Journal of Basic and Environmental Sciences

ISSN
Online: 2356-6388
Print: 2536-9202

Research Paper Open Access

Effect of rhizosphere bacteria to reduce Fusarium infection in tomato plant

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Abstract:

Fusarium Saprophytic fungi, which are widely distributed in the soil, are recognized to form associations with plants and cause a variety of plant diseases, including tomato *Fusarium* wilt. In order to mitigate the impacts of *Fusarium* wilt, this study examined the effects of rhizosphere bacteria on tomato plant growth parameters, including shoot length, root length, and fresh and dry weight of the shoot and root. Based on the impact of bacterial treatments on growth metrics, infected plants treated with isolated three bacteria, particularly B1, showed the highest growth parameters. the notable improvement in growth characteristics of tomato seedlings treated exclusively with bacteria as opposed to the control group. The diseased tomato seedlings, on the other hand, showed the lowest growth metrics. Additionally, the application of isolated bacteria reduces the stress caused by fungal infection. This was demonstrated by the observation that proline concentrations were low in plants treated with bacteria and high in plants infected with *Fusarium*.

Keywords: Electrolyte leakage; *Fusarium* wilt; Proline; Tomato plant

Introduction

With an annual production of over 115.95 million tons, tomatoes are the second most significant vegetable in the world economically (1). One of the most important tomato diseases worldwide, affecting both fields and greenhouses, is *Fusarium* wilt, which is caused by *Fusarium* in tomatoes (2). This disease causes output losses ranging from 25% to 55% and decreases the quality of tomato fruit. Among the distinguishing signs

include wilting, chlorosis, and stunted seedlings (3,4). Consequently, the contaminated plant perishes. Due to a number of limitations, such as the pathogen's soil-dwelling nature and polyphagous nature, crop rotation is ineffective (5).

Wounds enable the pathogen's entry through the root tips into the plant system following conidial germination in soil. Within the tomato-*Fusarium* pathosystem, the pathogen proliferates widely throughout the root tissues, resulting in significant damage such as cell disarray caused by the colonization of the roots by *Fusarium* hyphae and modifications to the cell wall (6), which might be attributed to the synthesis of plant chitinases. Every infection then spreads intercellularly across the root cortex and quickly expands into the xylem, the tissue that conducts water (7). The generated microconidia encroach on the pseudostem's xylem, preventing water and nutrients from moving upward. Sieve cells in the stem impede the conidia from further spread, and, as this happens the spores germinate and expand through the sieve cells where they continue to germinate until the complete xylem system is blocked. The water supply to the leaves is cut off as a result of the plugging and collapsing vessels, which results in wilting symptoms. The diseased xylem's inability to supply the plant with the necessary amount of water re1sults in the death of the tomato plant (8). The spores expand into the surrounding tissues upon plant death, when they produce chlamydospores that return to the soil (9). Both polluted soil and diseased plant material can spread the illness or infection. Additional ways for the disease to spread include people walking through the contaminated area or using irrigation water and tools that were previously used on an infected crop (10).

Fusarium wilt-affected plants initially exhibit stunted seedlings and a yellowing of the lowest leaves, which is sometimes confined to one side of the plant or a single branch. Afterwards, the older leaves begin to defoliate. The impacted foliage withers and withers. Wilting advances along the stem until the foliage dies and the stem begins to decompose (11). Even though these stems stay solid and green on the outside, brown vascular deterioration can be seen in stem tissue cross sections close to the soil line (Fig. 9). Even when afflicted plants do not die, their yields are frequently severely decreased and they are frequently stunted. As seedlings emerge from the earth, they may dampen off in areas with significant inoculum pressure. A major sign of pathogens that cause vascular wilt disease, such as *Fusarium* wilt pathogens, is the browning of the interior stem vascular tissue (Figure 9). Diseases of the root and stems

often present with yellowing, wilting, and stunting. Inside the stem, if the stem is sliced open lengthwise, the reddish brown staining of the xylem vessels can be seen as lines or spots. It is possible to observe white, pink, or orange fungal growth on the exterior of afflicted stems, particularly under damp circumstances (10).

This disease is currently being managed with fungicides, but without adequate control. The use of chemicals may affect the environment and cause development of fungicide-resistant pathogen isolates, and is expensive (12). Certain fumigants, such chloropicrin and methyl bromide, can be used to control *Fusarium*. However, beyond the depth of effective fumigation, this approach is ineffective against pathogens in soil layers (13,14). Chemical use is not cost-effective or environmentally beneficial (15). Soil solarization and flooding can be used for the control of *Fusarium* fungi but these methods are expensive and consumed time (16,17).

Tomato resistance can be overcome by new pathogen races, but the most economical and environmentally friendly method of disease control is to use resistant cultivars (15). Therefore, researchers studying biological control have been prompted by the challenge of managing *Fusarium* wilt. Many fungal and bacterial species have been employed as biological control agents to shield tomato plants from wilt illnesses (18, 19). Non-pathogenic isolates of *Fusarium* are known to be potent antagonists and to significantly reduce illness among the biocontrol agents (20). Biological control, or biocontrol (5), is the use of live organisms to lessen the density or impact of a certain pest organism, making it less common or destructive than it would otherwise be.

Material and methods

Isolation of *Fusarium* sp.

The pathogenic fungus was isolated from an infected tomato plant which was collected from Qulubia government then its infected roots were cut, washed by distilled water and soaked in 1% sodium hypochlorite for 2 min then rewashed by distilled sterilized water. The roots were placed on Potato Dextrose Agar and left for incubation at 35°C until the growth of colonies became visible (5 days). *Fusarium* isolates were identified molecularly through the examination of their macroconidia, phialids, microconidia, chlamydospores, and colony growth features (10).

Identification of the *Fusarium* isolate

Isolated fungi were sent to the regional center of Mycology and Biotechnology at Al-Azhar University for morphological identification.

Isolation of rhizosphere bacteria

A soil sample was collected from tomato field in Qulubia government Egypt. A sample of the soil was taken around healthy plants, ranging in depth from 1 to 10 cm, and stored in sterile plastic bags. For the purpose of isolating the soil using the soil dilution technique, a 0.5 mm screen was used to remove bigger particles from the soil sample, such as stones and plant debris. Ten milliliters of sterile distilled water were added to one gram of dirt, and the mixture was violently agitated. A dilution series up to 10^{-5} was prepared using one milliliter of the soil suspension, and one milliliter of each dilution 10^{-4} and 10^{-5} was uniformly dispensed onto PDA material, with three replicates. For five to seven days, the PDA plates were incubated at 37±1°C until there was evidence of colony growth (21).

Antagonism test

The Petri dish containing the pathogen causing tomato wilt and the isolated bacteria was left to develop together. The separated bacteria were arranged equally apart from one another, with the fungus situated in the center of the Petri dish. The petri dishes were incubated at 37°C, and the interaction was noted by the fungus either growing too quickly or forming an inhibitory zone between them. Every day, the plates were inspected, and the inhibition zones were noted (5).

Soil preparation

To increase the soil texture, sand was combined with soil that was sampled from a farm where tomatoes had previously been grown in a 3:1 ratio. After a day, the process was repeated using an autoclave set to 121°C for three hours to steam sterilize the soil. For a week, the soils were left to let any volatile harmful compounds created during the sterilization process to escape. Then the soil was placed into disposable 500 ml pots. The *Fusarium* inoculum was made in the following way: On Potato Dextrose Agar (PDA) at 25°C, the isolated *Fusarium* was cultivated for four days. The inoculum was generated in a shake culture of potato-dextrose broth at 150 rpm for one week at 25°C ± 2°C. PDA cultivation of *Fusarium* disks of a 7 mm diameter were used to seed the media in the flasks. A double layer of sterile gauze was used to filter the cultures (22). The suspension contains 107 spores per milliliter.

After the soils in the 500 ml plastic pots were sterilized, 100 ml of this *Fusarium* isolate were added three times, with each infestation resulting in 105 spores per milliliter of substrate. The isolate was then left to establish itself in the soil for ten days. (22).

As seen in photo (1), the fungus grows on the soil's surface as a white hypha layer. After that,

the soil was utilized in the green house to cultivate tomato seedlings.

photo (1): fugus growth

Pot experiment

A greenhouse study investigated the effectiveness of isolated bacteria in controlling a wilt pathogen in tomato seedlings. Twenty-eight-day-old tomato seedlings were transplanted into plastic pots infested with the pathogen. Eight treatments were applied: isolated Fungus, bacteria, fungus control, and a blank. The plants were watered daily and treated with specific bacteria daily. After six weeks, three seedlings were harvested.

Electrolyte leakage

The technique outlined by Sullivan and Ross 1979 (23) was used to measure the total amount of inorganic ions that seeped out of the leaves. Ten centiliters of deionized water were placed in a boiling tube with twenty leaf discs inside. The conductivity meter was used to measure the EC of the tubes after they were heated for 30 minutes at 45ºC (EC_a) and 55ºC (EC_b) in a water bath. After 10 minutes of boiling at 100 degrees Celsius, the contents' EC was once more measured as EC_C The formula was used to calculate the electrolyte leakage:

$$\text{Electrolyte leakage (\%)} = \frac{\text{ECb} - \text{ECa}}{\text{ECC} \times 100}$$

Estimation of proline

The free proline calculation technique was based on Bates *et al.* 1973 (24) Create an acid ninhydrin reagent by heating 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid, stirring until dissolved. Store at 4°C and keep chilled. Throughout the day, the reagent doesn't change.

A gram of macerated fresh tissue was homogenized in sulfosalicyclic acid, filtered, and mixed with glacial acetic acid and acid ninhydrin reagent. Toluene was used to extract the mixture, and the absorbance at 520 nm was measured. A standard curve was used to calculate the proline concentration on a dry matter basis.

Results

Isolation of *Fusarium* sp.

The isolated fungus has a white colony and doesn't produce any pigments as shown in photo (2). Its identification shows this Sp. is *Fusarium Tumidum* as shown in photo (3).

photo (2): Isolated fungus on PDA.

THE REGIONAL CENTER FOR MYCOLOGY AND BIOTECHNOLOGY

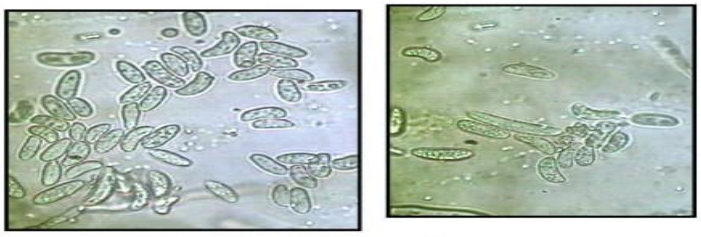
CULTURE COLLECTION AND IDENTIFICATION UNIT

IDENTIFICATION REPORT

Specimen Code :F-21-

Character	Examination
* Culture Exam.: Growth characteristics	Colonies on PDA attaining a diameter of 7.0 cm in 4 days. Mycelium cottony in white to buff colour. Reverse brown.
* Microscopic Exam.:	
Micro-conidia	Micro-conidia, oval, 1- celled 12 X4.5 µm.
Macro-conidia	Macroconidia 1-5 septata; 22.0X5.0 µm.
Chlamydospores	Rarely observed.

N.B. : The data are expressed as means of multiple measurements & only the diagnostic features were given here.



Accordingly the specimen is:

Fusarium tumidum

Investigator Director

photo (3): Fungus identification result.

Isolation of Bacteria

Four species of bacteria were isolated from rhizosphere of healthy tomato plants and purified for antagonism test as shown in photo (4).

photo (4): Isolated bacteria

Antagonism test

Only three species of bacteria give positive result against the isolated *Fusarium* (B1, B3&B4) while the fungus grow over the B2 specie as shown in photo (5). These three bacterial strains were selected for pots experiment.

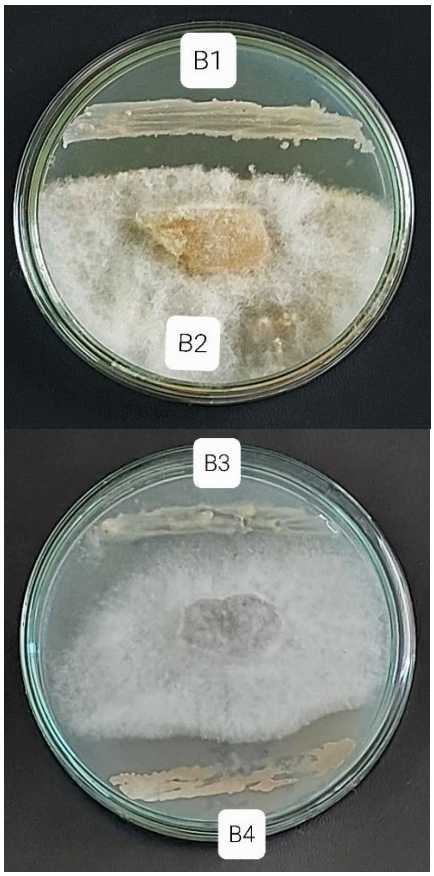


photo (5): the antagonism between isolated fungus and bacteria

Pot experiment

Tomato wilt disease in pots is characterized by the above-ground portion of the plant drooping, and brown staining of the vascular region apparent in sections tangential to the xylem and in cross sections of infected stems or roots (25). As seen in photo (6), the seedlings in the control pot displayed signs of wilt illness after 32 days.

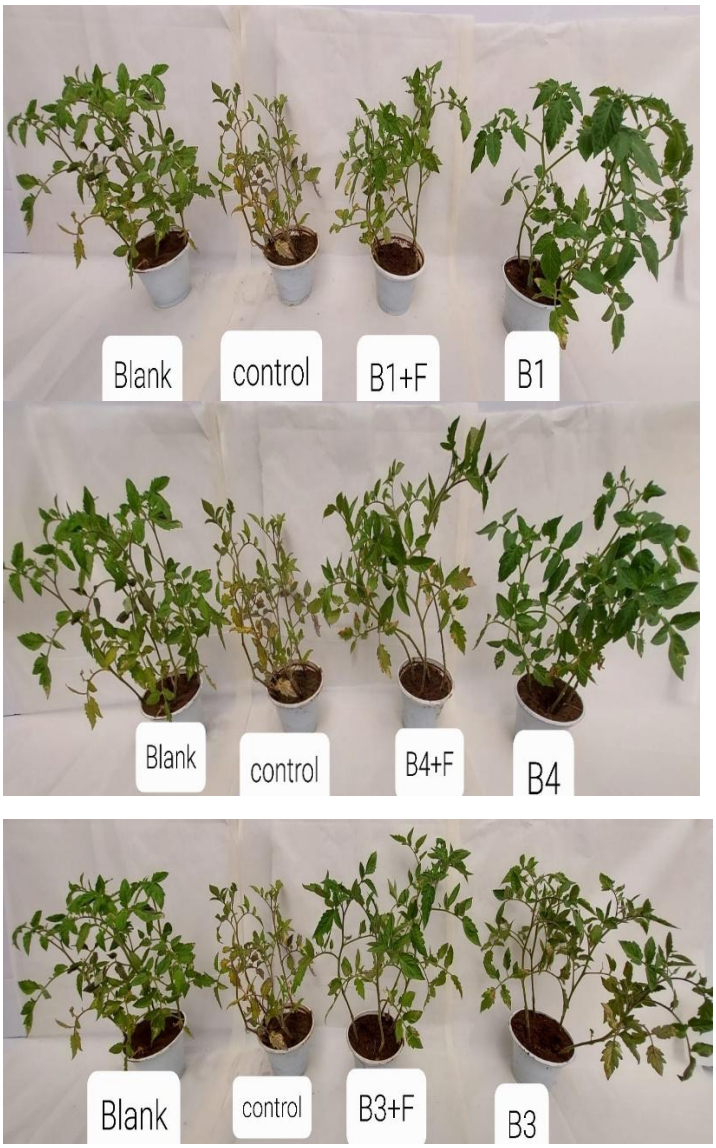


photo (6): treatment of plants with isolated bacteria

Shoot and root length

Figure (1,2)'s results demonstrated that the aver age length of the shoot and root was significantly impacted by bacterial treatments.

When comparing the average length of the roots and shoots of plants treated with bacterialisolates to other plants infected with fungus, this character istic showed a significant difference. On the other hand, plants treated with bacteria only showed a higher root and shoot length than plants treated with bacteria after infection by fungus.

Fresh and Dry weight for Shoot and Root

Data represented in the figures (3,4,5&6) clarify significant effect related to the application of three isolated bacteria on *Fusarium* infected plants. The effect of these treatments has extended to fresh and dry weight of shoot and root compared to the plants just infected with *Fusarium* which recorded the lowest weight. The highest weight was found in plants treated with bacteria only. These outcomes were in line with earlier research that showed the administration of the three bacterial treatments had considerably extended the length of the roots and shoots.

proline contents

Data in figure (7) showed that the higher proline accumulation was found in plants infected by *Fusarium*, while plants treated with bacteria after infection introduce low proline concentration. Plants not infested by *Fusarium* also introduce low proline concentration like control which not infected.

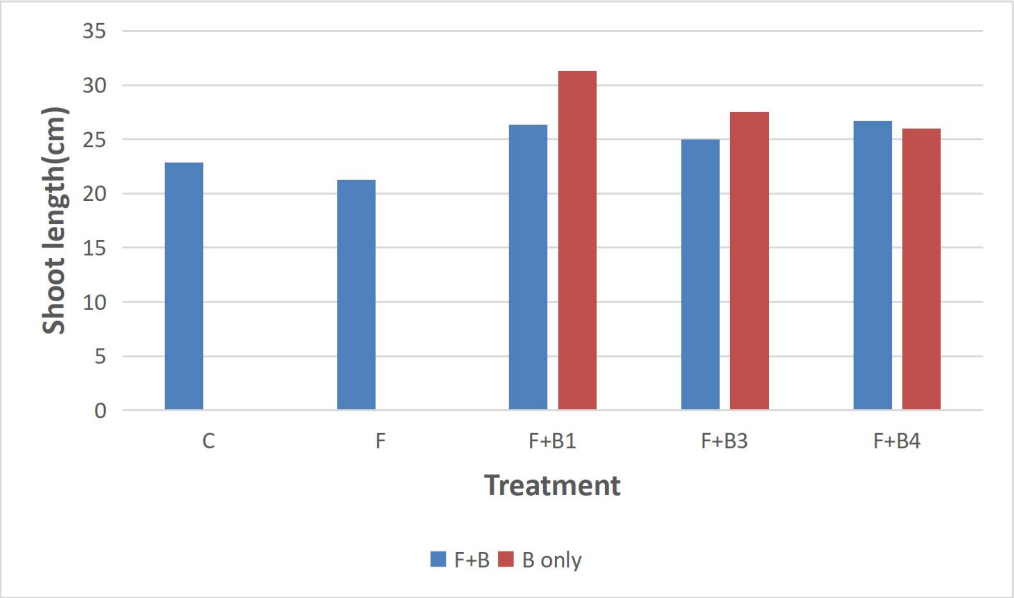


Figure 1: shoot length

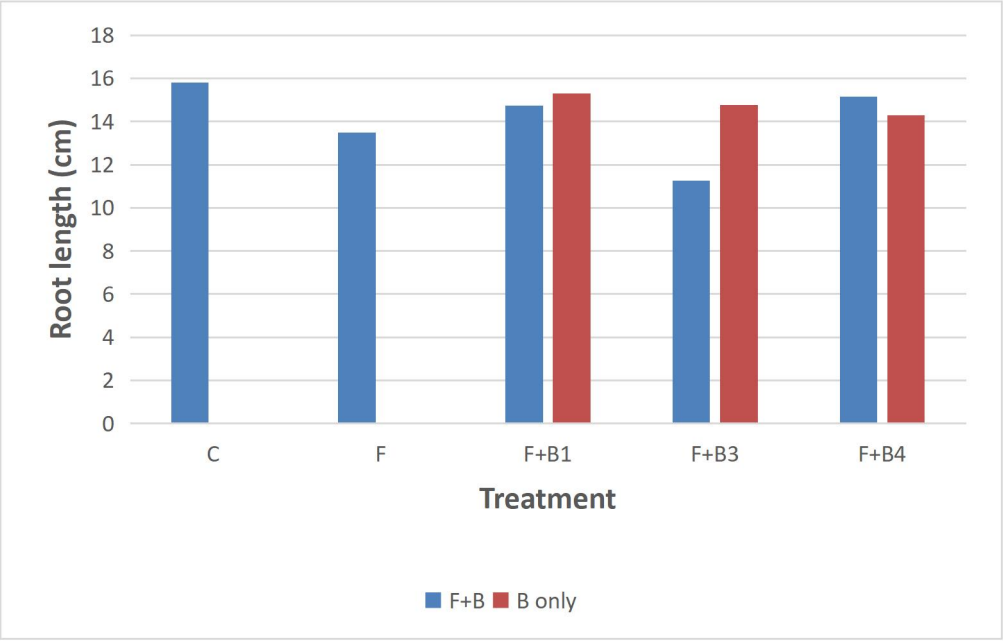


Figure 2: root length

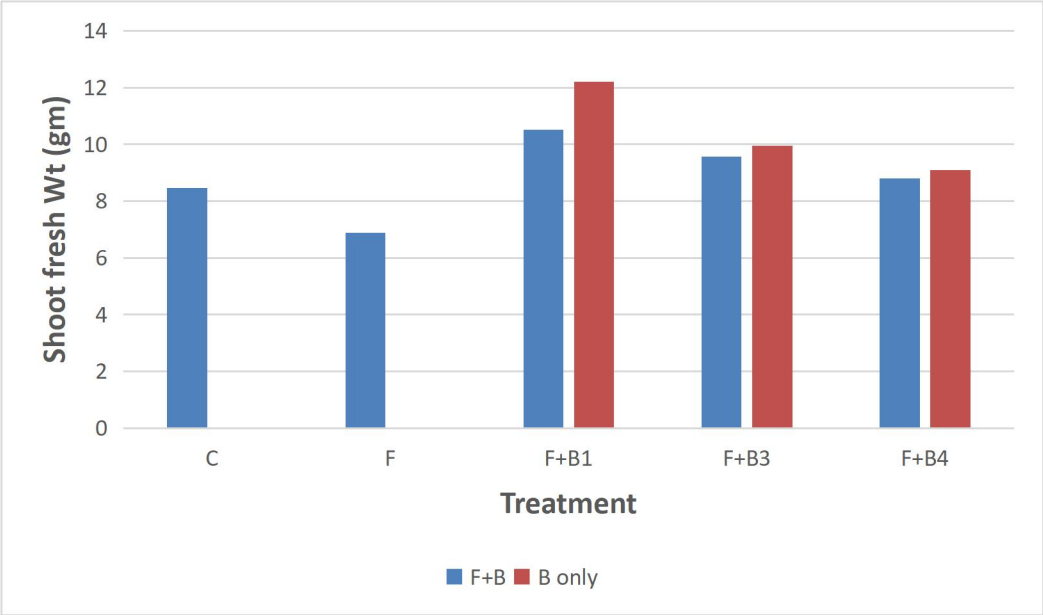


Figure 3: shoot fresh weight

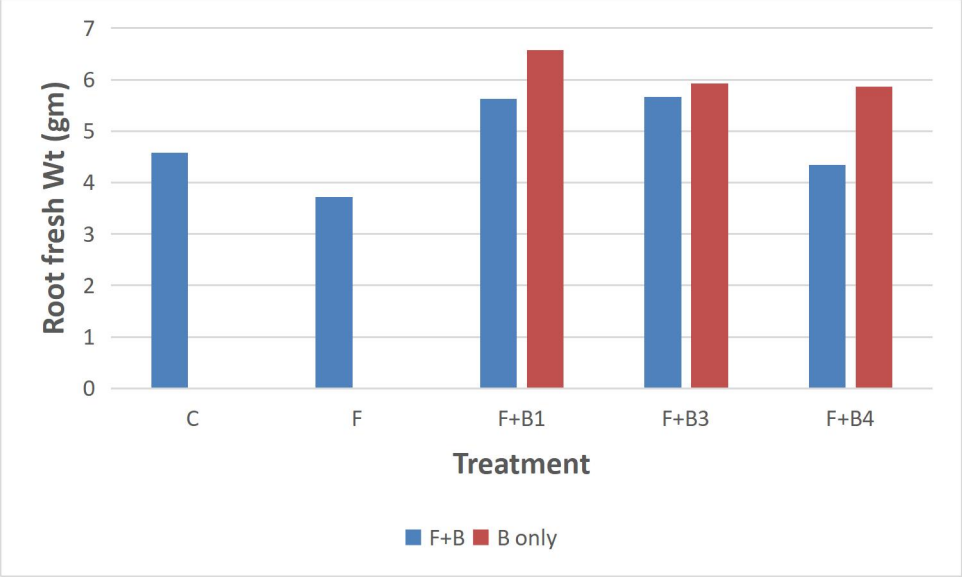


Figure 4: root fresh weight

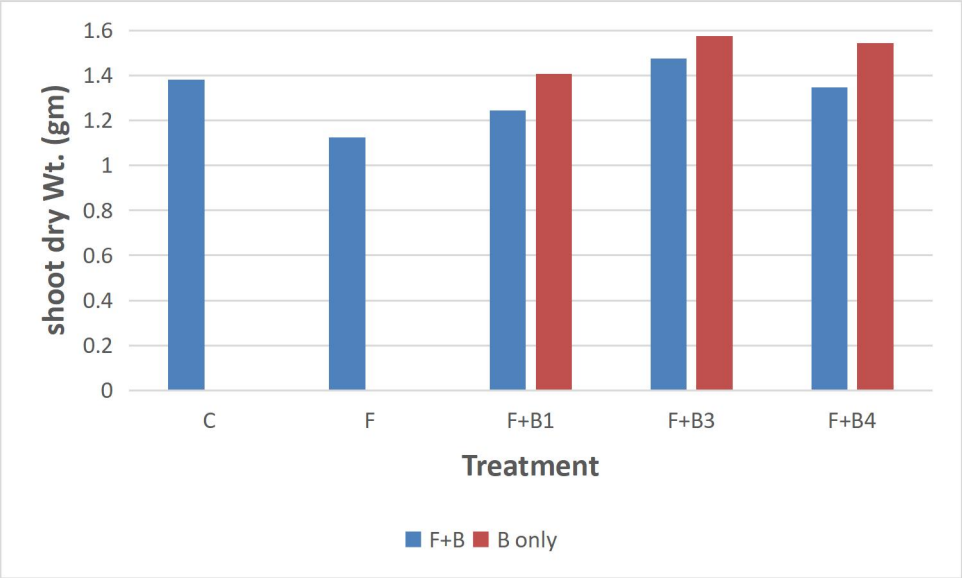


Figure 5: shoot dry weight

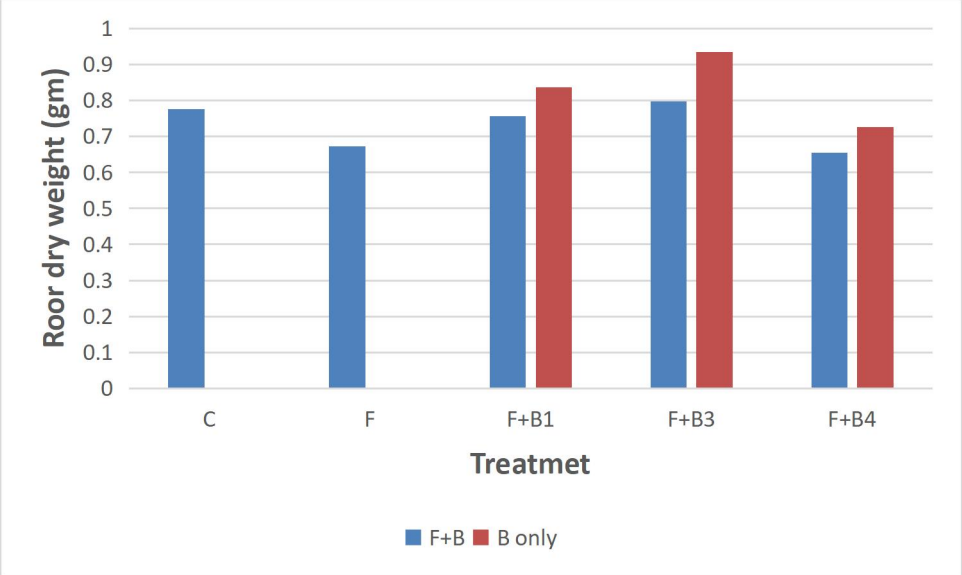


Figure 6: root dry weight

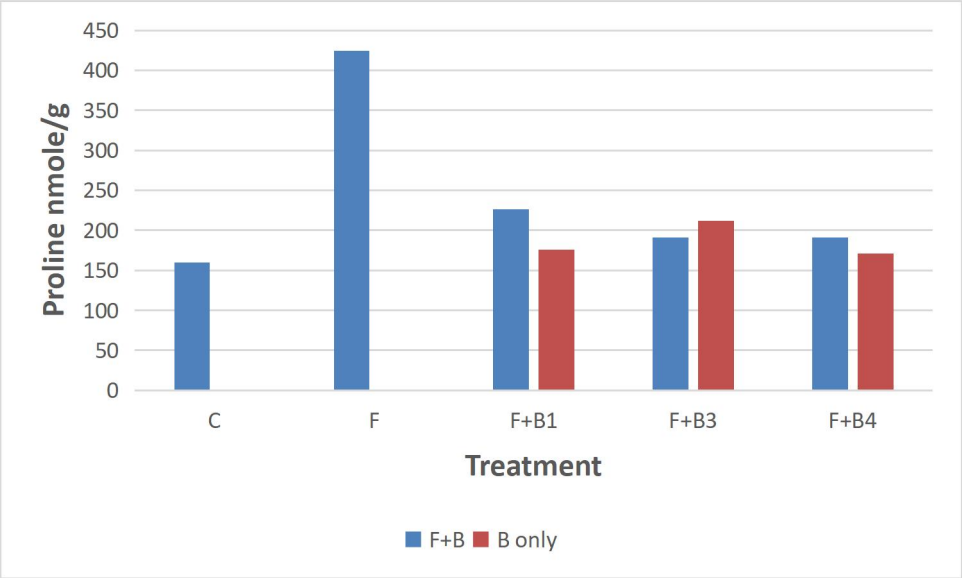


figure (7): proline estimation of all treatments

Electrolyte leakage

The EL was significantly lower in the plants treated with isolated bacteria after infection compared with plants just infected by *Fusarium* figure (8). Plants treated with bacteria only introduce a lower electrolyte leakage than *Fusarium* infected plants.

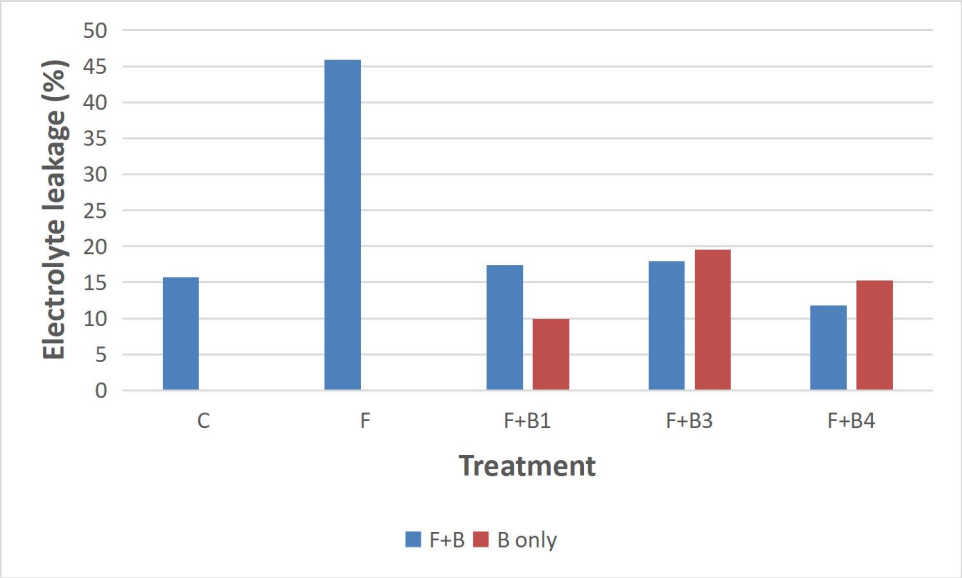


figure (8): electrolyte leakage % estimation of all treatments

Discussion

A study (26) that looked at how different fungus infection affected growth characteristics, such as shoot and root length and fresh and dry weight, found that when stress intensity increased, all of the growth metrics decreased. Recording a high growth parameter after bacterial treating confirm the ability of isolated bacteria in decreasing the effect of fungal infection.

The results of this experiment are consistent with the influence that bacterial isolates have on tomato plant growth and nutrient uptake, as demonstrated by the increased growth of inoculated seedlings compared to control ones. The aforementioned study's growth was significantly higher than that of control and infected plants due to the expansion of growth caused by isolated bacterial species. In general, the tomato seedlings inoculated with isolated bacteria have the highest growth parameters; hence, treatment by isolated biocontrol agents could decrease the effect of *Fusarium* infection.

Eubacteria, protozoa, marine invertebrates, and plants all accumulate proline under a variety of stressors. According to reports, proline accumulation in plants can happen as a result of exposure to salt, drought, extreme heat or cold, heavy metals, pathogen infection, anaerobiosis, nutrient shortage, air pollution, and UV radiation (27, 28&29). Plant stress tolerance is thought to be adaptively influenced by proline accumulation. According to certain theories, proline can store carbon and nitrogen and functions as a suitable osmolyte (27). Proline accumulation may offer a means of buffering cytosolic pH and balancing cell redox status. It has also been suggested that proline serves as a molecular chaperone, stabilizing the structure of proteins. Lastly, proline accumulation may be a stress signal influencing adaptive responses (30). Plants frequently accumulate proline as a reaction to stress. However, proline from an external source is hazardous under controlled circumstances. It is debatable if P5C, the breakdown product of proline, or proline itself is the hazardous agent (31, 32, 33 & 34). Plants that are exposed to both Pro and P5C experience cell death (35). According to Botta et al. (36) the absence of the pathogen in infected leaflets indicated that a phytotoxin was the source of the leakage, which led to an increase in electrolyte leakage in potato leaves treated with a *Fusarium* eumartii filtrate.

The apoplastic sap of the infected plants showed an increase in electrical conductance (ions) and leaf electrolyte leakage, indicating damage to the leaf plasma membrane. Damage to the cell membrane, which permits electrical conductivity to enter the apoplastic space, is

the origin of these occurrences (37). This work concludes that, compared to plants treated with isolated bacteria following infection, infected plants cause more serious damage to their cell membranes. Therefore, this can be employed as an environmentally sustainable approach.

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