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EFFICIENCY OF SALICYLIC ACID IN INDUCE SYSTEMIC RESISTANCE IN POTATO PLANTS AGAINST *PECTOBACTERIUM CAROTOVORUM*

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Abstract

The aim of this study was to test the efficacy of salicylic acid at 0.05 and 0.1% by immersing potato tubers and using salicylic acid at 2 mmol sprays on the leaves of Plants in induce systemic resistance in potato plants against *Pectobacterium carotovorum* caused Soft Rot Disease by determining the effectiveness of peroxidase enzyme And the accumulation of phenols. The Results showed the most effectiveness treatment was (salicylic acid at 0.1% + *P. carotovorum* + Spraying salicylic acid at 2 mmol) On the 10th day of plants life, in increasing the effectiveness of the peroxidase enzyme (30.66 min/gm soft weight) with a significant difference from the control treatment (distilled water only) (18.07 minutes/g soft weight). The same treatment also showed an increase in the content of potato plants from total phenols (209.22 mg/g soft weight) and no significant difference from the treatment of the use of (salicylic acid at 0.1% + *P. carotovorum*) and treatment of the use of (salicylic acid at 0.05% + *P. carotovorum* + Spraying salicylic acid at 2 mmol) Reached (204.25 and 202.46 mg / gm soft weight) respectively.

Key words: Salicylic acid, Soft rot, systemic resistance and Potato

Introduction

Solanum tuberosum L. is one of the most important vegetable crops in the Arab world and globally as well, because it is a good food source that is rich in energy compared to other starchy crops. Every 100 grams of potato tubers contains 22 grams of the dry matter giving about 76 calories. Tubers may be used for direct human consumption and also used indirectly after undergoing transitional manufacturing processes such as freezing or drying (Boras *et al.,* 2006). It can be cultivated widely around the world and is internationally ranked in terms of cultivated area and productivity where the cultivated area was estimated to be 19 million hectares (FAO, 2016). In Iraq potato is one of important corps, which can contribute positively in addressing the food gap in the country (Al Mashhadani, 2005) the productivity was estimated to be 165.6 tons yearly (for both spring and autumn seasons) (Central Statistical Organization, 2019). Many important diseases can infect potato, however, the soft rot caused by *P. carotovorum* can be considered the most widely recognized spread of diseases (des essarts *et al.,* 2016). The bacterial soft rot of potato is one of the most significant factors which restricting the productivity around the world (kapsa *et al.,* 2005) and influence economically in productive the potato (Nykyri, 2013). Induced systemic resistance is one of the most promising strategies for effective plant disease management, which is controlled by genes that encode to produce many pathogenic proteins (Prasannath, 2017). Chemicals have been used as inducers to induce resistance in plant hosts against plant pathogens on a large scale. Many of these compounds have been developed and successfully used under laboratory and field conditions. These compounds are non-toxic to humans, plants and animals and have no negative effect on plant growth, in addition; it has a low economic cost, makes good profits for the producers and gives long plant protection (Kuc, 2001) one of These inducers is salicylic acid (Miura and Tada, 2013).

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Materials and methods

Collection of samples

Samples of potato tubers showing symptoms of soft rot were collected from the stores of Radwaniya, Abu

Gharib, Yusifiyah and Jamella, which are located in Baghdad governorate and local markets in Kirkuk governorate. During the 2018 season, other samples were collected (infected tubers, diseased stems) from the fields, of Abu Gharib, Salamiyat and Yusifiyah towns in the province of Baghdad, Suwairah in Wasit province, Muelha in the province of Babylon, Amriyat Al-Fallujah in Anbar province and the Pema Rapper fields in the province of Arbil. The samples were placed in polyethylene bags and brought to the laboratory of Biotechnologies in Plant Protection Directorate in Baghdad and a process of isolation and purification of pathogens was conducted.

Isolation and purification of pathogens

Doolotkeldieva *et al.,* (2016) method was used to isolate the Pathogen with minor modifications. Infected potato samples were cut into small pieces of 1-2 cm length, sterilized the surfaces of pieces with 3% sodium hypochlorite for 2-3 minutes with three successive washings in distilled water. The sterilized pieces were placed on Petri dish plates containing a nutrient agar (NA) medium (20 ml/dish). Incubated at 28^0C for 24-48 hours.

Diagnosis of the pathogen

The bacteria was identified by the bacteriology lab/ plant protection directorate/ministry of agriculture according to the phenotypic traits (shape, color, and edges of bacterial colonies), microscopy (spores formation and cell response to gram dye), and Biochemical tests (Indole test, methyl red test, voges-proskauer test, catalase test, oxidase test, citrate utilization test), they were confirmed by molecular diagnosis using polymerase chain reaction (PCR).

Pathogenicity tests

The test of the pathogenicity was carried out for all isolates which is isolated from the infected tubers with soft rot and infected stems with black leg. The Kamysz *et al.,* (2005) method was used on potato tuber slices. The potato tubers were not shown to have symptoms of the disease or any visual mechanical damage. Surface sterilization was achieved using hypochlorite Sodium concentrate 1% for 5 minutes and then the samples were washed with distilled water twice. The tubers were cut into 10 mm thick homogeneous slices and placed on the sterile plastic containers 12×18 cm on wet filter paper to ensure moisture. A 5mm pit was placed in the middle of each slice and Pits was inoculation with100 µl of the bacterial suspension that concentration of 10^6 CFU. The containers were incubated at 28°C. The development was observed daily for 6 days and the results were recorded. Test the ability of contrast between several types of bacteria and pathogenic bacteria.

Induce systemic resistance in potato plants using salicylic acid against the soft rot disease caused by bacteria *P. carotovorum*

Field experience

Healthy and homogeneous potato (El Mundo. V) were used in the field experiment, sterilized with 10% sodium hypochlorite solution for 15 minutes, then washed with sterile distilled water and left to dry. Three separate wounds were then created in each tuber, using a sterile scalpel. The following treatments were performed by Lopez *et al.,* (2001) with minor changes:

1- Immerse the potato tubers with distilled water only for 30 minutes.

2- Immerse the potato tubers with pathogenic *P. carotovorum* only for 30 minutes.

3- spraying plants at the age of 10 days with salicylic acid at a concentration of 2 mmol

4- Immerse the potato tubers in the pathogenic bacteria *P. carotovorum* for 30 minutes and then spraying plants at the age of 10 days with salicylic acid at a concentration of 2 mmol

5- Immerse potato tubers with salicylic acid at a concentration of 0.05% for 30 minutes and then leave for 3 days at room temperature.

6- Immerse potato tubers with salicylic acid at a concentration of 0.05% for 30 minutes and leave it for 3 days at room temperature and then spray the plants at the age of 10 days with salicylic acid at a concentration of 2 mmol.

7- Submerge potato tubers with wire acid at a concentration of 0.05% for 30 minutes then leave for 3 days at room temperature and then treated with pathogenic bacteria *P. carotovorum*.

8- Immerse potato tubers with salicylic acid at a concentration of 0.05% for 30 minutes and then leave for 3 days at room temperature and then treated with pathogenic bacteria *P. carotovorum* and then spray the plants at the age of 10 days with salicylic acid at a concentration of 2 mmol.

9- Immerse potato tubers with salicylic acid at a concentration of 0.1% for 30 minutes and then leave for 3 days at room temperature.

10- Immerse potato tubers with salicylic acid at a concentration of 0.1% for 30 minutes and leave it for 3 days at room temperature and then spray plants at the age of 10 days with salicylic acid at a concentration of 2 mmol.

11- Immerse potato tubers with salicylic acid at 0.1% for

30 minutes, then leave for 3 days at room temperature and then treated with pathogenic *P. carotovorum*.

12- Immerse potato tubers with salicylic acid at a concentration of 0.1% for 30 minutes and then left for 3 days at room temperature and then treated with pathogenic bacteria *P. carotovorum* and then spray the plants at the age of 10 days with salicylic acid at a concentration of 2 mmol.

Field experiment was conducted using the Randomized Complete Block Design (RCBD) with 12 treatments, each treatment consist of three replicates, each replicate represented by 10 plants. Potato tubers were planted on the rows (length 2.5 m) distance between tuber and another 25 cm. Drip irrigation system was used to irrigate the plants.

The efficacy of peroxidase and phenol accumulation was estimated to be an indicator of the efficacy and effectiveness of salicylic acid in induce systemic resistance in potato plants against soft rotting disease.

Determination of Peroxidase (POD)

Peroxidase activity was assayed as described by Hammerschmidt *et al.*, (1982) by taking 1g of potato plant leaves and add 2 ml of phosphate buffer 0.01 M (pH 6.5) at 4°C. The homogenate was centrifuged at 6,000 rpm for 20 minutes at 4°C and the supernatant collected, 100 µl of enzyme extract was mixed with 1.5 ml of Pyrogallol (0.05M) solution and putted in the Spectrophotometer tubes produced by Biotech Engineering Management CO. LTD. (UK). 100 µl of 1% hydrogen peroxide was added to start the reaction. The absorbance of spectrophotometer read at 420 nm for 5 minutes intervals 30 seconds. The amount of peroxidase was defined with the increase of one unit of absorbance per min^{-1} mg^{-1} of fresh matter.

$$\text{Change in absorption} = \frac{\Delta A / \Delta T}{Soft\ weight\ /\ g}$$

ΔA = change in light absorption

ΔT = time change/min

Determination of total phenol activity

The total phenol activity was determined using the Saikia *et al.*, (2004) method. 1g of leaves are crushed in a ceramic mortar with 10 ml of 80% methanol and then placed in the test tubes, which are subsequently into a water bath at 70°C for 15 min with continuous stirring. 1 ml of filtrated with 5 ml sterile distilled water and 250 µl of Folin–Ciocalteu reagent placed in a sterile glass tube, and the solution is incubated at 25°C for 30 minutes. The total phenol (on milligrams of phenol per grams of soft plant tissue) was calculated according to spectrophotometer absorbation at 725 nm, catechol used as a standard material.

Results and Discussion

Diagnosis of the pathogen

The results of the diagnosis of isolated bacteria from potato tubers confirmed that the bacteria causing soft rotting disease are *Pectobacterium carotovorum*.

Testing the pathogenicity of *P. carotovorum*

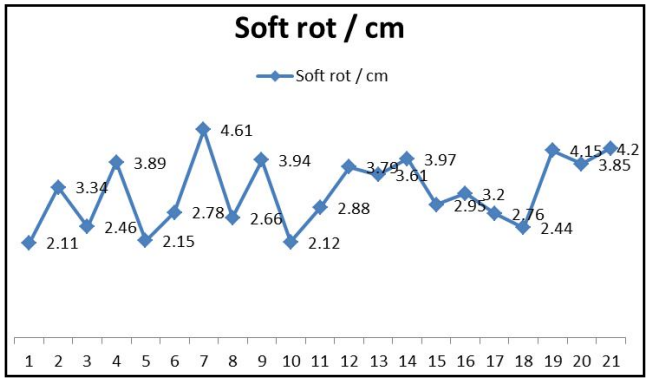
The results showed that all isolates of *P. carotovorum* tested on healthy potato tubers were able to cause the disease through tissue decomposition and foul odor. The isolates exhibited differences in their pathogenicity (shape 1). The most severe pathogenicity isolates were selected for subsequent experiments.

Effectiveness of the peroxidase enzyme

The results in Table 1 showed increase in the level of peroxidase in potato plants treated with Salicylic acid. The treatment of Salicylic acid 0.1% + pathogen + spraying salicylic acid 2 mmol was superior in absorption rate (30.66 min/gm of soft tissue), with significant difference from all other treatments. The fifth day after spraying salicylic acid showed superiority in the effectiveness of the enzyme peroxidase as it reached 24.30 min/gm of soft tissue, and a significant difference from the tenth day after spraying salicylic acid which amounted to 22.27 min/gm soft tissue.

Phenolic activity

The results showed the plants treated with salicylic acid increase in phenol accumulation rate. The highest phenol accumulation rate was obtained when treating salicylic acid 0.1% + the pathogen + spraying with 2mmol salicylic acid was 209.22 mg/gm of soft tissue, without significant difference from the treatment of (salicylic acid 0.1% with the pathogen) and treatment of (salicylic acid



Shape 1: Results of pathogenicity ability of isolates which causing soft rot on potato tubers.

Table 1: Effect of potato tubers treated with salicylic acid on the effectiveness of peroxidase.

Treatments		After 5 days	After 10 days	Average
T1	Water	18.31	17.83	18.07
T2	P. carotovorum	22.01	19.73	20.87
T3	Salicylic acid	19.91	17.95	18.93
T4	*P. carotovorum* + Salicylic acid	25.59	22.29	23.94
T5	Salicylic acid 0.05 only	21.16	20.01	20.59
T6	Salicylic acid 0.05 + Salicylic acid	22.96	21.78	22.37
T7	Salicylic acid 0.05 + *P. carotovorum*	25.99	23.01	24.50
T8	Salicylic acid 0.05 + *P. carotovorum* + Salicylic acid	28.06	25.41	26.73
T9	Salicylic acid 0.1 only	23.30	21.90	22.60
T10	Salicylic acid 0.1 + Salicylic acid	24.25	23.32	23.78
T11	Salicylic acid 0.1 + *P. carotovorum*	27.42	25.26	26.34
T12	Salicylic acid 0.1 + *P. carotovorum* + Salicylic acid	32.62	28.71	30.66
Average		24.30	22.27	
LSD at 0.05 to treatments		1.336		
LSD at 0.05 to times		0.546		
LSD at 0.05 to times and treatments		1.890		

Table 2: Effect of Potato Tubers Treatment on salicylic acid in the accumulation of phenols.

Treatments		After 5 days	After 10 days	Average
T1	Water	18.31	17.83	18.07
T2	P. carotovorum	22.01	19.73	20.87
T3	Salicylic acid	19.91	17.95	18.93
T4	*P. carotovorum* + Salicylic acid	25.59	22.29	23.94
T5	Salicylic acid 0.05 only	21.16	20.01	20.59
T6	Salicylic acid 0.05 + Salicylic acid	22.96	21.78	22.37
T7	Salicylic acid 0.05 + *P. carotovorum*	25.99	23.01	24.50
T8	Salicylic acid 0.05 + *P. carotovorum* + Salicylic acid	28.06	25.41	26.73
T9	Salicylic acid 0.1 only	23.30	21.90	22.60
T10	Salicylic acid 0.1 + Salicylic acid	24.25	23.32	23.78
T11	Salicylic acid 0.1 + *P. carotovorum*	27.42	25.26	26.34
T12	Salicylic acid 0.1 + *P. carotovorum* + Salicylic acid	32.62	28.71	30.66
Average		24.30	22.27	
LSD at 0.05 to treatments		1.336		
LSD at 0.05 to times		0.546		
LSD at 0.05 to times and treatments		1.890		

0.05% + the pathogen + spraying with 2mmol salicylic acid) which reached 204.25 and 202.46 mg/gm of soft tissue, respectively. with a significant difference from all other treatments. All treatments exceeded the phenolic accumulation rate on the tenth day after salicylic acid spraying which was 199.86 mg/gm of soft tissue and a significant difference from the fifth and fifteenth day, which reached 166.08 and 171.34 mg/gm of soft tissue, respectively. Table 2.

The results of Table 1 show the high level of peroxidase enzyme and Table 2 increase the accumulation of phenols in potato plants due to treatment with wire acid concentration of 0.05 and 0.1% by submerging potato tubers and concentration of 2 mmol spray method on potato plants at the age of 10 days and the pathogen. The increase in peroxidase and phenolic enzyme to the effectiveness of salicylic acid in stimulating plant reactions against the pathogen. Hassan *et al.*, (2007) have reported that the effectiveness of the enzyme peroxidase is directly correlated with the induced resistance in the host against pathogenic pathogens during exposure to it and is also involved in stimulating the production of phytoaloxins by oxidizing phenols and converting them into more toxic pathogens. Fernandez and Heath (1989) also stated that the rapid accumulation of phenols restricts pathogens at their point of entry. Antoniw and White (1980) stated that the role of salicylic acid in induce systemic resistance in treated plants may be attributed to the transfer of acid

inside the plant and its spread by vessel vectors to all parts of the plant and thus to stimulate resistance genes in plant cells. Leeman *et al.*, (1996) reported that many researchers reported that when injecting salicylic acid into a plant stem, spraying it or treating roots, it stimulates systemic resistance in the plant and stimulates the production of proteins associated with the disease PR-Protein.

Conclusion

Soft rot disease is an important bacterial disease that affects potato tubers and accompanies them from the field to the stores. One method of resistance to soft rot is to induce systemic resistance to plants. Salicylic acid is one of the systemic resistance inducers that can be used to treat potato tubers or spray it on potato plants in the field. The results showed that the salicylic acid at 0.05 and 0.1% concentrations were effective in inducing resistance by increasing the effectiveness of the enzyme peroxidase and the rate of phenol accumulation which are indicators and clear evidence of induce Systemic resistance.

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