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Evaluation of the Efficiency of Some Rhizobial Strains in Colonizing the Roots of Zucchini Squash (*Cucurbita pepo* L.) Plant

Hosam A. Sayed[1,*], Hend M.A. El-Egami[2] and Soad Y.S. El-Sayed[2]

[1]Gene Bank, Agricultural Research Center (ARC), P.O. Box 12619, Giza, Egypt.
[2]Department of Agricultural Microbiology, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt.

[*]**Corresponding author:** hosam722000@yahoo.com

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Abstract: The use of *Rhizobium* strains as plant growth promoting rhizobacteria (PGPR) is rapidly expanding in vegetable crops in recent years. In the current study, three *Rhizobium* strains were examined as a PGPR in two experiments with zucchini squash (*Cucurbita pepo* L.) cultivated in the spermosphere model and greenhouse soil respectively. The spermosphere model to ensure that microbial cells enter the zucchini root. This was verified by counting rhizobia cells in a specialized environment. After that, an electron microscope was used to observe the changes that bacteria make when penetrating the plant roots walls. All rhizobial strains were assessed for features that promote plant growth. All strains synthesized indole-3-acetic acid (IAA), with the greatest concentration seen in *R. leguminosarumbv. trifolii* (61.18 µg mL^{-1}), which also exhibited peak siderophore production (21.58%), phosphate solubilization (92.00 ppm), and ammonia synthesis. All strains exhibited the synthesis of protease and hydrogen cyanide. The results demonstrate that the evaluated rhizobia exhibit various PGPR characteristics and have promise for application as biofertilizers in sustainable agriculture. Additionally, seed inoculation with different *Rhizobium* strains enhanced both fresh and dry shoot biomass of zucchini in comparison to the uninoculated control and significantly increased the phosphorus concentration, demonstrating the potential of rhizobia as biofertilizers.

Key words: *Rhizobium*, colonization, vegetable plants, spermosphere, transmission electron microscope.

1. Introduction

Crop yields are surely impacted by interactions among plants and rhizobacteria, microorganisms found in the rhizosphere. The crucial initial stage in the interaction of advantageous microbes with plants is root colonization **(Kloepper and Beauchamp, 1992).**

The proliferation of microorganisms in, on, or near roots is known as root colonization. It involves the migration of

microorganisms from an inoculum source to the actively growing root as well as their growth or multiplication in the rhizosphere. Although it has been demonstrated that physical, chemical, and biological aspects of the soil can influence root colonization, few phenotypic characteristics of plants and microorganisms that support successful root colonization have been identified **(Parke, 1991)**.

Rhizobia are symbiotic bacterial partners forming nitrogen fixing nodules on legumes. Nodule creation is the result of a complicated biochemical communication amongst rhizobia and legumes in a symbiotic relationship. This interaction involves the release of nod-gene activating signaling compounds by the plant and the production of lipo-chito-oligosaccharide nodulation components by the bacteria **(Jaiswal *et al*., 2021).**

The goal of using rhizobia with non-legume plants is to expand the benefits of this beneficial microbe to a broader range of crops. This could lead to increased crop yields, improved soil fertility, and rhizobia working in concert with synthetic fertilizers **(Dent and Cocking, 2017)**.

In addition to providing symbiotic nitrogen, certain rhizobia also display physiologically beneficial features like the synthesis of phytohormones that promote plant growth, such as lumichrome, Nod factors, riboflavin, gibberellins, cytokinins, indole-3-acetic acids (IAA), etc. all of which have different functions in augmenting the productivity and growth of plants **(Dakora and Phillips, 2002 and Berg, 2009)**. Conversely, IAA is a significant auxin family member that regulates multiple physiological processes in plants, such as cell division and expansion, tissue distinction, and reactions to light as well as gravity **(Teale *et al*., 2006; Shokri and Emtiazi,2010)**.

Thus, rhizobia give plants more benefits than only the N they provide through symbiotic relationships. The creation of several metabolites and enzymes that are either directly or indirectly induced by rhizobia and plants through the development of nodules mediates the advantageous effects of rhizobia. Plant growth promotion has been suggested to be facilitated by the production of IAA, riboflavin, lumichrome, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and protons for phosphate dissolution, among other plant growth-promoting substances **(Li *et al*., 2000; Matiru and Dakora, 2005; Gravel *et al*., 2007; Bal *et al*., 2013 and Dakora *et al*., 2015)**. An enzyme called ACC deaminase helps higher plants become more productive by reducing harmful ethylene levels.

The use of rhizobia in non-legume crops is a relatively recent field of research that looks into the potential benefits of developing symbiotic relationships between these rhizobia species and non-legume plants. The idea behind applying rhizobia to non-legume plants is to increase the beneficial organism's benefits to a wider variety of crops. This may result in higher crop yields, healthier soil, and rhizobia that complement chemical fertilizers. **(Dent and Cocking, 2017).**

Promoting plant growth by beneficial bacteria called rhizobacteria, or PGPR, inhabit plant root systems and promote the growth and well-being of those plants **(Bhattacharyya and Jha, 2012)**. A varied group of bacteria known as PGPR improves nutrient uptake, suppresses plant infections, and fosters tolerance for stress, all of which are helpful to plant development and health **(Díaz-Valle *et al*., 2019).** Plant growth-promoting rhizobacteria (PGPRs) can be classified into two groups based on their degree of connection to plant root cells: intracellular PGPRs, which are found inside plant tissue, and extracellular PGPRs, which are found in the soil surrounding plant roots **(Martínez-Viveros *et al*., 2010).** Endophytic PGPR has the ability to colonize plant tissue and offer advantages to the plant, including enhanced nutrient uptake, enhanced resistance to both abiotic and biotic stress, and growth stimulation. They are known to generate enzymes like phytase and protease, which can aid in the uptake of nutrients, as well as growth-promoting substances including gibberellins (GA_3) and indole acetic acid (IAA). PGPRs in the rhizosphere, whereas, interaction with plant roots can offer advantages to the plant, including bettering plant development, nitrogen uptake, and the structure of the soil. They can lessen viruses' capacity to infect the plant by competing with them for nutrition and space. By creating antibiotics or other substances that prevent the growth of infections, they can also function as biocontrol agents. **(Goswami, *et al*., 2016 and Vessey, 2023).**

Rhizobia have been shown to stimulate non-leguminous plant growth through a combination of direct and indirect mechanisms **(Mehboob, *et al*., 2009 and Mehboob, *etal*., 2012)**. Direct techniques include the manufacture of vitamins and phytohormones, the inhibition of ethylene synthesis in plants,

the development of stress tolerance, and enhancements in nutrient uptake (such as the solubilization and mineralization of inorganic and organic phosphorus). Conversely, indirect techniques involve the production of antibiotics and/or fungicidal agents through biosynthesis, competition for supplies (for example, by siderophore generation), and the development of systemic resistance against infections. These techniques mainly decrease or stop the harmful effects brought on by microbial infections. Additionally, the bacteria can aid in the indirect growth of non-leguminous crops by interacting with other beneficial microorganisms. **(Antoun *et al*., 1998)**.

Under lab circumstances, the spermosphere model facilitates the study of natural associations between plants and bacteria **(Thomas-Bauzon *et al*., 1982)**. The model has chosen unique, effective genotypes of nitrogen-fixing bacteria that can be used to inoculate perennial plants. The model's justification is that a wide variety of bacteria with diazotrophic properties compete with one another to colonize the roots of annual plants in the field; the more suited bacteria eventually emerge over the less effective ones and colonize the roots. When placed in xenobiotic circumstances, like the spermosphere model, these best-adapted potential PGPRs are those that eventually produce significant population numbers on mature plants and demonstrate the greatest efficiencies of N-fixation **(Ivan and Abu, 2002).**

Additionally, for the reason to utilize only microorganisms safe for human health—not just for customers or end users, but also for handlers during the biofertilizer manufacturing process—it is vital to be aware of the positive and negative impacts of bacteria before using them as biofertilizers. Currently available for sale as biofertilizers for non-legumes, strains of *Azospirillum, Gluconacetobacter, Bacillus, or Azotobacter* have not been shown to have any negative effects on people. However, after decades of legume inoculation demonstrating their safety for people and their promise as non-legume plant growth boosters, industrial biofertilizers based on rhizobia are still nonexistent.In particular, it is commonly known that rhizobia can stimulate the growth of cereals like rice, canola, and barley as well as other plants whose seeds are used to make edible oil. However, rhizobia are particularly important in the biofertilization of raw eaten non-legumes because of their safety for human health. As of right now, data regarding the growth promotion of crops edible as raw leaves, like lettuce, and raw roots, such radishes, are available. Data on how rhizobia affectsnon-legumes, however, is lacking, making it difficult to consume raw fruits **(Paula Garcı´ *et al*.,2012)**.

Due to its high nutritional content, zucchini squash (*Cucurbita pepo* L.), a member of the *Cucurbitaceae* family, is a highly valued vegetable that is popular throughout the world **(Liu *et al*., 2020)** and has significant economic implications **(Martínez-Valdiviesо*et al*., 2015)**. In the Mediterranean region, zucchini squash is frequently grown in fields and greenhouses **(Liopa-Tsakalidi*et al*., 2010)**. It is also grown in desert climate zones, which are marked by scarce water resources, hot temperatures, and poor soil organic matter content. **(Farid *et al*., 2022)**. The objective of this research was to investigate the impact of Rhizobium inoculation on zucchini, a vegetable of considerable commercial importance whose fresh fruits are widely consumed globally.

2. Materials and Methods

2.1. Microbial cultures

The microbial strains utilized in this research were generously supplied by the Biofertilizers Production Unit within the Department of Agricultural Microbiology at the Soils, Water, and Environment Research Institute (SWERI), Agricultural Research Center (ARC), located in Giza, Egypt. These strains were cultivated in a yeast extract mannitol (YEM) broth medium, as described by **Vincent in 1970**. The cultures were incubated at a temperature of 28°C for duration of 3 to 5 days on a rotary shaker, allowing them to reach the early logarithmic phase and achieve a population density of 10^9cfu/ml. The inoculum for each strain was prepared in YEM. Seeds were stirred in 1 mL inoculum containing about 10^8cfu/mL and kept in the refrigerator for 12–24 h until used. Un-inoculated control seeds were treated in a similar way in media without bacteria. Three strains of rhizobia were selected *(Rhizobium leguminosarum* bv. *Viceae* strain (ICARDA 441), *Rhizobium leguminosarum* bv. *Trifolii*

and *Bradyrhizobium spp*. (strain USDA 3456)) and various tests were conducted on them as plant growth promoting rhizobacteria (PGPR).

2.2. *In vitro* Screening of *Rhizobium* strains for Plant Growth Promoting Activities

Rhizobium strains were cultivated using yeast extract mannitol (YEM) media as described by **Vincent (1970**). IAA production was quantitatively assessed using the method outlined by **Gordon and Weber (1951)**. Dicalcium phosphate agar plates were used to evaluate phosphate solubilization based on the method described by **Frioni (1990)**, and the quantitative measurement of phosphate solubilization was conducted according to **Pikovskaya (1948**) with phosphorus content determined as per **Watanabe and Olsen (1965)**. Siderophore assay was carried out following the procedure outlined by **Alexander and Zuberer (1991)**. The optical density was measured at 630 nm, and the siderophore content in the aliquot was calculated using the method presented by **Sayyed *et al*. (2005)**. **Bakker and Schippers (1987)** provided the methodology for determining the production of hydrogen cyanide (HCN). By adding 1 ml of Nessler's reagent to a 72-hour-old culture grown in peptone broth (Peptone-10g, NaCl-5g, distilled water-1lit), ammonia (NH_3) production was detected and the presence of a deep yellow to brown color was noted **(Bakker and Schippers, 1987)**. Proteolytic activity was measured by spotting the strains on a skim milk agar plate that contained 1.5% agar, 0.5% peptone, 0.3% beef extract, and 0.5% skim milk. For twenty-four hours, the infected plates were incubated at 37°C. According to **Dunne *et al*. (1997)**, Plates of *Rhizobium* were observed daily for a maximum of seven days, and they were incubated at 25^0C or 28^0C, respectively (see Table 1).

2.3. Estimation of Exopolysaccharides production EPS

The synthesis of exopolysaccharides by *Rhizobium* was quantitatively assessed gravimetrically using ethanol precipitation of culture supernatants, as outlined by **Somasegaran and Hoben (1994).** To extract cells, bacterial cultures were centrifuged at 8,000–10,000 rpm for 15–20 minutes at 4 °C. The supernatant was collected, and three liters of cooled 100% ethanol were gradually added while maintaining continual stirring. The solution was incubated overnight at 4 °C to facilitate EPS precipitation.

The precipitated EPS was isolated using centrifugation at 10,000 rpm for 20 minutes and subsequently washed twice with 70% ethanol. The EPS pellet was dehydrated at 60 °C until a stable weight was achieved.

2.4. Plant Material and Experiment

Two experiments were conducted at Biofertilizers Production Unit, Agric. Microbiology. Dept., Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt. The first experiment was conducted to spermosphere model and investigate the interaction between plants and *Rhizobium* strains using transmission electron microscopy (TEM), while in the second, a greenhouse experiment was used to assess some zucchini vegetative growth parameters.

2.5. Spermosphere model experiment

Under laboratory conditions, the spermosphere model facilitates the study of spontaneous relationships between plants and bacteria (**Thomas-Bauzon *et al*., 1982**).

Using a spermosphere model staining technique, patterns of systemic bacterial colonization were found on the roots of *Cucurbita pepo* (summer squash) seedlings grown under sterile conditions. This method is adopted by the Center of Pédologie in Nancy, France (**Omar *et al*., 2022**). The summer squash seeds were thoroughly rinsed in aseptic distilled water, sterilized on the surface with 70% ethanol for 30 seconds, and then soaked in 20% sodium hypochlorite for 15 minutes. The seeds were washed three more times in sterile distilled water, then spread out on filter paper in a Petri plate and left to dry for ten minutes prior to being cultivated. Aseptic germination of sterilized seeds was achieved by placing them in a Petri plate containing Watanabe medium, which is devoid of carbon sources. (Fig. 1).

2.6. Estimation of Root Colonization by *Rhizobium* strains

The roots were chosen at the conclusion of the spermosphere model experiment in order to measure the concentration of *Rhizobium* both inside and outside the roots. In the laboratory, **(Cappuccino & Sherman, 2014)** the summer squash roots were rinsed with sterile distilled water to remove any growth media. The roots were then divided into segments that were between one and two centimeters long. These segments were then placed in sterile tubes, and 10 milliliters of 0.01 M phosphate buffer solution (PBS) was added to the containers. To dilute the root homogenate, sterile tubes containing sterile PBS (10−1, 10−2, 10−3, etc.) were filled with known volumes of the homogenate. A volume of 0.1 mL from every dilution was poured onto distinct sterile agar plates containing YEM medium **(Vincent, 1970)** containing 0.2 g/l brilliant green. The incubated plates were then placed in an aerobic microbiology incubator at 37 °C for three days to facilitate the growth of the colonies. Following incubation, each plate was examined and the number of *Rhizobium* colonies was recorded.

2.7. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was utilized to observe how the PGPR that was utilized in this work interacted with zucchini seedlings. The task was carried out at Cairo University's TEM Lab FA-CURP, Faculty of Agriculture. Research Park. The method according to **Bozzola and Russell (1999)** was used. Samples of tissue were cut into pieces about 1 mm in size. After slicing the tissue, it was fixed in osmium tetroxide and glutaraldehyde, dried in alcohol, and then embedded in epoxy resin for transmission electron microscopy. Microtome sections were prepared using a Leica Ultracut UCT ultramicrotome, with a thickness of roughly 500-1000 µm. Tolodin blue (1X) was used to stain thin sections, which were then viewed with a camera (Lica ICC50 HD). Ultra-thin sections measuring nearly 75-90 µm in thickness were prepared, stained with lead citrate and uranyl acetate, and examined using a JEOL transmission electron microscope (JEM-1400 TEM). CCD camera model AMT with side mount configuration and 1632 x 1632 pixels was used to capture the images. **Dykstra and Reuss (2003)** state that this camera acquires data using a 1394 fire wire board.

2.8. Some zucchini vegetative growth parameters

To analyze the impact of *Rhizobium* inoculation of zucchini, the pot experiment was conducted at green house of Biofertilizers Production Unit, Agric. Microbiology. Dept., Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt., during summer 2021 using sandy soil from Ismailia Agric. Res. Station, Ismailia Governorate, Egypt, and the main charterers of used soil were shown in table (1). The following treatments were conducted:

T_1 – uninoculated plants (control).

T_2 - Inoculation with *Rhizobium leguminosarum* bv. *Viceae* (ICARDA 441).

T_3 - Inoculation with *Rhizobium leguminosarum* bv. *Trifolii*

T_4 - Inoculation with *Bradyrhizobium spp*. (strain USDA 3456)

All treatments received the recommended mineral fertilizer of NPK at a rate of 100 kg / fed. ammonium sulphate (20.5 % N), 150 kg / fed., super phosphate (15.5 % P_2O_5) and100 kg / fed. potassium sulphate (48 % K_2O), the rates were added according to the weight of soil in the pot (10 Kg). A randomized complete block design with three replicates was used.

A sample of five zucchini plants from each pot was collected 75 days post-sowing to assess plant height (cm), leaf count per plant, leaf area (cm^2) and shoot fresh and dry weight (g). The contents of N, P and K in shoots were assessed using the methodologies outlined by **Okalebo *et al.* (2002), Pregl (1945).**

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used for data analysis **(Snedecor and Cochran, 1980).**

Table (1). Some chemical, physical and biological properties of experimental soil

Property	Soil
Sand (%)	89.79
Silt (%)	2.48
Clay (%)	7.73
Texture	Sandy
pH	7.32
E.C (dS m^{-1}at 25ºC)	0.25
Organic matter (%)	0.25
Organic carbon%	0.15
Total nitrogen (%)	0.021
C/N ratio	7.14
Dehydrogenase activity*	264
CO_2-evolution rate**	13.24

*µg TPF/100g dry soil/24h. **mg CO_2/100g soil/24 h.

3. Results and Discussion

Table 2 shows information about the three distinct *Rhizobium* strains under investigation and their production of plant growth promoters. Every strain that was tested was found to produce IAA equivalents. The auxin values for the three strains that were tested were *Rhizobium leguminosarum* bv. *Viceae* strain (ICARDA 441), *Rhizobium leguminosarum* bv.*Trifolii*, and *Bradyrhizobium spp.* (strain USDA 3456), in that order: 42.98, 61.18, and 38.22 µg ml^{-1}.

The highest levels of siderophore production (21.58%) and phosphate solubilization (92.00 ppm) were recorded by *R. leguminosarum* bv. *Trifolii*. The production of protease enzymes is the same in all the strains under investigation. The best bv. of *R. leguminosarum* for producing NH_3 was *Trifolii*, which was identified by adding 1 milliliter of Nessler's reagent to the broth medium and noting the presence of a deep yellow to brown hue. Upon visual inspection of the plates, the same strains were found to be hydrogen cyanide producers (Table 2). These results were in align with **Paula *et al*. (2012)**, who found that Both TPV08 and PETP01 *Rhizobium* strains demonstrated the capacity to proliferate in JMM medium enriched with tryptophan, yielding comparable quantities of indole acetic acid (75 mg L^{-1} and 63 mg L^{-1}, respectively). Consequently, both strains exhibited a direct method for promoting plant development through indole acetic acid production, while the siderophore formation by strain TPV08 further indicates its capacity to facilitate iron acquisition.

Table (2). *Rhizobium* strains and its PGPR Mechanism of Action

	IAA ug/ ml	Phosphorous ppm	Protease	NH_3	HCN	Siderophores %
Rhizobium leguminosarum* bv. *Viceae	42.98	81.12	+	+	++	18.63
Rhizobium leguminosarum* bv. *Trifolii	61.18	92.00	+	++	+++	21.58
***Bradyrhizobium spp*. (strain USDA 3456)**	38.22	73.75	+	+	+	10.18

Exopolysaccharide Production by Different *Rhizobial* Strains

Significant variance was noted in exopolysaccharide (EPS) synthesis among the examined rhizobial strains cultured in Yeast Extract Mannitol Broth. All strains generated quantifiable EPS, yet the amounts varied among species. Table 3 revealed that EPS production by *Rhizobium leguminosarum* bv. *Trifolii* was markedly superior to that of the other strains, although *Bradyrhizobium spp.* strain USDA 3456. exhibited much lower EPS production. *Rhizobium leguminosarum* bv. *Trifolii* generated the greatest extracellular polysaccharide (EPS) concentration, averaging 1.78 mg mL^{-1}, followed by *Rhizobium leguminosarum* bv. *Viceae* at 1.53 mg mL^{-1}. The minimum EPS production was seen in *Bradyrhizobium spp*. (strain USDA 3456), with an average of 1.16 mg mL^{-1}.

Studies by **Kumar and Singh, 2016** on EPS production, plant growth promotion, and root colonization by *Rhizobium sp.* demonstrated increased EPS synthesis, greater seed germination, and overall improvement in plant development compared to the control and *R. meliloti* treatment. The EPS generation by Rhizobia strains may have multiple benefits it can improve bacterial adherence to root surfaces, offer protection against desiccation or osmotic stress, and promote successful colonization of host plants **(Kumar and Mody, 2009).**

Table (3). Exopolysaccharide production by rhizobial strains

Rhaizobial strains	EPS production (mg mL^{-1})
Rhizobium leguminosarum* bv. *Viceae	1.53
Rhizobium leguminosarum* bv.*Trifolii	1.78
***Bradyrhizobium spp*. (strain USDA 3456)**	1.16
L.S.D	**0.18**

Fig. (1) Point to the quantity of *Rhizobium* colonies that were counted on the root system of zucchini at the time of termination. When compared to untreated seeds, a notably higher percentage of data indicates plants growing from seeds treated with *Rhizobium* strains. The variations were very notable across all strains that were being studied.

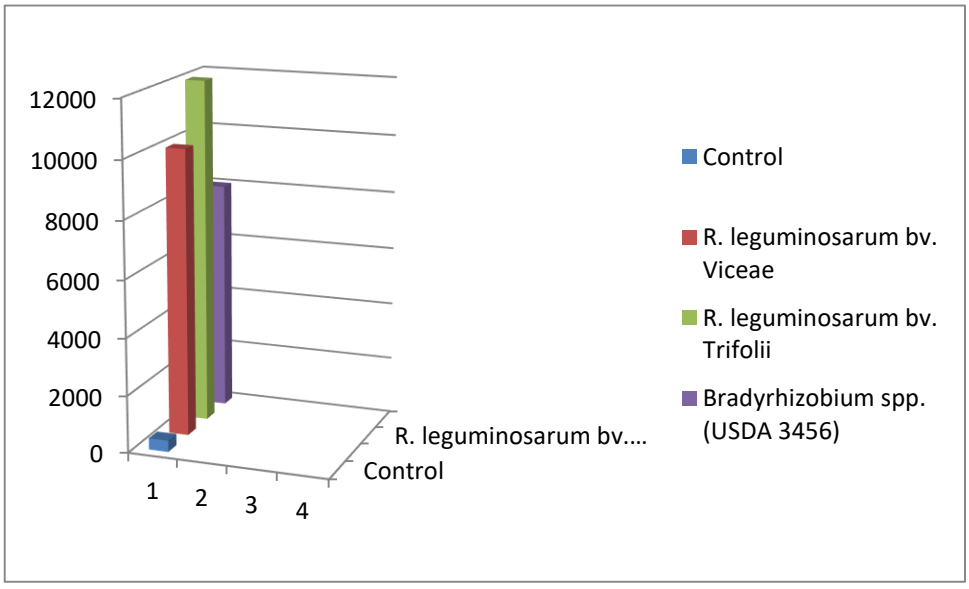

Fig. (1). CFU *Rhizobium colonies* counted on the root system of zucchini seeds planted under aseptic conditions

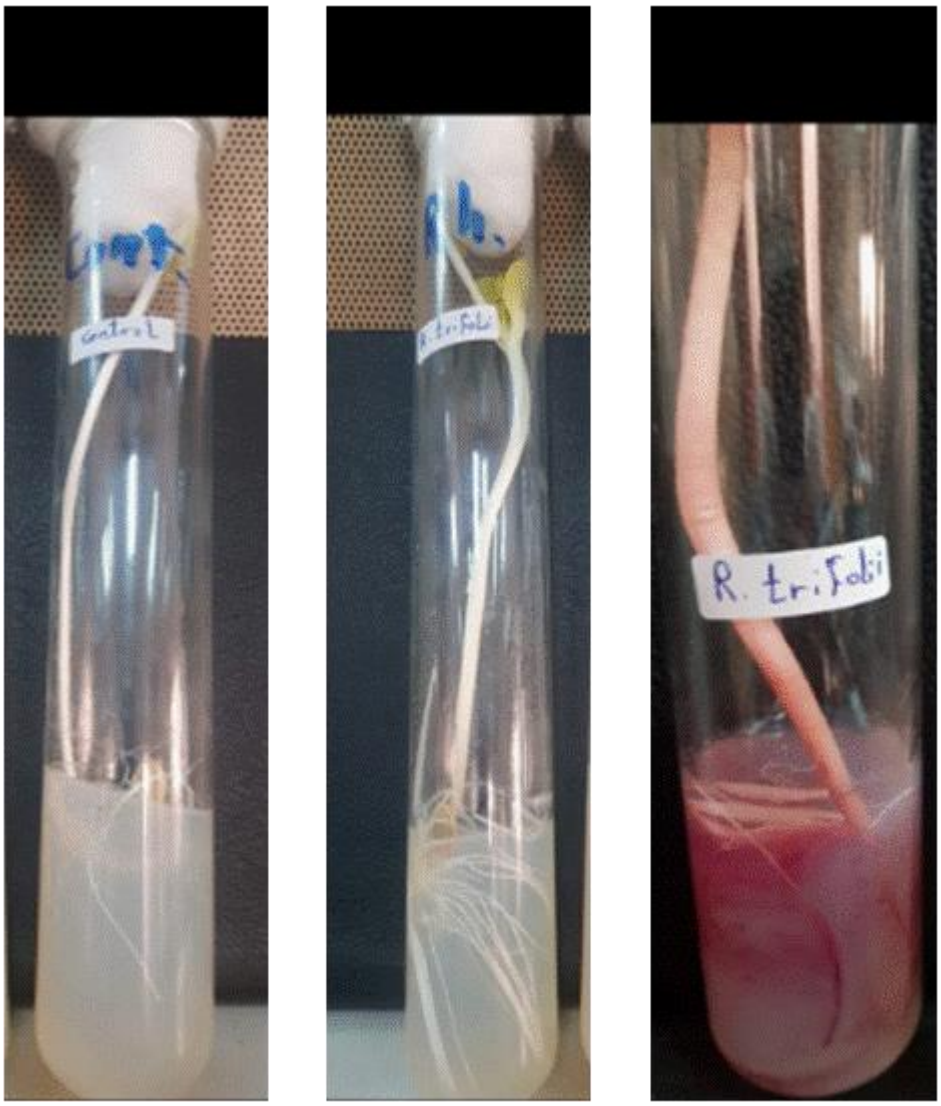

Fig. (2). Effect of *Rhizobium* inoculation of zucchini seedlings planted under aseptic conditions (Spermosphere model experiment).

The effectiveness of the *Rhizobium* strains may be predicted by examining the microbial colonization of zucchini roots using the staining technique in the spermosphere during the early stages of plant growth. *R leguminosarum*bv. *Trifolii* colonized zucchini seedlings (10 days old) in the spermosphere under aseptic conditions, as depicted in Fig 2. Based on the reduction of 2, 3, 5-triphenyletetrazolium chloride (TTC) to the red-colored formazan (TPF). (**Praveen and Tarafdar, 2003**). Compared to the *R leguminosarum* bv. *Viceae* strain (ICARDA 441), *Bradyrhizobium spp*. (strain USDA 3456), and the control treatment, the inoculated seedlings of *R leguminosarum* bv. *Trifolii* exhibited a strong intensity of red color. In this experiment, our initial evaluation was solely based on visual quantification. The activity of the mitochondrial respiratory chain is directly correlated with the reduction of TTC by seedling tissue **Markus and Brunner (2002)**; **Omar and Basilious(1998**). TTC can only be reduced to TPF by living tissue. This discovery explains why the inoculated treatments' increased color intensity was caused by bacterial colonization. Numerous intricate processes, including hormonal effects, N_2fixation, proton extrusion, and/or mineral uptake, interact as a result of inoculation. (**Geddie and Sutherland 1993; Han and Lee 2005).**

Transmission electron microscopy (TEM)

Light microscopic examination of root sections revealed that the *Rhizobium* strains used in this work had colonized zucchini seedlings (Fig. 3). The seedlings inoculated with the three *Rhizobium* strains (Figs. 3b, c, and d) clearly differed from the control treatment (Fig.3a). The roots of the zucchini seedling that had been inoculated with the *R. leguminosarum* bv. *Viceae* strain (ICARDA 441), *R. leguminosarum* bv. *Trifolii*, and *Bradyrhizobium spp.* (strain USDA 3456) showed that, the interaction between *Rhizobium* strains and plant roots using TEM clearly showed significant changes in root cells (Fig. 3 b, c and d), including, partial cell walls degradation found as Fig. 3 d and cytoplasmic confusion fig. 3 b and c whereas, Fig. 3 c exhibit increased bacterial colonization of roots bacteria surrounded by host cell organelles or fused with cell organelles. This result was in line with what **Su-Jung and Kremer (2005)** found when rhizobacteria colonized plant root seedlings degraded plasma membranes and partial cell wall and cytoplasmic disruptions. Additionally, the same finding was obtained by **Omar *et al*. (2022)** who inoculated wheat seedlings with *Bacillus thuringiensis* and *Azospirillumbrasilense* and TEM results revealed the dissolution in cell wall by colonized bacteria in the intercellular and intracellular spaces of plant root cells.

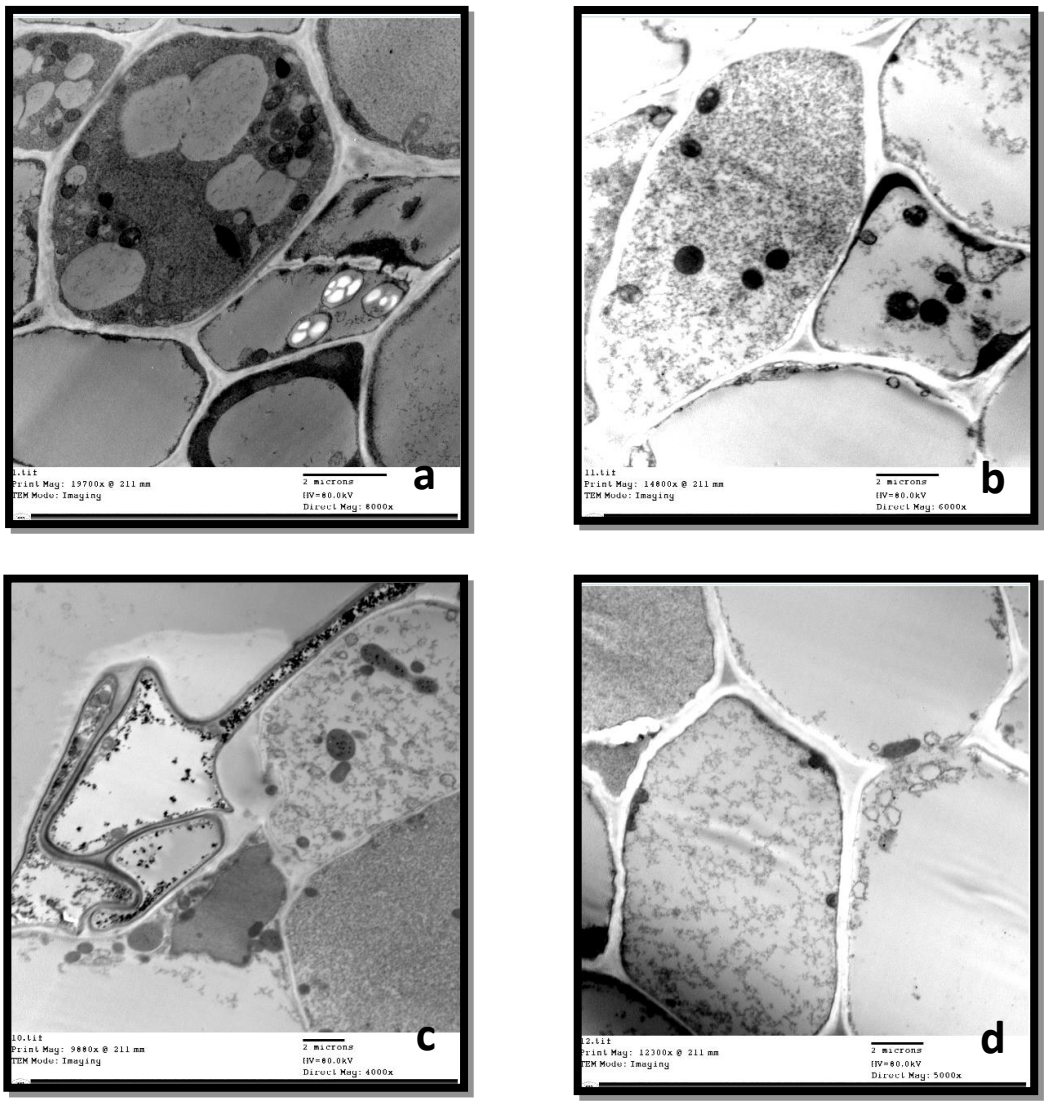

Fig. (3). SEM of root surface of zucchini squash seedlings through transmission electron microscopy (TEM)after seven days of inoculation. (a) Root surface of control (non-inoculated) seedlings (b)*R. leguminosarum*bv. *Viceae*(c)*R. leguminosarum*bv. *Trifolii*and (d)*Bradyrhizobium spp*

Vegetative growth

All treatments significantly improved all vegetative growth parameters (plant height, number of leaves per plant, leaf area and shoot dry weight) except for fresh weight in comparison to the control (untreated plants) (Table 3a). The fresh weight of shoots was not significantly influenced by any inoculation treatments; however, the highest numerical fresh weight (1.983 kg/plant) was recorded with *R. leguminosarumbv. Trifolii*. The treatments significantly influenced shoot dry weight. The maximum dry weight was observed in *R. leguminosarum bv. Trifolii* (160.3 g/plant), succeeded by *Bradyrhizobium spp*. (145.9 g/plant), *R. leguminosarum bv. Viceae* (130.2 g/plant), and the control group (118.5 g/plant). The results demonstrate that although fresh biomass accumulation was comparable among treatments, inoculation with *R. leguminosarum bv. Trifolii* significantly enhanced the dry matter content of zucchini shoots. **Paula *et al*. (2012)** demonstrated that seed inoculation with two strains of *Rhizobium leguminosarum* promoted the growth of tomato and pepper plants. Inoculated seedlings generated over double the dry biomass (combined shoots and roots) in comparison to uninoculated controls.

The impact of seed bacterization was consistently beneficial, as the seedlings of tomato and pepper exhibited greater length compared to uninoculated controls. These findings align with previous studies indicating that *Rhizobium* can enhance the shoot growth of plants as stated by**Alami *et al*. (2000).**

Table (3a). Impact of seed inoculation with rhizobium strain on Shoot fresh weight, Shoot dry weight, plant height, number of leaves and leaf area of zucchini squash in a greenhouse

Treatment	Shoot fresh weight Kg/plant	Shoot dry weight g/plant	Plant height (cm)	Number of leaves/plant	Leaf area (cm^2)
Control	1.322	118.5	40.15	10.33	20687.79
R. leguminosarum* bv. *Viceae	1.699	130.2	50.33	21.51	2311.62
R. leguminosarum* bv. *Trifolii	1.983	160.3	51.96	25.66	2349.89
***Bradyrhizobium spp*. (USDA 3456)**	1.784	145.9	49.15	22.81	2168.79
L.S.D	1.883	1.883	2.542	2.793	34.66

Nutrient contents

The inoculation of the zucchini seeds with different *Rhizobium* strains in the greenhouse had no impact on the shoot nitrogen and potassium levels but significantly increased the phosphorus concentration (Table 3b). Data in agreement with **Qureshi *et al*., 2019** who reported, rhizobial inoculum increased biomass and yield parameters of cotton plants. It might be attributed to hydrogen cyanide production **(Adnan *et al*., 2016)**, production of growth hormones **(Qureshi *et al*., 2013)** like IAA in the cotton rhizosphere which improved root morphology, root proliferation **(Hussain *et al*., 2009),** thus better root system for acquisition and uptake of mineral nutrients (N, P and K) as seen in case of rice seed and seedling inoculation with different *Rhizobium spp* (i.e. *Bradyrhizobium spp.* IRBG271, *R. leguminosarum* bv. *Trifolii* E11 and *Rhizobium sp*. IRBG74,) **(Biswas *et al*., 2000)** ultimately improving cotton yield.

Alami *et al*. (2000) also detected increases in the percentages of N, P, K, and Mg due to *Rhizobium* colonization, finding that the results correspond with those in lettuce and sunflower, where

inoculation with *R. leguminosarum* strains led to improved nitrogen and phosphorus uptake. Thus, *Rhizobium* strains function as exceptional biofertilizers for tomato and pepper across various production phases, leading to improved yield and quality.

Our results indicate that *Rhizobium* is capable of penetrating the roots of zucchini, thereby promoting plant growth at different stages of development. These results, in conjunction with findings from other studies, suggest that the application of rhizobia represents a reliable method for biofertilization in non-leguminous crops, meriting further exploration.

Table (3b). Impact of seed inoculation with rhizobium strain on N, P and K concentrations in shoot samples of zucchini squash greenhouse

Treatment	Shoot		
	N (mg g^{-1}d.wt.)	P (mg g^{-1}d.wt.)	K (mg g^{-1}d.wt.)
Control	3.22	2.73	25.22
R. leguminosarum* bv. *Viceae	3.29	3.21	27.12
R. leguminosarum* bv. *Trifolii	3.62	4.12	29.88
***Bradyrhizobium spp.* (USDA 3456)**	3.39	3.46	26.18
L.S.D	**1.959**	**1.783**	**1.883**

4. Conclusion

Rhizobia are particularly intriguing for the biofertilization of raw-consumed non-legumes due to their safety for human health. We currently possess data regarding the growth promotion of vegetables that are consumable as raw fruits such as zucchini.

The inoculation of rhizobia into non-legume crops has great promise in advancing agriculture by improving crop growth and soil fertility. By being mindful of the potential risks and benefits, it is possible to fully reap the benefits of this innovative technology and promote sustainable agriculture practices for the future.

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