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# Effect of Adding the Biological Fertilizer, Fulzyme Plus, and Spraying CaC12 on Efficiency of the Rhizoctonia Fungus, and the Qualitative of the Potato (*Solanum tuberosum* L)

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## Abstract

A experiment was conducted to study in the spring of 2020 in Anbar – Heet to investigate the effect of adding the biological fertilizer, Fulzyme Plus, and spraying using CaC12 on the qualitative features of the *Solanum Tuberosum* L cv. of potatoes (Arizona) produced by Nahar Al-Awrad company for Potatoes Trading and Agricultural Equipment. The potato tubers were planted on 31/1/2020. The experiment was designed according to Randomized Complete Block Design (R.C.B.D). They were planted as treatments randomly in three replications. The results showed a significant superiority of the treatment T7 for iron of the product. Also, it showed 17.40 % superiority in the percentage of the dry composition in the tubers, 8.303% protein, 1.0666 g/cm<sup>3</sup> qualitative density of the tubers, 11.50% starch, and 5.96.% T.S.S. The pathogenicity test showed the isolating *Rhizoctonia* fungus presented the lowest percentage, 3.57 of T7 treatment where a fertilizer and spraying were used in comparison with the comparison treatment that showed the highest percentage of pathogenicity (T2= 29.52) where *Rhizoctonia* fungus was used.

## 1.Introduction

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in Iraq and in the world. It belongs to the Solanaceae family, and considered as one of the most important vegetable crops in many regions of the world (Matlob,1989 ). It is the second vegetable crop after tomato according to the cultivated area and one of the most important exported crops. It is rich of nutrients but its production in Iraq is still very low. Potato is the world's fourth largest food crop where it plays



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an important role as a staple food in the Iraq. The crop occupied an overall area about 1 million hectares which produced 28 million tons of tubers (FAO.2005).

It is considered as a rich crop of nutrient substances and is consumed very large quantities as manufactured, each 100g of potato peeled tubers contain 79.80g. water, 76 calories, 2.10 gm protein, 0.1gm lipids, 17.1gm carbohydrates, 0.5gm fibers and 0.9gm ash as well as it contains a little quantity of nutrient elements and some vitamins, it contains 0.1 mg thiamin, 0.4 mg Riboflavin, 1.5 mg Niyasin and 20.0 mg Ascorbic acid (Hasan, 1999).

Potato crops wear out the soil, and they need large quantities of nutrients. Recently, health and nutritional awareness have increased. The risks of using mineral fertilizers have also increased as a result of the excessive use of hormones, pesticides, and chemical fertilizers that have contributed to increasing productivity, growth, and high negative impacts on the environment as well. This increased the need for the sustainability of clean agriculture, the introduction of new agricultural technologies, and the use of natural materials that are not harmful to humans and animals. This can be achieved by using bio-fertilizers that contain specific microorganisms and growth regulators which stimulate plant growth in plant treatment. Moreover, they increase the plant's ability to withstand harsh environmental conditions. Bio-fertilizers work through their vital activity to produce some nutrients in the medium of agriculture. Some of them help the plant by providing it with the nutrients needed for growth, and some other work to present a hormonal balance stimulating growth through the activity of microorganisms in which the bio-fertilizer is present. As for the non-biochemical compounds such as humic, fulvic, and ascorbic acids contribute to improving vegetative growth because they act as a growth stimulator (Hussein, 1998). Bio-fertilizers have two important roles: supplying the plant with nutrients and producing plant growth regulating substances (Yusuf, 2011).

As for the use of fertilizers in Iraq, we find that the cultivated areas do not match the quantities of fertilizers used. Moreover, it is noticed that phosphate and nitrogen fertilizers are more commonly used, while potassium fertilizers are of little use there despite their importance in terms of productivity (Al-Dhibibi, 2003). The researchers explained AL-Enzi and Almohammed(2020) The present study concluded that the potato cultivars response to organic fertilizer and biofertilizer NOVA-GR. Organic fertilizer and biofertilizer NOVA-GR help plant growth and give increase plant height, chlorophyll content and plant leaf area to capture more carbohydrate, then higher tuber yield) Likewise, calcium fertilizers are of little use in the country although they play a role in potato production, which is considered an important storage crop.

Calcium is considered an essential component of plant development and growth. It participates in the formation of the middle plate (Middle Lamella) as calcium pectate in the cell wall It is also important in maintaining the activity and permeability of cell membranes (Al-Sahaf, 1989).

Calcium maintains the integrity of cell membranes, reduces the rate of ethylene production, reduces the process of respiration, and holds adjacent cells in the cell wall. Calcium affects the dry composition by increasing it and accelerates building photosynthesis ( Giorano, et al., 1982).

## 2. Materials and Methods:

The experiment was done in a field in Anbar province – Heet in the spring of 2020 (13 plant-1). The researchers planted potato tubers of Arizona type (*Solanum tuberosum* L) produced by the Dutch Agrico Company (Elite) and imported by Nahar Al-Awrad for potato trade and agricultural equipment. Potatoes were planted during spring in lines of 4m x 0.75 cm. and 25 cm between each plant. Each tuber was planted in 10-12 cm deep in the ground. The treatments were randomly planted in three replications with an average of 16 plant-1. The soil was plowed orthogonally using a reversible-plow. Then, the soil was smoothed and leveled according to the potato production programs. The lines were done using a special tool according to the recommended distances. As for the drip irrigation network, it was distributed in proportion to the requirements of the potato crop. All crop service operations were conducted, including fertilization, irrigation, preventive control, and as recommended (Matlob, 1989).

Details of the symbols from T1 to T8 are to be mentioned in addition to the Rhizoctonia.

The treatments are as follows:

- 1- Control without adding any treatment.
- 2- Adding the Rhizoctonia fungus only.
- 3- The bio-fertilizer Fulzyme plus.
- 4- Spraying with CaCl<sub>2</sub> of 4000 mg. liter<sup>-1</sup> concentration.
- 5- Adding the Rhizoctonia fungus + the bio-fertilizer Fulzyme plus.
- 6- Adding the Rhizoctonia fungus + spraying with CaCl<sub>2</sub> of 4000 mg. liter<sup>-1</sup>.
- 7- The bio-fertilizer Fulzyme plus + spraying with CaCl<sub>2</sub> of 4000 mg. liter<sup>-1</sup>.
- 8- Adding the Rhizoctonia fungus + bio-fertilizer Fulzyme plus + spraying with CaCl<sub>2</sub> of 4000 mg. liter<sup>-1</sup>.

The Qualitative Features of the Corp

The Percentage of the Dry Composition in Tubers

Fresh pieces of the tubers (100 g) were dried in an electric oven at a temperature of 70 ° C until the weight stabilized for 72 hours (Al-Sahaf, 1989a). After that, the percentage was calculated as follows:

$$\text{Dry composition percentage} = \frac{\text{Dry weight}}{\text{Fresh Weight}} = \mathbf{X \ 100}$$

The Percentage of Starch

The starch percentage was calculated according to what was stated in A.O.A.C (1970) with the following equation:

% starch =  $17.55 + 0.891 (\text{dry composition percentage} - 24.182)$ .

### The Qualitative Density of Tubers

The qualitative density of tubers was calculated based on the percentage of the dry composition and as shown in the following equation:

$$\text{Qualitative Density} = 1.0988 + \frac{\text{Dry Composition Percentage} - 24.182}{211.04} \quad (\text{Hasan, 1999})$$

### Total Soluble Solids (T.S.S)

The percentage of total soluble solids was calculated by squeezing a piece of the tuber and taking several drops from it and placing it on a Hand Refractometer machine (Al-Ani, 1985).

### The Percentage of Protein

The percentage of protein = Nitrogen in tubers X 5.7

(Bruckner and Morey, 1988)

### Isolating Rhizoctonia Solani Fungus

#### Data Collection

Several field visits were made to fields planted with potato crops in Abu Ghraib and Anbar province including the fields and plant nurseries of the College of Agriculture, Abu Ghraib, and potato fields in different areas in Abu Ghraib, such as Al-Zaidan and its vicinity. Samples of potato tubers that show symptoms of infection (Rhizoctonia solani bodies), infected parts, and infected parts of the stalks were collected and placed in plastic bags, and transported to the laboratory. Then, they were stored in the refrigerator to make the isolation process.

#### Isolating R. solani Fungus from the Infected Plants

Samples were brought from the fields to the laboratory and the isolation was done on the next day. Parts of the stems and other parts showing ulcers were taken and washed with running water for 30 minutes to remove the clays of mud attached to them and were cut into small parts 0.5 cm long. Then, they were sterilized by immersing them for 3 minutes in sodium hypochlorite solution (0.5% free chlorine). After that, they were washed with sterile distilled water for 2 minutes and dried with sterile filter paper, transferred with sterile forceps. They were planted in plates of 9 cm diameter and four pieces were planted in each. They were put in 15-20 cm<sup>3</sup> of the chosen culture medium which

included a liter of water, 18 gm of Agra, 100 mg of Streptomycin sulfate, and 100 mg Penicillin - G - sodium salt (Guttierrez, et al. 2001). Antibiotics were added to the culture medium after sterilizing it at 161° C and a pressure of 1.5 kg / cm<sup>2</sup> for 30 minutes. The plates were kept at 1 ± 22 ° C for 24 hours, after which the growth of the *R. solani* fungus was examined and purified by transferring pieces from the ends of the mycelium to the medium, potato sucrose agar PSA( 200 gm potato, 10 gm sucrose and 10 gm agar in a liter of water. The plates were kept at 1 ± 25 ° C for three days and kept in tubes containing the culture medium, potato carrot agar (PCA), formed of potatoes, carrots, and agar (20 gm of each ) in a liter of water to be used in subsequent tests.

#### Isolating *R. Solani* Fungus from the Infected Tubers

Infected tubers that have sclerotia were taken from the field and washed with running water to remove the mud from them. Then, they were sterilized for one minute using by immersing it for one minute in sodium hypochlorite solution (5% free chlorine). After that, they were washed with sterile distilled water and four sclerotia bodies were separated from different areas of the tuber surface with a sterile needle and transferred to the culture medium, PSA, that has the 100 mg/L of antibiotic streptomycin sulfate. The plates were kept under 25± 1 C for three days, then the fungus was purified and stored in tubes containing PCA medium to be used in the following tests.

#### Diagnosing *R Solani* Fungus

Rhizoctonia fungus was diagnosed after the appearance of the fungal growth, depending on the taxonomic traits mentioned by (Parameter, 1970 and Blazier and Conway, 2004).

#### 1- Preparing the Fungal Vaccine for Rhizoctonia Solani

To prepare the vaccine, one-liter beakers were prepared containing 250 gm of millet seeds with 50 cm<sup>3</sup> of distilled water. Next, they were sterilized with an autoclave at a temperature of (121 ° C) under the pressure of 15 kg cm<sup>2</sup> for (20) minutes. Then, they were cooled and placed in an incubator for two days at a temperature of (28 C) to make sure of sterilization. A part of the fungal culture, *Rhizoctonia solani*, was added to the beakers. After one week, the beakers were put in an incubator for 8 days taking into account the daily stirring to activate the fungus.

$$\text{Percentage of Infection} = \frac{\text{Number of infected tubers}}{\text{The total number of tubers}} \times 100$$

### 3.Results and Discussion:

Figure (1)Percentage of the dry composition of tubers

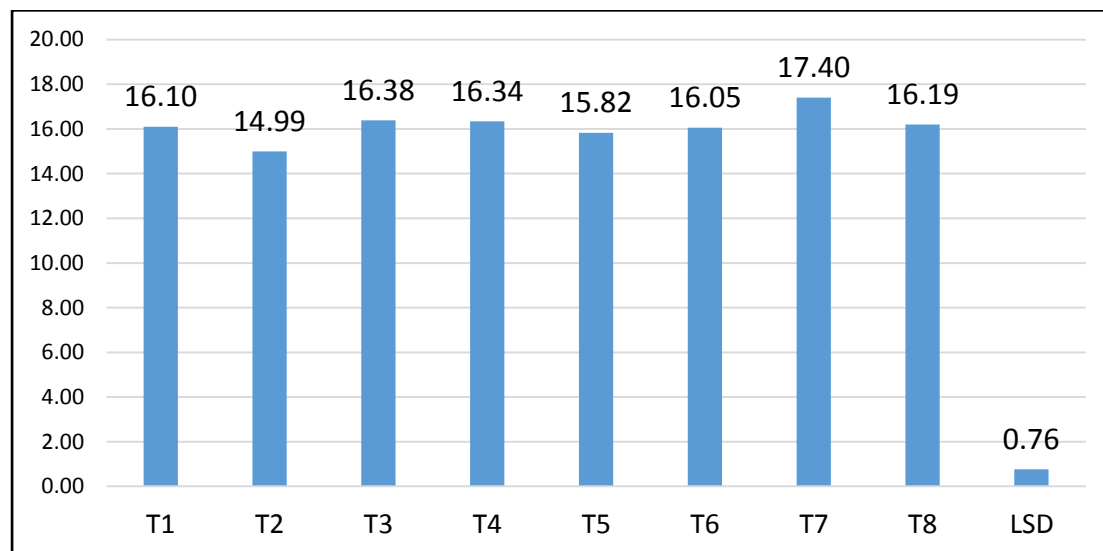


Figure (1) shows the impact of the used treatments where T7 gives the highest percentage of the dry composition (17.40) using (a fertilizer and spraying) In comparison with the lowest dry composition percentage of the T2 (14.99) where the Rhizoctonia fungus was used.

The interaction between the bio-fertilizer, fulzym plus, and spraying with Calcium T7 showed a significant increase in the percentage of dry composition in tubers which was 14.40% compared to the treatment of infection T2 which was 14.99%. The treatment T8 (spraying with calcium, biological fertilizer, and the pathogen Rhizoctonia) showed a significant increase in the percentage of dry composition in tubers, which was 16.19.

Figure (2) The percentage of Starch in the Tubers

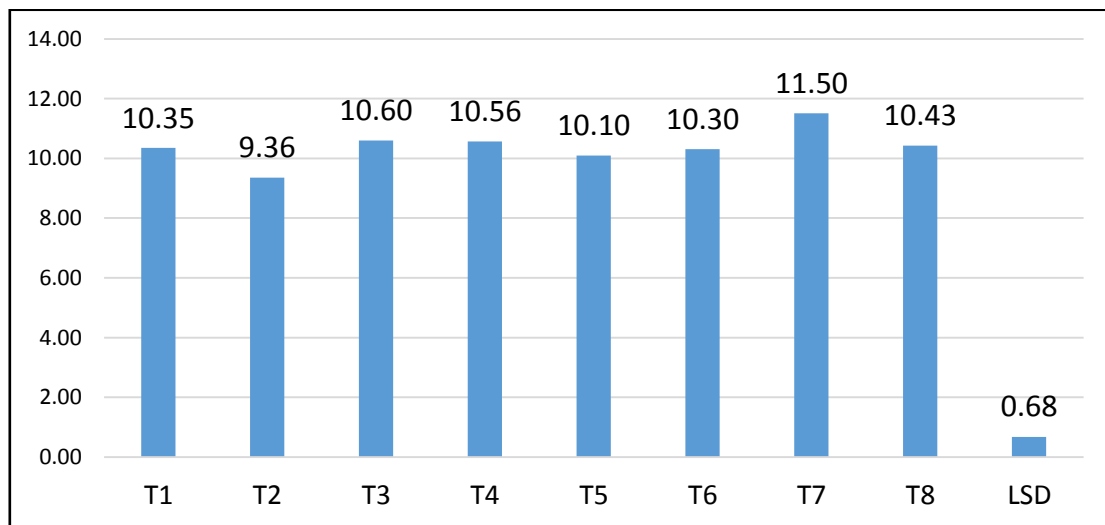


Figure (2) presents the effect of the treatments used, as the treatment (T7) shows the highest percentage of starch with (fertilizer + spray). It reached 11.50 compared to the lowest percentage of starch in the treatment T2 which reached 9.36 in which the *Rhizoctonia* fungus was used.

The results of the interaction between the bio-fertilizer fulzym plus and the spray with calcium T7 showed the highest percentage of starch, reaching 11.50%, compared to the treatment of infection and disease T2, which reached 9.36%. The treatment T8, spraying with calcium, bio-fertilizer, and the pathogen *Rhizoctonia*, showed a significant increase in the percentage of starch, which reached 10.43%.

Figure (3) Qualitative Density of the Tubers

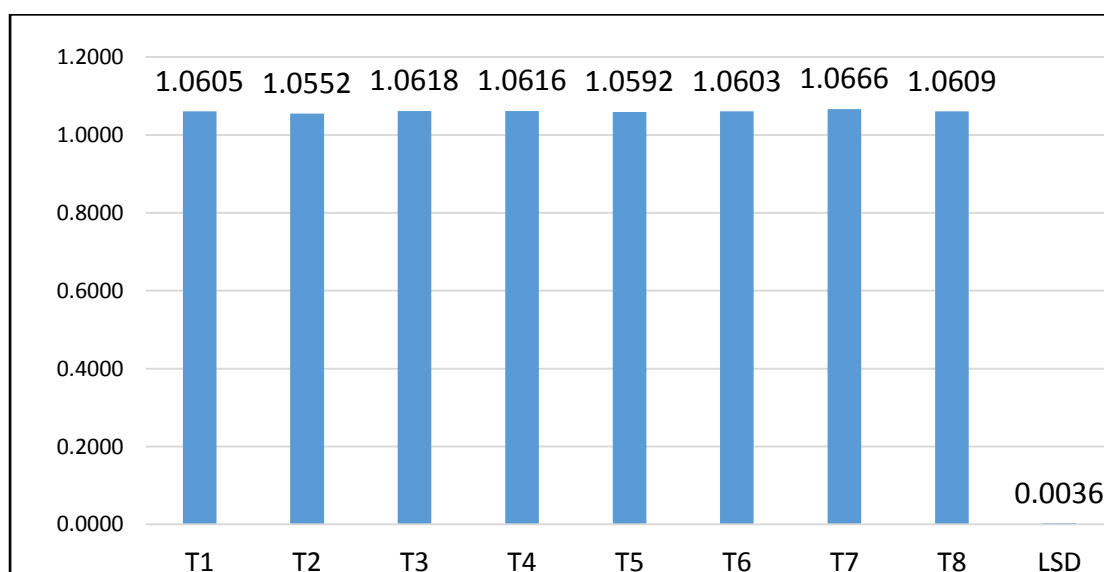




Figure (3) explains the effect of the used treatments, as the treatment (T7) shows the highest qualitative density of tubers with (fertilizer + spray) and reached 1.0666 compared to the lowest increase, which was 1.0552 in the treatment (T2) in which the *Rhizoctonia* fungus was used.

The results of the interaction between the bio-fertilizer fulzym plus and the spraying with calcium (T7) showed a significant increase in the qualitative density of 1.0666% (g. Cm3) compared to the treatment of infection and disease (T2), which reached 1.0552% (gm cm3). The treatment T8 (spraying with calcium, bio-fertilizer, and the pathogen *Rhizoctonia*) showed a qualitative increase, which reached 1.0669% (g.Cm3).

Figure (4) The Percentage of Total Soluble Solids (T.S.S)

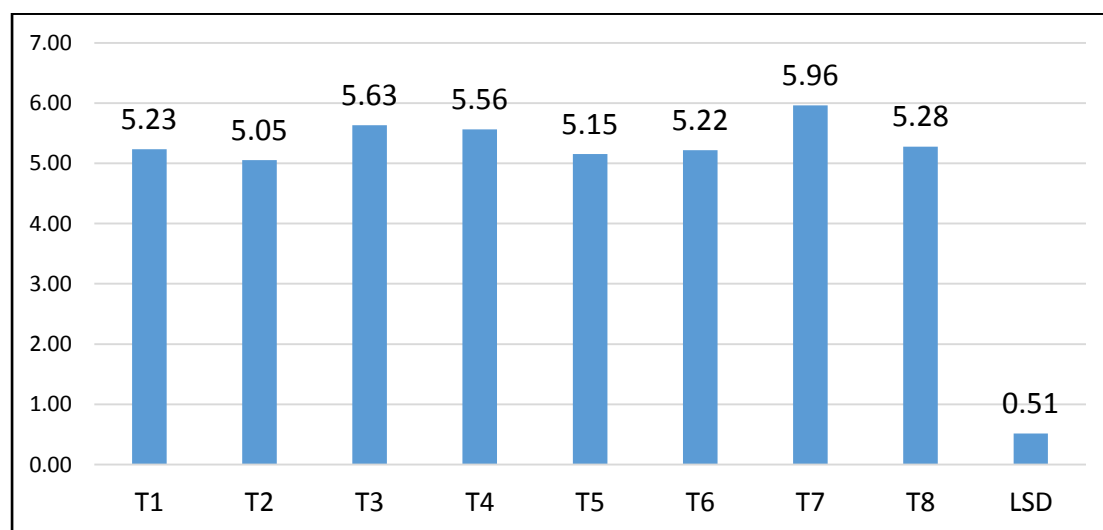


Figure (4) shows the effect of the used treatments, as treatment (T7) presented the highest percentage of total soluble solids with (fertilizer + spraying). It reached 5.96 compared to the lowest percentage of total dry solid, which was 5.05 in T2 using the *Rhizoctonia* fungus.

The results of the interaction between the bio-fertilizer, fulzym plus, and spraying with calcium (T7) showed a qualitative increase in the percentage of total soluble solids (5.96) compared to the treatment of infection and disease (T2), which reached 5.05. The treatment T8 (spraying with calcium, bio-fertilizer, and the pathogen *Rhizoctonia*) showed a qualitative increase, which reached 5.28.

Figure (5) The Percentage of Protein in the Tubers

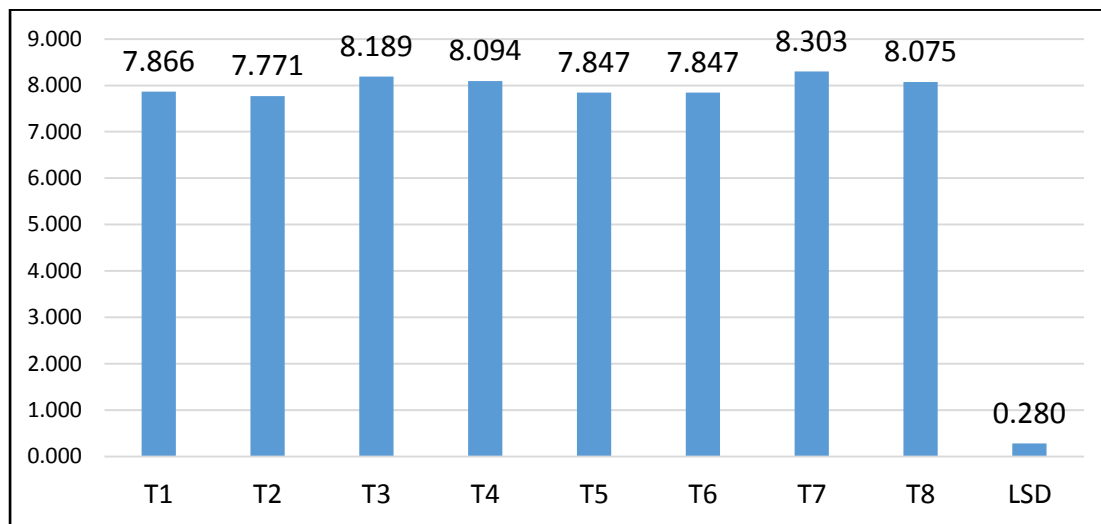


Figure (5) shows the effect of the used treatments, where treatment (T7) presented the highest percentage of protein in the tubers with (fertilizer + spraying). It reached 8.303 compared to the lowest percentage of protein in the tubers, which was 7.771 in T2 using the *Rhizoctonia* fungus.

The results of the interaction between the bio-fertilizer, fulzym plus, and spraying with calcium (T7) showed a qualitative increase in the percentage of protein in the tubers which was (8.303%) compared to the treatment of infection and disease (T2), which reached 7.771%. The treatment T8 (spraying with calcium, bio-fertilizer, and the pathogen *Rhizoctonia*) showed a qualitative increase, which reached 8.075.

Figure (6) Percentage of Infection

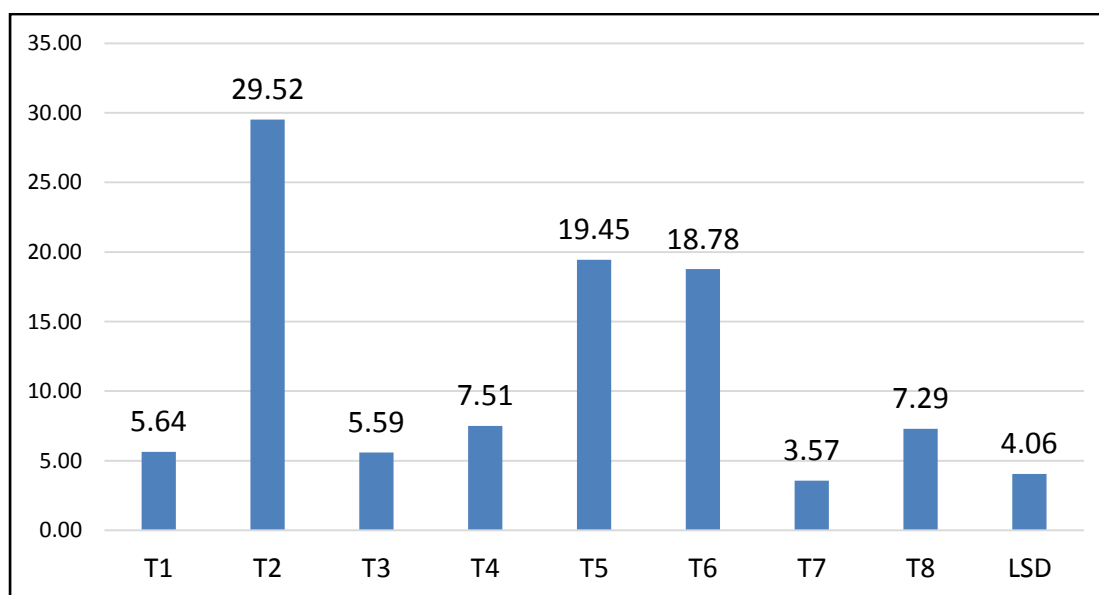


Figure (6) shows the effect of the used treatments, where treatment (T7) presented the lowest percentage of infection with (fertilizer + spraying). It reached 3.57 compared to the highest percentage, which was 29.52 in T2 using the *Rhizoctonia* fungus.

The results of the interaction (T7 and T8) also showed that their use led to a significant reduction in the percentage of infection which was 3.57% and 7.29% respectively, compared to the treatment of pathogen T2, in which the infection percentage was 29.52%.

#### 4. Discussion

The results of Figures (1-6) showed the effect of the treatments on the dry characteristics, the percentage of dry composition, the percentage of protein, the percentage of starch, the percentage of total soluble solids, and the qualitative density. They also showed the positive effect of the treatments on the vegetative growth characteristics and the percentages of nutrients content in tubers and leaves, because of the biological secretions of growth regulators, vitamins, and amino acids. They effectively contributed to preparing and facilitating phosphorus and nitrogen, as well as freeing iron to be absorbed by siderophores. (Al-Hasan, 2008, Al-Sahaf and Ati, 2007, Hassan et.al, 2017)

Several studies indicated that biological nitrogen-fixing and phosphorous solvent fertilizers play a significant role in secreting hormones, vitamins, free compounds, and organic acids, which work to free iron, nitrogen, phosphorus, and potassium, to stimulate growth. They have a positive effect on increasing the percentage of dry composition, protein, and starch that are directly associated with nitrogen (Salehi et al., 2014).

Qualitative features are the result of nutrients and compounds resulting from biological processes, such as forming carbohydrates and amino acids stored in tubers and proteins. The dry composition of the tubers is associated with an increase in carbohydrates and proteins stored in the tubers, which is attributed to the increased absorption of nutrients. The enhancement of the qualitative characteristics is due to the continuous and slow processing of nutrients with the help of biological processes that are reflected in the starch and protein content of the tubers (AL-muhamadi, and AL-essawi, 2015, Gosavi et al, 2010 Titova and Chudokvasoff, 2019).

The increasing leaf surface in the table (8) may be reflected in the amount of carbohydrates stored and manufactured in the tubers (Willmitzer and Alsiair, 2001).

If the balanced absorption of phosphorous, nitrogen, and potassium elements is achieved, the dry composition amount of tubers and starch will be increased (Chan, 2006, Das and Prasad 2005) agree with (Sarhan, 2008, Thyab, 2012).

T.S.S represents the soluble extract where any increase in solutes increases the value of T.S.S to which the increase in its value is attributed, in addition to the aforementioned effect of biological treatments on increasing nutrients (Chowdhury, 2017).

The increased percentage of starch and dry composition in the tubers reflects the tubers' ability to store nutrients (Al-Ameer and Abu-Henna, 2016).

As declared by (Harrison et al, 1982, and Locascio et al, 1992), it is noted that there is no significant effect of calcium chloride on the qualitative characteristics. They add that there is no significant effect of adding calcium to soil on the specific weight of tubers. Moreover, (Al-Sahaf and Abdulrasul, 1993, and Nogueira et al, 1996) claim that spraying calcium chloride on plants and adding gypsum to the soil have no significant effect on the percentage of dry composition and starch in the tubers.

The fungus, *Rhizoctonia solani*, is one of the most dangerous soil-dwelling fungal pathogens that infect a wide range of economic plant types, like the cucurbit, leguminous, and cruciferous plants (Mezian, 1970, Holliday, 1980, Aljibouri, 1998, Aegertor, 2000, and Jubeer, 2000).

Some ornamental plants, like Poinsettia, have been infected by the *R. solani* fungus (Hwang, 2002, Miniature, and Pryatmoje, 2001). The *R. Solani* fungus causes serious diseases to many plants where it infects them in various stages of their growth. It infects the seeds and causes them to rot. It also affects seedlings, stems, vines, tubers, and all parts that grow in the soil or on their surface (Agrios, 1997). The *R. Solani* fungus remains in the soil, either in the form of Sclerotia bodies or in the form of fungi that are active on organic wastes. So, they can resist hard conditions (Andresom, 1977). The infection efficiency of *R. Solani* fungus increases when the plants are exposed to toxic substances in

the soil, there is a lack of nutrients or the environmental stress factors that affect the plants (Garrett, 1977). Lucas (1980) believes that grown plants are more resistant to fungus infection. According to (Al-Baldawi, 1983, and Moe, 1987), the R. Solani fungus differs in its pathogenicity on different plants, ranging from high pathogenicity to weak pathogenicity.

The fungus is also distinguished by its high ability to resist harsh environmental conditions. As noticed by Sumner (1987), fungal filaments retain their vitality for nine months in the soil that is not intended for cultivation.

This fungus, R. Solani, is the fastest plant-killing pathogen. This property was studied in a laboratory by Murphy (1984), and Dillard (1987), and they noticed that there were a group of enzymes that helped break down cell walls like Pectin lyase, Cellulase, and Phosphatase which were associated with the fungus. The R. solani fungus secretes toxins. Some of them have glycoside or quinoloid properties, and some toxic substances are believed to be related to the fungus-like P- and hydroxy derivatives, and Actic phenyl acids (Dickson, 1993).

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