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# TEST SENSITIVITY OF SOME POTATO VARIETIES CULTIVATED IN IRAQ TO SOFT ROT DISEASE CAUSED BY *PECTOBACTERIUM CAROTOVORUM* AND ITS RELATION WITH CONTENTS OF DRY MATTERS, STARCH AND CALCIUM OF TUBERS

**Nazar Rashid Merzah[1] and Jamal Hussein Kadhim[2]**

[1]Plant Protection Directorate, Ministry of Agriculture, Iraq.
[2]Plant Protection Dept., College of Agriculture, University of Kufa, Iraq.

## Abstract

This study was included testing sensitivity of 23 varieties of potatoes which cultivate in Iraq to the bacterial soft rot which caused by *pectobacterium carotovorum* and the effect of dry matters content starch and calcium the results showed that the variety Elmondo was the most sensitive to the infection with 17.783 moldy tissue /ml of water with significant differences on all the varieties followed by sifra variety 14.250 moldy tissue /water ml. the most resistant varieties were Amarin, Lady rossita without significant differences to Banba, Melanto, Diamond, Sinora, Labadia, Arezona, Hermis, Vogu, Elbida, Agria and Everest as well as, the variety lady rossita showed the highest content of dry matters content and starch 25.78 % and 19.34% respectively without significant differences to Hermis and Sinora, while Amarin showed the highest calcium content 1.590% without significant differences to Hermis 1.450% with significant differences comparing with all the other varieties.

***Key words:*** potato varieties, Soft Rot, *Pectobacterium carotovorum*, Dry Matters, Starch, Calcium.

## Introduction

A potato can be considered one of the important vegetables both in Arabic and universally due to the high content of energy comparing with other crops. Each 100 gm of potato contains 22 gm dry matters which can give around 76 calories, Tubers might be utilized for direct human consumption and furthermore 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, 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). Cultivation of resistant varsities is one of the important methods of controlling the disease, it can be considered one of the best and effective in resisting the plant diseases, Where it reduces the losses that caused by plant diseases, reduce the costs of workers, cost of spraying and reduces significantly the environmental pollution (Agrios .1997). So this study was aimed to test the sensitivity of numerous varieties of potato which cultivates in Iraq to the causal agent of soft rot *P. carotovorum* and estimate the total content of dry matters, starch and calcium, and association with sensitivity to infection of soft rot.

## Materials and methods

### Collection of samples

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

***Author for correspondence :** E-mail: nazar.rashid@yahoo.com, jamal.alsharmani@uokufa.edu.iq

Gharib, Yusifiyah and Jamella, which are located in Baghdad governorate and local markets in Kirkuk governorate. During the 2017-2018 season, other samples were collected (infected tubers, diseased stems) from the fields in the towns of Abu Gharib, Salamiyat and Yusifiyah in the province of Baghdad, the fields of Suwairah in Wasit province, the fields of Muelha in the province of Babylon, the fields of 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**

Two methods were used to isolate and purify the pathogen:

Doolotkeldieva *et al.*, (2016) with minor modifications. Infected samples were cut into small pieces of 1-2 cm length and their surfaces sterilized with 3% sodium hypochlorite for 2-3 minutes with three successive items of washing in distilled water. The sterilized pieces were placed on Petri plates containing a nutrient agar (NA) medium (20 ml/dish). The plates were incubated at $28^0$C for 24-48 hours.

The second purification method used, as described by Goszczynska and Serfontein (1998), the infected samples were taken and their surfaces sterilized with 3% sodium hypochlorite for 3 minutes, washed with sterilized distilled water twice. Then small Pieces were taken by sterile scalpel and placed in a petri dish containing 10 ml sterile distilled water for 15 minutes, taking into consideration the movement of the dish from time to time. Next, the loop is filled with the solution and Placed on the Nutrient agar medium and incubated at 28°C for 24-48 hours, after which time, individual colonies were selected for breeding.

**Diagnosis of the pathogen**

The bacteria were identified based on the phenotypic traits (shape, colour, and edges of bacterial colonies), microscopy (spore 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 of the isolated bacteria from the infected tubers with soft rot and infected stems with a 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 inoculated with 100 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.

**Sensitivity testing of varieties**

Testing sensitivity of 23 varieties of potato which cultivates in Iraq (ElMundo, Sifra, Fandango, Burren, Clasico, Revera, Synergy, Antracteca, Elctra, Antarctica, Electra, Nectar, Everest, Agria, Elbida, Vogue, Hermes, Arizona, Labada, Sinora, Diamond, Melanto, Banba, Lady rositta, Amarin) were conducted by following (Pasco *et al.*, 2006 ) that prescribed by (Lap wood and gans, 1984) with little modifications; 20 tubers choose and everyone cuts into two homogenous parts with making bore, 0.5 cm in the middle of tuber 30 minutes before inoculation the tubers put in 12 × 18 cm plastic cons which Contain wetted Filter paper to insure the humidity. 50 µl of bacterial suspension was added into each bore with shaking slightly the plates to avoid the cells precipitation. Some sterilized water was added to the filter paper, then the plastic cons were closed and stored in a dark place after 6 days of incubation at 20°C, the decayed tissues were removed carefully by small spoon the bores were filled out by water the size of ratting tissues was calculated by filling out the bores by water through using a pipette.

**Percentage of dry matter**

Pieces of tubers were dried by oven at 70°C for 72 hours up to persistence of weight (Al-sahaf, 1989) the samples were weighed, and the percentage calculated by using the equation below:

$$\% \text{ of dry matter} = \frac{a\ dry\ weight\ of\ the\ sample}{fresh\ weight\ of\ the\ sample} \times 100$$

**Percentage of starch in tubers**

The percentage was calculated according the equation below:

% of starch = 17.55 + 0.891 (% of dry matter -24 - 182) (A.O.A.C, 1970).

### Calcium estimate

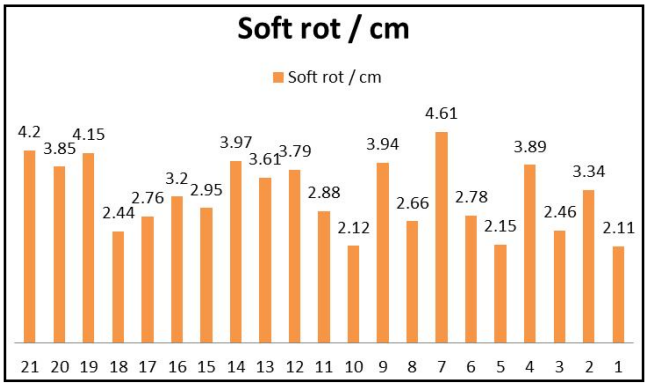
Their replication of each variety was used to estimate the calcium the tubers washed, cut and dried by oven on 70°C for 72 hours up to persistence of weight. The samples grinded by mill 0.2 gm were put in digestion flasks three ml of $H_2So_4$ had added then wait for 24 hours. 1 ml of a mixture of $H_2So_4$ and $HCIO_4$ (1:1) was added. The flasks were put on a hot plate white fumes were noticed and the colour has changed gradually up to getting a clear colourless solution. A while of waiting to be cooled the samples under the laboratory environment. The size of each sample was amended up to 50 ml by adding sterilized water (Cresser and parsons 1979). Calcium estimated by spectrophotometer in accordance with brown and Lilliand (1946).

## Results and discussion

### Isolation and Purification of Pathogen

The Two used methods showed high efficiency in isolation The bacteria which causing soft rot of potato.

### Identification of Pathogen:




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

The results showed that the causal agent of soft rot of potato is the bacteria *P. carotovorum*

### Pathogenicity test of *P. corotovorum*

All the tested isolates showed ability to infect the tubers through break down the tissues and releasing smelly gas. The isolates showed differences in pathogenicity (shape 1). The most severe isolates were chosen to conduct the next experiments.

### Sensitivity of varieties

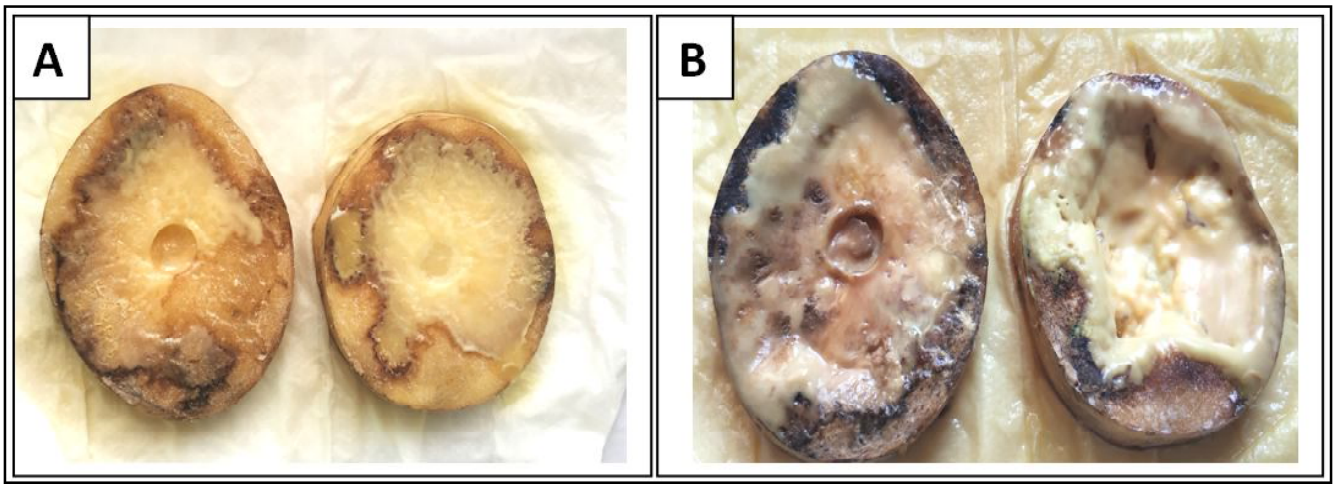
The results showed that the variety Elmundo was the most sensitive to the infection with 17.783 moldy tissue/ ml water, with significant deference to all varieties , followed by sifra 14.250 moldy tissue / ml water. While the most tolerant Verities were Amarin and Lady Rositta without significant differences to Banba, Melanto, Diamond, Sinora, Labadia, Arezona, Hermis, Vogu, Elbida, Agria and Everest , as showen in table 1.

### Dry matter percentage:

The results showed the high content of dry matters was in the variety Lady Rositta with 25.78% without significant differences with Hermis and Sinora 23.71% and 22.36% respectively. While the variety Fandanco was the lowest content of dry matter 12.37 without significant differences with synergy, Revera and Elmundo 13.07%, 14.41% and 14.57% respectively.

### Starch percentage

The result showed that the variety Lady Rositta was the highest percentage of starch with 19.34% without significant differences to Hermis and Sinora 17.01 and 15.49 respectively, while the lowest starch percentage was in the variety Fandanco 4.29% without significant differences to synergy, Revera and Elmundo 5.07 %, 6.58% and 8.32% respectively.




**Shape 2:** Showing sever pathogenicity of one bacterial (A) showing pathogenicity after 3 days, (B) showing the development of pathogenicity after 6 days.

**Estimation of calcium**

The results showed that Amarin variety was the highest in calcium content with 1.590% without significant difference Hermis 1.450% with significant deference to the variation. The variety of Lady Rositta showed the lowest percentage of calcium at 0.277%.

## Discussion

The results obtained showed that there was an unclear relationship between the content of dry matter and sensitivity to infection of soft rot, maybe there is an effect on sensitivity of varieties to infection might be shown that the highest content varieties of the dry matter being the lowest exposure to infection in soft rot.

It is shown that there is no fixed relationship between the content of calcium and sensitivity to infection of soft rot.

The results agreed with what mentioned in previous studies referred to pagel and Heitefuss (1989) that no fixed relationship between calcium content and sensitivity to infection as well as they noticed a high level of relationship between the cell walls content and resistance. Weber (1983) mentioned that the content of cell walls 5-10 % of weight the dry matter in the tuber, this might be affecting the relationship between dry matter and sensitivity to soft rot. Its referred by Tzeng *et al.,* (1990) that the high content of dry matter in tubers might reduce the possibility of infection in comparing with the lowest content tubers. Chang *et al.,* (2013) also mentioned that each variety has different genetic factors affect the control of soft rot the factors can include electrolyte composition membrane permeability reducing sugar levels dry matter content and calcium levels.

**Table 1:** Showing sensitivity of varieties to the causal agent of soft rot *P. carotovorum* and content of dry matter, starch and calcium.

| | Varieties | Soft rot vol. / ml water | dry weight percent | starch percent | Ca percent |
|---|---|---|---|---|---|
| 1 | Elmundo | 17.783 | 14.57 | 8.23 | 0.390 |
| 2 | Sifra | 14.250 | 18.28 | 11.37 | 0.383 |
| 3 | Fandanco | 12.400 | 12.37 | 4.29 | 0.517 |
| 4 | Burren | 8.733 | 17.42 | 8.96 | 1.020 |
| 5 | Clasico | 8.433 | 17.80 | 10.38 | 0.797 |
| 6 | Revera | 8.250 | 14.41 | 6.58 | 0.567 |
| 7 | synergy | 4.153 | 13.07 | 5.07 | 0.470 |
| 8 | Antarcteca | 2.667 | 18.50 | 11.17 | 0.497 |
| 9 | Elctra | 2.533 | 18.34 | 10.99 | 0.447 |
| 10 | Nectar | 2.183 | 21.84 | 14.91 | 0.747 |
| 11 | Everest | 1.283 | 18.35 | 11.00 | 0.310 |
| 12 | Agria | 1.250 | 20.58 | 13.51 | 0.763 |
| 13 | Elbida | 1.183 | 16.42 | 8.83 | 0.427 |
| 14 | Vogu | 1.183 | 16.94 | 9.42 | 0.633 |
| 15 | Hermis | 1.133 | 23.71 | 17.01 | 1.450 |
| 16 | Arezona | 0.767 | 18.49 | 11.16 | 0.430 |
| 17 | Labadia | 0.600 | 18.47 | 11.13 | 0.457 |
| 18 | Sinora | 0.583 | 22.36 | 15.49 | 0.393 |
| 19 | Diamond | 0.583 | 18.47 | 11.13 | 0.520 |
| 20 | Melanto | 0.567 | 21.00 | 13.98 | 0.780 |
| 21 | Banba | 0.583 | 19.37 | 12.15 | 0.840 |
| 22 | Lady Rositta | 0.550 | 25.78 | 19.34 | 0.277 |
| 23 | Amarin | 0.550 | 20.43 | 13.33 | 1.590 |
| 24 | Control | 0.000 | - | - | - |
| | LSD at 0.05 | 1.1095 | 3.666 | 4.230 | 0.3939 |

## Conclusion

Bacterial soft rot disease can be considered one of the most important diseases which infect potato and accompany the tubers from the field to store using the resistant varieties can be considered one of the important methods of controlling the disease so 23 varieties which cultivate in Iraq chose to test their sensitivity to the mentioned disease. Elmondo variety was the most sensitive to infection, as well as there is a middle and law sensitive to infection. The relationship of dry matter percentage starch and calcium and effect on sensitivity to soft rot *P. carotovorum.*

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