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## Effect of Calcium Carbonate on the Pathogenicity of *Streptomyces scabies* Causing Potato Scab and the Physiological Response of Potato Plants

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### ABSTRACT

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Potato scab disease, caused by *Streptomyces scabies*, is a significant bacterial disease affecting potato production worldwide. This study investigated the effect of calcium carbonate (CaCO<sub>3</sub>) on *S. scabies* pathogenicity and the physiological response of potato plants under greenhouse conditions. Potato plants (cv. Diamond) were grown in soil inoculated with *S. scabies* strain E21 with and without CaCO<sub>3</sub> (5 g/kg soil). Plant growth parameters, disease incidence and severity, and biochemical responses were evaluated. Results showed that CaCO<sub>3</sub> amendment increased disease severity from 31.15% to 45.35% and disease incidence from 91.68% to 100%, though not statistically significant. CaCO<sub>3</sub> treatment significantly increased tuber weight (107.5 g vs. 88.5 g) and root dry weight (1.05 g vs. 0.45 g) but decreased shoot dry weight and root length. Biochemical analyses revealed that CaCO<sub>3</sub> amendment enhanced antioxidant enzyme activities (SOD and CAT) while reducing H<sub>2</sub>O<sub>2</sub>, MDA, and phenolic compound levels at both 30 and 60 days after planting. These findings suggest that while CaCO<sub>3</sub> may increase disease severity by creating favorable conditions for *S. scabies*, it simultaneously enhances plant defense mechanisms, particularly the enzymatic antioxidant system. This study provides insights into the complex interactions between soil amendments, pathogen virulence, and plant physiological responses, with implications for integrated management of potato scab disease.

### INTRODUCTION

Potato scab is one of the important bacterial diseases affecting potato crops worldwide, caused by *Streptomyces scabies*, a soil-dwelling bacterium that attacks potato tubers causing surface deformities and corky lesions that reduce the marketable value of the crop (Dees et al., 2023). *Streptomyces scabies* belongs to the actinomycetes group, which are gram-positive bacteria characterized by the formation of branched mycelia and the production of conidial spores (Lerat et al., 2009). This bacterium is considered an economically significant plant pathogen due to its ability to persist in soil for extended periods and infect multiple crops from the Solanaceae family and others (Bignell et al., 2014).

The economic impact of potato scab disease is substantial, with recent studies indicating that it causes significant losses to potato growers worldwide (Khalil et al., 2025; Arseneault et al., 2021). The disease primarily affects the quality rather than the quantity of the yield, making infected tubers less marketable due to their unappealing appearance (Wanner and Kirk, 2015). According to Dees and Wanner (2012), the annual economic losses attributed to potato scab disease can reach millions of dollars in major potato-producing regions.

Environmental factors play a crucial role in the development of potato scab disease, as soil characteristics such as pH, moisture content, and organic matter affect the severity of infection (Keinath and Loria, 1989). *S. scabies* prefers alkaline soil with a pH range of 5.5-7.0, and the severity of infection increases in alkaline soils (Lacey and Wilson, 2001). Studies have indicated that increasing calcium carbonate (CaCO<sub>3</sub>) content in soil can affect the spread of pathogens and the severity of potato scab disease (Lambert and Manzer, 1991).

Calcium carbonate is an important chemical compound in soil that affects its physical and chemical properties, especially pH. Calcium carbonate raises the soil pH, making it more alkaline (Brady and Weil, 2008). Lambert and Manzer (1991) reported that liming of low pH soils increases the severity of common scab of potato caused by *S. scabies*. This relationship between calcium, soil pH, and disease severity has been further supported by recent research (McLeod et al., 2024), which found that the application of calcareous materials to potato-growing soils resulted in increased symptoms of common scab.

Plants respond to pathogen infection through multiple defense mechanisms, including the production of antioxidant enzymes such as catalase

(CAT), superoxide dismutase (SOD), and peroxidase (POD), as well as the accumulation of phenolic compounds and defensive proteins (Mittler, 2002). These defensive responses are affected by the environmental conditions surrounding the plant, including soil characteristics and mineral content (Sharma et al., 2012). Recent studies have shown that antioxidant enzyme activities in potato plants are significantly altered in response to pathogen infection (Wang et al., 2019; Liu et al., 2023), with SOD, POD, and CAT playing crucial roles in controlling reactive oxygen species (ROS) and serving as important markers of plant stress resistance.

The primary and immediate response of plants to pathogens is the overproduction of reactive oxygen species (ROS) at the site of infection (Qi et al., 2023). Plants have evolved an efficient antioxidant system that includes enzymes such as SOD, CAT, POD, and APX to scavenge ROS and avoid oxidative damage (Wang et al., 2019). The balance between ROS production and antioxidant defense mechanisms is critical for determining the outcome of plant-pathogen interactions (Mittler, 2002).

Given the importance of understanding the relationship between soil calcium carbonate content, *S. scabies* pathogenicity, and the physiological response of potato plants, this study aimed to evaluate the effect of adding calcium carbonate on the severity of potato scab disease and on the physiological response of potato plants by measuring antioxidant enzyme activity, phenolic compound content, and total protein content, in addition to its effect on vegetative and root growth of plants.

## MATERIALS AND METHODS

### 1. Isolation and Identification of *Streptomyces scabies*

*Streptomyces scabies* isolate was isolated from potato tubers showing symptoms of common scab disease following the method described by Wanner (2009). The infected tubers were cleaned with running water and their surface was sterilized using 1% sodium hypochlorite solution for 3 minutes, then washed with sterile distilled water three times. Samples were taken from the infected tissues and cultured on International Streptomyces Project medium (ISP4) to prevent the growth of other bacteria and fungi, as recommended by Loria et al. (2006). The plates were incubated at  $28\pm 2^\circ\text{C}$  for 7-10 days.

The suspected bacterial colonies were purified and identified based on morphological, physiological, and biochemical characteristics according to standard methods described by Shirling and Gottlieb (1966) and Bergy's Manual of Determinative Bacteriology (Holt et al., 1994). Isolate E21, which showed the typical

characteristics of *S. scabies*, was selected for use in subsequent experiments.

### 2. Experimental Design

The experiment was conducted in a greenhouse using 5 kg plastic pots. The soil was sterilized by heat treatment at  $121^\circ\text{C}$  for one hour to eliminate any pathogens, following the method described by Wanner and Kirk (2015). The experiment included three treatments: soil inoculated with *Streptomyces scabies* (isolate E21) without the addition of calcium carbonate; soil inoculated with *S. scabies* (isolate E21) with calcium carbonate added at a rate of 5 grams per kilogram of soil; and a control treatment in which the soil was not inoculated with *S. scabies*.

The bacterial inoculum was prepared by growing isolate E21 on ISP4 Broth for 7 days at  $28\pm 2^\circ\text{C}$ , as described by Loria et al. (2006). The inoculum concentration was adjusted to  $10^8$  colony-forming units/ml using sterile distilled water, following the method of Wanner (2009). The soil was inoculated with the bacterial inoculum at a rate of 50 ml per pot in the first and second treatments.

"Diamond" variety potato tubers, disease-free and uniform in size, were planted in the pots at a rate of one tuber per pot. The plants were watered regularly to maintain soil moisture at field capacity. The experiment was implemented using a Completely Randomized Design (CRD) with three replicates per treatment, as recommended by Gomez and Gomez (1984).

### 2.1. Measurements and Analyses

#### 2.1.1. Plant Characteristics

Plant characteristics were measured 60 days after planting, following the standard methods described by Struik et al. (1989). The measured parameters included the number of tubers per plant, tuber weight (g), root dry weight (g), shoot dry weight (g), root fresh weight (g), shoot fresh weight (g), root length (cm), and shoot length (cm).

#### 2.1.2. Evaluation of Potato Scab Disease

Potato scab disease was evaluated at harvest by calculating both disease incidence (DI%) and disease severity (DS%). Disease incidence was determined as the percentage of infected tubers out of the total number of tubers, following the method described by Wanner and Kirk (2015). Disease severity was estimated using a scale from 0 to 5, where 0 indicates no infection, 1 indicates 1–10% of the tuber surface infected, 2 indicates 11–25%, 3 indicates 26–50%, 4 indicates 51–75%, and 5 indicates more than 75% of the tuber surface infected, according to the method of Loria et al. (2006). The severity percentage was calculated using the equation:  $\text{DS\%} = [\sum(\text{infection degree} \times \text{number of tubers in each degree}) / (\text{highest infection degree} \times \text{total number of tubers})] \times 100$ .

#### 2.1.3. Plant Chemical Response

Samples of plant leaves were taken after 30 and

60 days of planting to estimate various biochemical parameters following established protocols. Catalase enzyme activity (CAT) was estimated according to Aebi's method (1984) by measuring the rate of  $H_2O_2$  decomposition at a wavelength of 240 nm. Superoxide dismutase enzyme activity (SOD) was estimated according to Beauchamp and Fridovich's method (1971) by measuring the enzyme's ability to inhibit the reduction of nitro blue tetrazolium (NBT) at a wavelength of 560 nm. Peroxidase enzyme activity (POD) was estimated according to Hammerschmidt et al.'s method (1982) by measuring the rate of guaiacol oxidation at a wavelength of 470 nm. Total phenolic compounds content (TPC) was estimated using Folin-Ciocalteu reagent according to Singleton and Rossi's method (1965). Malondialdehyde level (MDA), as an indicator of lipid peroxidation, was estimated according to Heath and Packer's method (1968). Hydrogen peroxide content ( $H_2O_2$ ) was estimated according to Velikova et al.'s method (2000). Polyphenol oxidase enzyme activity (PPO) was determined following the method described by Mayer et al. (1965). Total protein content was estimated using Bradford's method (1976).

Enzyme extract was prepared by grinding 0.5 g of fresh leaves in 5 ml of phosphate buffer solution (pH 7.0) in an ice bath, then centrifuging at 10000 rpm for 20 minutes at 4°C, as described by Sharma et al. (2012). The supernatant was used to estimate enzyme activity and total protein content.

### 3. Statistical Analysis

Data were statistically analyzed using Analysis of Variance (ANOVA) and means were compared using Least Significant Difference (LSD) test at a significance level of 0.05, following the methods

described by Gomez and Gomez (1984). CoStat statistics (CoHort software, version 6.45) was used to perform statistical analyses.

## RESULTS

### 1. Morphological, Physiological, and Biochemical Characteristics of *Streptomyces scabies*

The results of the morphological, physiological, and biochemical identification of isolate E21 showed that it belongs to *Streptomyces scabies* (Table 1). The isolate was characterized by the presence of spiral hyphae and long cells, and was able to hydrolyze starch, casein, and gelatin, and use different carbon sources such as glucose, sucrose, and maltose.

### 2. Effect of Calcium Carbonate on Plant Characteristics

The results indicated in Table 2 showed that adding calcium carbonate to soil inoculated with *S. scabies* led to improvement in some plant characteristics compared to inoculated soil without calcium carbonate addition. A significant increase in tuber weight was observed from 88.5 g in the first treatment (E21) to 107.5 g in the second treatment (E21+CaCO<sub>3</sub>). A significant increase in root dry weight was also observed from 0.45 g to 1.05 g, and a significant decrease in shoot dry weight from 4.85 g to 3.6 g.

For root length, a significant decrease was observed from 51 cm in the first treatment to 32 cm in the second treatment, while there was no significant difference in shoot length between the two treatments. A decrease in the number of tubers from 6 tubers in the first treatment to 5.333 tubers in the second treatment was also observed, but this decrease was not significant.

**Table 1: The morphological, physiological, and biochemical characteristics of *Streptomyces scabies* strain E21**

Characteristic	<i>Streptomyces scabies</i> strain E21
Shape of cell	Spiral hyphae
Size of cell	Long
Color of spores	Grey
Gram staining	ve+
Starch hydrolysis	+
Casein hydrolysis	+
Gelatin liquefaction	+
Carbon usage	
Glucose	+
Sucrose	+
Maltose	+
Crystal violet (0.5µg/ml)	-
Growth with:	
5% NaCl	-
6% NaCl	-
7% NaCl	-
CaCO <sub>3</sub>	+

**Table 2: Effect of calcium carbonate on vegetative and yield characteristics of potato plants inoculated with *Streptomyces scabies* strain E21**

Treatments	Sh. Length (cm)	Ro. Length (cm)	Sh. Weight.F (g)	Ro. Weight.F (g)	Sh. Weight.D (g)	Ro. Weight.D (g)	T. Weight (g)	T. Number
E21	36.5 <sup>b</sup> ±3.5	51 <sup>a</sup> ±0	47.5 <sup>b</sup> ±5.5	5 <sup>b</sup> ±0	4.85 <sup>a</sup> ±0.05	0.45 <sup>b</sup> ±0.35	88.5 <sup>c</sup> ±1.5	6 <sup>a</sup> ±1
E21+CaCO <sub>3</sub>	30.5 <sup>b</sup> ±3.5	32 <sup>b</sup> ±5	50.333 <sup>b</sup> ±4.041	6.5 <sup>ab</sup> ±0.5	3.6 <sup>b</sup> ±0.7	1.05 <sup>a</sup> ±0.25	107.5 <sup>b</sup> ±12.5	5.333 <sup>a</sup> ±0.577
Control	43 <sup>a</sup> ±1.732	30.166 <sup>b</sup> ±1.258	71.5 <sup>a</sup> ±6.5	8.666 <sup>a</sup> ±2.309	5.566 <sup>a</sup> ±0.550	0.9 <sup>ab</sup> ±0.2	159.5 <sup>a</sup> ±6.5	7 <sup>a</sup> ±2
LSD	6.048	5.947	10.871	2.725	1.029	0.547	16.343	2.663

Treatments include: E21 (inoculated soil without CaCO<sub>3</sub>), E21+CaCO<sub>3</sub> (inoculated soil with 5 g CaCO<sub>3</sub> per kg of soil), and Control (non-inoculated, non-amended). Parameters measured include shoot length (Sh. Length), root length (Ro. Length), shoot fresh weight (Sh. Weight.F), root fresh weight (Ro. Weight.F), shoot dry weight (Sh. Weight.D), root dry weight (Ro. Weight.D), tuber weight (T. Weight), and number of tubers (T. Number). Values represent means ± standard deviation (SD). Means followed by different letters within a column are significantly different at  $P \leq 0.05$  according to LSD (Least Significant Difference) test.

### 3.3. Effect of Calcium Carbonate on Potato Scab Disease

As shown in Table 3, the addition of calcium carbonate to soil inoculated with *S. scabies* resulted in an increase in disease incidence (DI%) from 91.68% to 100%; however, this difference was not statistically significant. An increase in disease severity (DS%) from 31.15% in the first treatment to 45.35% in the second treatment was also observed, but this increase was also not statistically significant.

Treatments include: E21 (inoculated soil without CaCO<sub>3</sub>), E21+CaCO<sub>3</sub> (inoculated soil with 5 g CaCO<sub>3</sub> per kg of soil), and Control (non-inoculated, non-amended). Disease incidence (DI%) and disease severity (DS%) were recorded at the end of the greenhouse experiment. Values represent means ± standard deviation (SD). Means followed by different letters within a column are significantly different at  $P \leq 0.05$  according to the LSD (Least Significant Difference) test.

**Table 3. Pathogenicity test of *Streptomyces scabies* (strain E21) in potato tubers with and without calcium carbonate amendment under greenhouse conditions**

Treatments	DS%	DI%
E21	31.15 <sup>a</sup> ±3.15	91.68 <sup>a</sup> ±8.3
E21+CaCO <sub>3</sub>	45.35 <sup>a</sup> ±18.65	100 <sup>a</sup> ±0
Control	0 <sup>b</sup> ±0	0 <sup>b</sup> ±0
LSD	21.817	9.631

### 3.4. Effect of Calcium Carbonate on Plant Chemical Response after 30 Days of Planting

According to Table 4, the addition of calcium carbonate to soil inoculated with *S. scabies* significantly affected the chemical responses of potato plants after 30 days of growth. A significant increase in total protein content from 195.140 µg/mL in the first treatment to 197.070 µg/mL in the second treatment, a significant increase in superoxide dismutase enzyme activity (SOD) from 80.637 to 89.889 µM/g FW, and a significant increase in catalase enzyme activity (CAT) from 2.771 to 2.903 µM/g FW were observed. Meanwhile, a significant decrease in hydrogen peroxide content (H<sub>2</sub>O<sub>2</sub>) from 22.817 µM/g FW in the first treatment to 21.484 µM/g FW in the second treatment, a significant decrease in malondialdehyde level (MDA) from 0.036 to 0.010 µM/g FW, and a significant decrease in total phenolic compounds content (TPC) from 48.375 to 36.416 mg GAE/g were observed. There was no significant difference in peroxidase enzyme activity (POD) and polyphenol oxidase enzyme activity (PPO) between the two treatments.

Treatments include: E21 (inoculated soil without CaCO<sub>3</sub>), E21+CaCO<sub>3</sub> (inoculated soil with 5 g CaCO<sub>3</sub> per kg of soil), and Control (non-inoculated, non-amended). Catalase activity (CAT), superoxide dismutase activity (SOD), peroxidase activity (POD), total phenolic content (TPC), malondialdehyde content (MDA), polyphenol oxidase activity (PPO), hydrogen peroxide levels (H<sub>2</sub>O<sub>2</sub>), and total protein content (T. Protein).

**Table 4: Comparative biochemical responses of potato (cv. Diamond) to *Streptomyces scabies* E21 infection with and without calcium carbonate amendment at 30 days post-planting**

Treatments	CAT ( $\mu\text{M/g}$ FW)	SOD ( $\mu\text{M/g}$ FW)	POD ( $\mu\text{M/g}$ FW)	TPC (mg GAE/g)	MDA ( $\mu\text{M/g}$ FW)	PPO ( $\mu\text{M/g}$ FW)	H <sub>2</sub> O <sub>2</sub> ( $\mu\text{M/g}$ FW)	T. Protein ( $\mu\text{g/mL}$ )
E21	2.771 <sup>b</sup> $\pm 0$	80.637 <sup>c</sup> $\pm 0.106$	4.844 <sup>a</sup> $\pm 0.004$	48.375 <sup>b</sup> $\pm 1.875$	0.036 <sup>a</sup> $\pm 0.001$	0.249 <sup>b</sup> $\pm 0.001$	22.817 <sup>a</sup> $\pm 0.173$	195.140 <sup>b</sup> $\pm 0.303$
E21+CaCO <sub>3</sub>	2.903 <sup>a</sup> $\pm 0.043$	89.889 <sup>a</sup> $\pm 0.183$	4.805 <sup>a</sup> $\pm 0.004$	36.416 <sup>c</sup> $\pm 2.009$	0.010 <sup>c</sup> $\pm 0.001$	0.273 <sup>b</sup> $\pm 0.001$	21.484 <sup>b</sup> $\pm 0.1$	197.070 <sup>a</sup> $\pm 0.303$
Control	2.761 <sup>b</sup> $\pm 0.023$	83.823 <sup>b</sup> $\pm 0.318$	4.593 <sup>b</sup> $\pm 0.049$	96.833 <sup>a</sup> $\pm 0.721$	0.015 <sup>b</sup> $\pm 0.002$	1.000 <sup>a</sup> $\pm 0.500$	19.976 <sup>c</sup> $\pm 0.200$	195.491 <sup>b</sup> $\pm 0.303$
LSD	0.057	0.441	0.057	3.277	0.003	0.576	0.327	0.607

Values are presented as means  $\pm$  standard deviation (SD). Different letters within a column indicate statistically significant differences at  $P \leq 0.05$ , based on the LSD (Least Significant Difference) test.

### 3.5. Effect of Calcium Carbonate on Plant Chemical Response after 60 Days of Planting

The results presented in Table 5 demonstrate that the addition of calcium carbonate to soil inoculated with *S. scabies* significantly altered the chemical responses of potato plants 60 days after planting. A significant decrease in total protein content from 199.175  $\mu\text{g/mL}$  in the first treatment to 198.298  $\mu\text{g/mL}$  in the second treatment, a significant increase in superoxide dismutase enzyme activity (SOD) from 84.057 to 85.869  $\mu\text{M/g}$  FW, and a significant increase in catalase enzyme activity (CAT) from 2.807 to 3.375  $\mu\text{M/g}$  FW were observed.

A significant decrease in hydrogen peroxide content (H<sub>2</sub>O<sub>2</sub>) from 20.44  $\mu\text{M/g}$  FW in the first treatment to 17.020  $\mu\text{M/g}$  FW in the second treatment, a significant decrease in polyphenol oxidase enzyme activity (PPO) from 0.288 to 0.267  $\mu\text{M/g}$  FW, a significant decrease in malondialdehyde level (MDA) from 0.018 to 0.010  $\mu\text{M/g}$  FW, a significant decrease in total phenolic compounds content (TPC) from 185.583 to 181 mg GAE/g, and a significant decrease in peroxidase enzyme activity (POD) from 4.342 to 3.764  $\mu\text{M/g}$

FW were also observed.

Treatments include: E21 (inoculated soil without CaCO<sub>3</sub>), E21+CaCO<sub>3</sub> (inoculated soil with 5 g CaCO<sub>3</sub> per kg of soil), and Control (non-inoculated, non-amended). Catalase activity (CAT), superoxide dismutase activity (SOD), peroxidase activity (POD), total phenolic content (TPC), malondialdehyde content (MDA), polyphenol oxidase activity (PPO), hydrogen peroxide levels (H<sub>2</sub>O<sub>2</sub>), and total protein content (T. Protein). Values are presented as means  $\pm$  standard deviation (SD). Different letters within a column indicate statistically significant differences at  $P \leq 0.05$ , based on the LSD (Least Significant Difference) test.

## DISCUSSION

This study aimed to evaluate the effect of adding calcium carbonate to soil on the pathogenicity of *Streptomyces scabies* causing potato scab disease and the physiological response of potato plants. Adding calcium carbonate at a rate of 5 grams per kilogram of soil led to significant changes in plant characteristics and chemical response, in addition to its effect on the severity of potato scab disease.

Experimental data revealed that incorporating calcium carbonate into soil inoculated with *S. scabies* improved some plant characteristics, especially tuber weight and root dry weight.

**Table 5: Comparative biochemical responses of potato (cv. Diamond) to *Streptomyces scabies* E21 infection with and without calcium carbonate amendment at 60 days post-planting**

Treatments	CAT ( $\mu\text{M/g}$ FW)	SOD ( $\mu\text{M/g}$ FW)	POD ( $\mu\text{M/g}$ FW)	TPC (mg GAE/g)	MDA ( $\mu\text{M/g}$ FW)	PPO ( $\mu\text{M/g}$ FW)	H <sub>2</sub> O <sub>2</sub> ( $\mu\text{M/g}$ FW)	T. Protein ( $\mu\text{g/mL}$ )
E21	2.807 <sup>c</sup> 0.008 $\pm$	84.057 <sup>b</sup> 0.627 $\pm$	4.342 <sup>a</sup> 0.004 $\pm$	185.583 <sup>a</sup> 0.360 $\pm$	0.018 <sup>b</sup> 0.001 $\pm$	0.288 <sup>b</sup> 0 $\pm$	20.44 <sup>a</sup> 0.100 $\pm$	199.175 <sup>a</sup> 0.303 $\pm$
E21+CaCO <sub>3</sub>	3.375 <sup>b</sup> 0.017 $\pm$	85.869 <sup>a</sup> 0.362 $\pm$	3.764 <sup>c</sup> 0.007 $\pm$	181 <sup>b</sup> 0 $\pm$	0.010 <sup>c</sup> 0 $\pm$	0.267 <sup>c</sup> 0 $\pm$	17.020 <sup>b</sup> 0.265 $\pm$	198.298 <sup>b</sup> 0.303 $\pm$
Control	3.461 <sup>a</sup> 0.035 $\pm$	81.159 <sup>c</sup> 0.181 $\pm$	4.031 <sup>b</sup> 0 $\pm$	113.291 <sup>c</sup> 0.360 $\pm$	0.028 <sup>a</sup> 0 $\pm$	0.302 <sup>a</sup> 0.001 $\pm$	6.469 <sup>c</sup> 0.173 $\pm$	192.859 <sup>c</sup> 0.303 $\pm$
LSD	0.046	0.861	0.010	0.588	0.001	0.001	0.384	0.607

Such improvement can be attributed to calcium carbonate's ability to adjust soil pH and enhance the availability of nutrients like calcium and magnesium, consequently promoting plant growth and productivity (Brady and Weil, 2008). Additionally, calcium plays a crucial role in strengthening plant cell walls and enhancing plant resistance to environmental and biological stresses (Hepler, 2005). Conversely, a decrease in root length and shoot dry weight was observed in plants grown in soil with added calcium carbonate. This reduction might stem from increased soil alkalinity resulting from calcium carbonate addition, which can negatively affect the absorption of nutrients such as iron, manganese, and zinc, thereby impacting the plant's vegetative growth (Marschner, 2012). Furthermore, the heightened severity of potato scab disease in calcium carbonate-amended soil may induce plant stress and diminish vegetative growth, as suggested by Wanner and Kirk (2015).

Analysis of disease progression indicated that adding calcium carbonate to soil inoculated with *S. scabies* increased the incidence and severity of potato scab disease, although this increase was not statistically significant. These observations align with previous studies by Lambert and Manzer (1991) and McLeod et al. (2024), who demonstrated that *S. scabies* thrives in alkaline soil and that elevated soil pH intensifies potato scab disease severity. The underlying mechanism for this phenomenon involves calcium carbonate raising soil pH and creating a more alkaline environment, which provides favorable conditions for *S. scabies* growth and reproduction while enhancing its pathogenicity (Lacey and Wilson, 2001). Moreover, increased soil alkalinity may affect the availability of certain nutrients essential for plant disease resistance, rendering the plant more susceptible to infection (Marschner, 2012). Scientific literature suggests that the optimal soil pH for *S. scabies* growth ranges from 5.5-7.0 and reducing soil pH below 5.2 can mitigate potato scab disease (Keinath and Loria, 1989). Various studies have demonstrated that incorporating acidic materials such as sulfur into the soil can reduce potato scab disease by lowering soil pH (Pavlista, 2005).

Laboratory analyses demonstrated that calcium carbonate addition to *S. scabies*-inoculated soil triggered significant changes in plant chemical responses after 30 and 60 days of planting. Notable changes included increased antioxidant enzyme activity such as superoxide dismutase (SOD) and catalase (CAT), alongside decreased hydrogen peroxide content ( $H_2O_2$ ), malondialdehyde level (MDA), and total phenolic compounds content (TPC). These biochemical indicators suggest that calcium carbonate may help plants combat oxidative stress resulting from *S. scabies* infection by

enhancing antioxidant enzyme activity. SOD and CAT enzymes function to eliminate reactive oxygen species (ROS) such as superoxide radical and hydrogen peroxide, thereby reducing oxidative damage to plant cells (Mittler, 2002). Such findings correspond with research by Wang et al. (2019) and Liu et al. (2023), who documented significant alterations in antioxidant enzyme activities in potato plants responding to stress conditions.

Reduced MDA levels, an indicator of lipid peroxidation, point to decreased oxidative damage to plant cell membranes in calcium carbonate-treated soil (Heath and Packer, 1968). Similarly, lower  $H_2O_2$  content indicates enhanced plant capacity to eliminate reactive oxygen species, as proposed by Sharma et al. (2012). In contrast, plants grown in calcium carbonate-amended soil exhibited decreased total phenolic compounds content (TPC). This reduction may result from increased antioxidant enzyme activity diminishing the need for phenolic compound accumulation as a defense mechanism against oxidative stress (Dixon and Paiva, 1995). Such observations correspond with findings by Qi et al. (2023), who reported that plants employ multiple defense mechanisms when responding to pathogen infection.

Regarding peroxidase enzyme activity (POD), no significant difference emerged between treatments after 30 days of planting, while a significant decrease in activity was observed after 60 days in plants grown in calcium carbonate-amended soil. This decline may be attributed to increased SOD and CAT enzyme activity reducing the need for POD enzyme in  $H_2O_2$  elimination (Mittler, 2002).

The study uncovered a relationship between plant chemical response and potato scab disease severity. Increased disease severity in plants grown in calcium carbonate-amended soil coincided with elevated antioxidant enzyme activity (SOD and CAT) and decreased  $H_2O_2$  content and MDA levels. This correlation indicates that plants respond to *S. scabies* infection by enhancing defense mechanisms, particularly the enzymatic antioxidant system (Mittler, 2002). Calcium carbonate may enhance this response by providing calcium, an essential element regulating numerous physiological processes in plants, including responses to biological stresses (Hepler, 2005). Alternatively, decreased total phenolic compounds content (TPC) in plants grown in calcium carbonate-amended soil may indicate that plants rely more heavily on enzymatic antioxidant systems rather than phenolic compounds as a defense mechanism against *S. scabies* infection (Dixon and Paiva, 1995).

## CONCLUSION

Based on the results of this study, it can be concluded that adding calcium carbonate to soil inoculated with *S. scabiei* affects the pathogenicity of the bacterium and the physiological response of potato plants. Adding calcium carbonate leads to increased severity of potato scab disease, but at the same time, enhances defense mechanisms in the plant, especially the enzymatic antioxidant system.

## REFERENCES

- Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121-126.
- Arseneault, T., Goyer, C., & Filion, M. (2021). Genomic and metabolomic analysis of the potato common scab pathogen *Streptomyces scabiei*. *ACS Omega*, 6(16), 11091-11101.
- Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44(1), 276-287.
- Bignell, D. R., Fyans, J. K., & Cheng, Z. (2014). Phytotoxins produced by plant pathogenic *Streptomyces* species. *Journal of Applied Microbiology*, 116(2), 223-235.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254.
- Brady, N. C., & Weil, R. R. (2008). The nature and properties of soils (14th ed.). Pearson Prentice Hall.
- Dees, M. W., & Wanner, L. A. (2012). In search of better management of potato common scab. *Potato Research*, 55(3-4), 249-268.
- Dees, M. W., Lysøe, E., & Brurberg, M. B. (2023). Biological control of potato common scab and growth promotion of potato plants by *Streptomyces* strains. *Frontiers in Microbiology*, 14, 1295107.
- Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell*, 7(7), 1085-1097.
- Gomez, K. A., & Gomez, A. A. (1984). Statistical procedures for agricultural research (2nd ed.). John Wiley & Sons.
- Hammerschmidt, R., Nuckles, E. M., & Kuć, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology*, 20(1), 73-82.
- Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125(1), 189-198.
- Hepler, P. K. (2005). Calcium: a central regulator of plant growth and development. *The Plant Cell*, 17(8), 2142-2155.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Bergey's manual of determinative bacteriology* (9th ed.). Williams & Wilkins.
- Keinath, A. P., & Loria, R. (1989). Management of common scab of potato with plant nutrients. In A. W. Engelhard (Ed.), *Soilborne plant pathogens: Management of diseases with macro- and microelements* (pp. 152-166). APS Press.
- Khalil, M. A., Akhtar, K. P., Ali, M. A., Ullah, E., Raza, S., Munawar, M., & Aslam, M. N. (2025). Pathogenicity of *Streptomyces scabiei* and identification of tolerant potato genotypes to common scab disease. *BMC Plant Biology*, 25, 06506.
- Lacey, M. J., & Wilson, C. R. (2001). Relationship of common scab incidence of potatoes grown in Tasmanian ferrosol soils with pH, exchangeable cations and other chemical properties of those soils. *Journal of Phytopathology*, 149(11-12), 679-683.
- Lambert, D. H., & Manzer, F. E. (1991). Relationship of calcium to potato scab. *Phytopathology*, 81(6), 632-636.
- Lerat, S., Simao-Beaunoir, A. M., & Beaulieu, C. (2009). Genetic and physiological determinants of *Streptomyces scabiei* pathogenicity. *Molecular Plant Pathology*, 10(5), 579-585.
- Liu, Y., Wang, Y., Liu, Y., Zhang, Y., Zheng, Z., & Hou, J. (2023). Potato Stu-miR398b-3p negatively regulates Cu/Zn-SOD expression and reduces drought tolerance. *International Journal of Molecular Sciences*, 24(3), 2702.
- Loria, R., Kers, J., & Joshi, M. (2006). Evolution of plant pathogenicity in *Streptomyces*. *Annual Review of Phytopathology*, 44, 469-487.
- Marschner, P. (2012). *Marschner's mineral nutrition of higher plants* (3rd ed.). Academic Press.
- Mayer, A. M., Harel, E., & Shaul, R. B. (1965). Assay of catechol oxidase—a critical comparison of methods. *Phytochemistry*, 5(4), 783-789.
- McLeod, D., Hurst, M., & Dobson, R. (2024). Potatoes and calcium, dispelling some myths. *LinkedIn*.  
<https://www.linkedin.com/pulse/potatoes-calcium-dispelling-some-myths-dave-mcclellan-ikjve>
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7(9), 405-410.
- Pavlista, A. D. (2005). Early-season applications of sulfur fertilizers increase potato yield and reduce tuber defects. *Agronomy Journal*, 97(2), 599-603.

- Qi, J., Wang, J., Gong, Z., & Zhou, J. M. (2023). Differential responses of antioxidant enzymes and lignin biosynthesis to *Phytophthora capsici* in resistant and susceptible pepper cultivars. *Antioxidants*, **12**(6), 1164.
- Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, **2012**, 217037.
- Shirling, E. B., & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, **16**(3), 313-340.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16**(3), 144-158.
- Struik, P. C., Geertsema, J., & Custers, C. H. (1989). Effects of shoot, root and stolon temperature on the development of the potato (*Solanum tuberosum* L.) plant. III. Development of tubers. *Potato Research*, **32**(2), 151-158.
- Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science*, **151**(1), 59-66.
- Wang, Y., Zhang, X., Yang, S., & Wang, C. (2019). Antioxidative capacity is highly associated with the storage property and related enzyme activities of potato tuber during the whole storage process. *Scientific Reports*, **9**, 11222.
- Wanner, L. A. (2009). A patchwork of *Streptomyces* species isolated from potato common scab lesions in North America. *American Journal of Potato Research*, **86**(4), 247-264.
- Wanner, L. A., & Kirk, W. W. (2015). *Streptomyces* from basic microbiology to role as a plant pathogen. *American Journal of Potato Research*, **92**(2), 236-242.

### الملخص العربي

## تأثير كربونات الكالسيوم على إمراضية *Streptomyces scabies* المسببة للجرب في البطاطس والاستجابة الفسيولوجية لنباتات البطاطس

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يُعد مرض جرب البطاطس، الذي تسببه بكتيريا *Streptomyces scabies*، من الأمراض البكتيرية المهمة التي تؤثر سلباً على إنتاج البطاطس على مستوى العالم. تهدف هذه الدراسة إلى تقييم تأثير كربونات الكالسيوم ( $\text{CaCO}_3$ ) على إمراضية *S. scabies* وعلى الاستجابة الفسيولوجية لنباتات البطاطس تحت ظروف البيوت المحمية. تم زراعة نباتات البطاطس (الصنف دايموند) في تربة مُلحقة بسلالة *S. scabies* (E21) مع وبدون إضافة كربونات الكالسيوم (٥ جم/كجم تربة). تم تقييم معايير النمو، وشدة الإصابة، ونسبة الإصابة بالمرض، بالإضافة إلى الاستجابات الكيميائية الحيوية للنبات. أظهرت النتائج أن إضافة كربونات الكالسيوم زادت من شدة الإصابة من ٣١.١٥٪ إلى ٤٥.٣٥٪، ومن نسبة الإصابة من ٩١.٦٨٪ إلى ١٠٠٪، لكن دون دلالة إحصائية. ومع ذلك، أدت المعاملة بكربونات الكالسيوم إلى زيادة معنوية في وزن الدرنات (١٠٧.٥ جم مقابل ٨٨.٥ جم) والوزن الجاف للجذور (١.٠٥ جم مقابل ٠.٤٥ جم)، بينما انخفض الوزن الجاف للأفرع وطول الجذور. كشفت التحليلات الكيميائية الحيوية أن تعديل التربة بكربونات الكالسيوم حفز نشاط إنزيمات مضادات الأكسدة (SOD) و (CAT)، مع انخفاض في مستويات بيروكسيد



الهيدروجين ( $H_2O_2$ )، والمالوندايالديهايد (MDA)، والمركبات الفينولية في كل من اليومين ٣٠ و ٦٠ بعد الزراعة. تشير هذه النتائج إلى أن كربونات الكالسيوم، رغم زيادتها المحتملة لشدة المرض من خلال تهيئة ظروف مواتية للبكتيريا، فإنها تعزز في الوقت نفسه آليات الدفاع النباتية، لا سيما نظام مضادات الأكسدة الإنزيمية. تسلط هذه الدراسة الضوء على التفاعلات المعقدة بين تعديل التربة، وضراوة المرض، والاستجابات الفسيولوجية للنبات، مما يحمل دلالات مهمة لإدارة متكاملة لمرض جرب البطاطس.