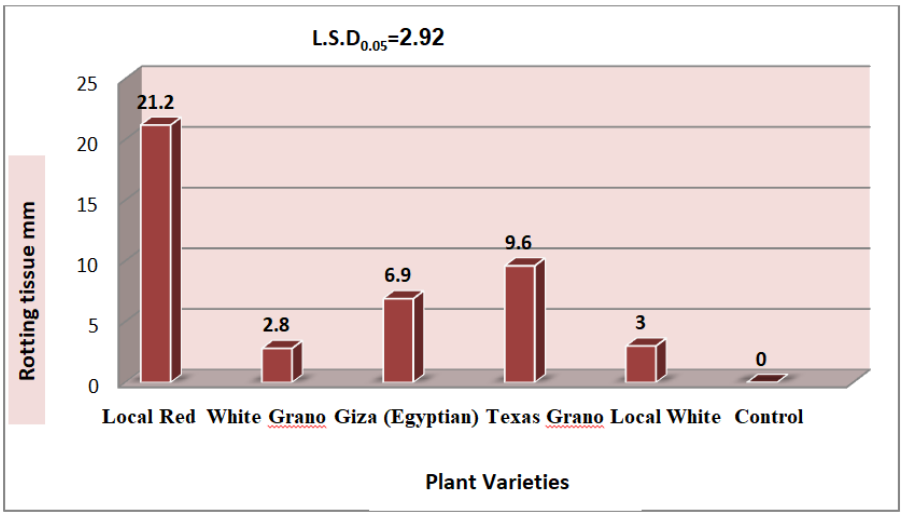

Figure (4): Rotted Tissue Volume in Onion Varieties Infected by *Pectobacterium carotovorum* subsp. *carotovorum*

Control of *P. carotovorum* subsp. *carotovorum* (Pcc7)

Evaluation of Melatonin and Glutathione

Melatonin and glutathione showed potent inhibitory effects on *P. carotovorum* subsp. *carotovorum* (Pcc7) under in vitro conditions Figure (5), achieving 100% inhibition at a concentration of 1.0 mg/L.

The results indicated a positive correlation between melatonin concentration and its inhibitory effect. This could be attributed to its structural similarity to biological compounds like tryptophan and serotonin, potentially interfering with bacterial metabolic pathways. Melatonin also acts as a signaling molecule in plants, activating defense pathways dependent on salicylic acid and jasmonic acid and mitigating oxidative stress by neutralizing reactive oxygen species (ROS) [14, 15].

Glutathione similarly demonstrated potent antibacterial activity. As a major antioxidant in plants, it protects cells from oxidative damage by scavenging ROS. It also contributes to the synthesis of defense-related metabolites and activates systemic acquired resistance. Recent studies highlight glutathione's role as a signaling molecule in long-term plant immune [16,17, 18].

At lower concentrations, glutathione slightly outperformed melatonin, suggesting that their mechanisms of action may be complementary. Melatonin primarily regulates and stimulates antioxidant defenses, while glutathione is directly involved in detoxification and immediate oxidative stress management [19, 20].

These findings support the promising potential of melatonin and glutathione as eco-friendly biocontrol agents for managing bacterial soft rot in onion, offering sustainable alternatives to synthetic chemical pesticides.

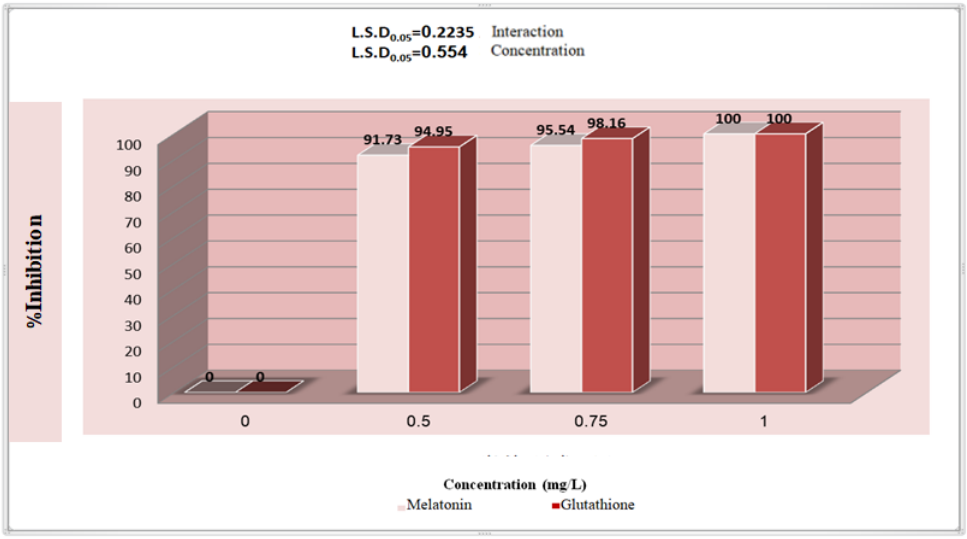

Figure (5): Inhibitory Effect of Different Concentrations of Melatonin and Glutathione Against *P. carotovorum* subsp. *carotovorum* in NA Medium

Evaluation of Chemical Pesticides

The in vitro assessment of four chemical pesticides—Beltanol, Ganger, Oxyride, and Nordox—against *P. carotovorum* subsp. *carotovorum* (Pcc7) showed varying degrees of inhibition Figure (6). All treatments significantly Inhibition bacterial colony growth compared to the control.

Beltanol and Ganger showed the highest inhibitory effect, achieving complete bacterial growth inhibition (100%) at 1.0 mL/L. This was attributed to their shared active ingredient, hydroxyquinoline sulfate (50% w/v), which has been documented to suppress a wide range of plant pathogens [21, 22].

Oxyride demonstrated high inhibitory activity as well, reaching 96.04% at the highest concentration. Its efficacy is due to its high copper oxide content (60%), which acts via Cu^{2+} ions, damaging multiple bacterial cell components and interfering with enzymatic activity [23, 24].

Nordox recorded the lowest inhibition rate (67.01%), which, although statistically significant compared to the control, was notably lower than the other pesticides.

These results support the superior performance of pesticides containing hydroxyquinoline sulfate or copper oxide, with Beltanol and Oxyride identified as the most promising candidates for future application-based experiments [25].

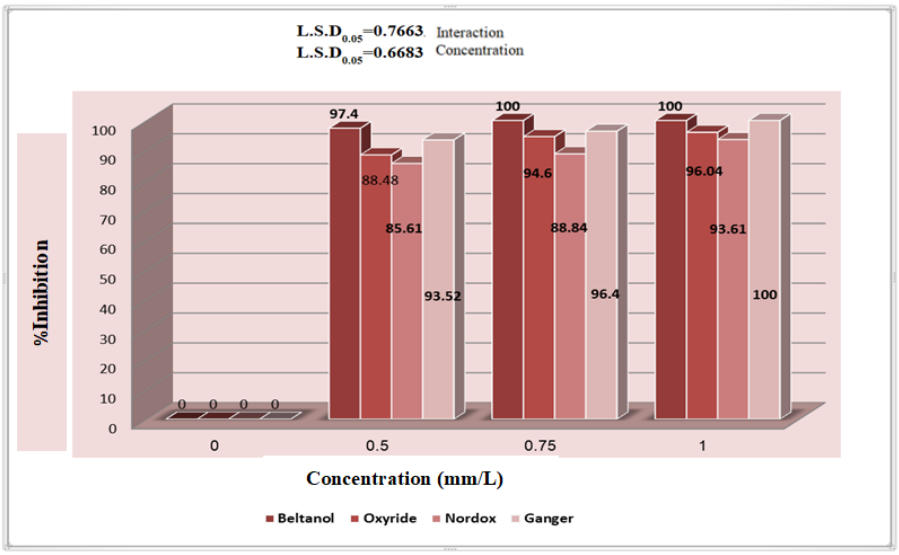

Figure (6): Inhibitory Effect of Chemical Pesticides on *P. carotovorum* subsp. *carotovorum* (Pcc7) in NA Medium

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