

Influence of a Mixture of Microbiology (Bacteria and Fungi) and Marine Alga Extracts on Some Growth Characteristics of Strawberry (cv. Roby Gem)

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ABSTRACT

KEY WORDS: Strawberry , microbiology, bacteria and fungi, Marine Alga Extract**.**

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Throughout the 2021 season, an experiment was conducted to observe the impact of different mixtures on strawberry growth. The mixtures consisted of equal levels of microorganisms, specifically (20mlL-1 of Rhizobium combined with 20mlL⁻¹ of Arbuscular mycorrhizal, and (20ml.L⁻¹ of Bacillus subtilis combined with 20 ml.L⁻¹ of Arbuscular mycorrhizal).(20 mlL⁻¹ of Rhizobium combined with 20mlL-1 of *Trichoderma harzianum*), (20ml.L-1 of Bacillus subtilis combined with 20mlL-1 of *Trichoderma harzianum*). Additionally, Marine Alga Extracts (MAE) at concentrations of 0.5 and 1 mlL-1 were included, along with a control treatment without any spray. The results revealed that treating the seedlings with 20 mil^{-1} of Bacillus bacteria and 20 mil^{-1} of Arbuscular mycorrhizal fungus resulted in a height of 11.02 cm. Similarly, treating the seedlings with 20 ml. L^{-1} of Rhizobium bacteria and 20 ml.L⁻¹ of tricho fungus showed a significantly higher height of 11.13 cm compared to the control treatment , which only reached 2.20 cm. All combinations of microorganisms demonstrated a significant increase in the diameter of the seedlings. The diameter measurements were 2.73, 2.50, 3.13, and 2.01 mm for the different mixtures, respectively. Furthermore, the treatment with Rhizobium bacteria and tricho fungus exhibited a superior leaf area of 94.333 cm², while the treatment with Bacillus bacteria and Arbuscular mycorrhizal fungus had a leaf area of 88.866 cm² . In contrast, the control treatmenthad a leaf area of 68.666 cm². The average weight of the fruit was significantly higher with the MAE treatment, with an average of 71.37 g. In contrast, the comparison treatment had the lowest average fruit weight of 22.873 g. On the other hand, the Microorganism treatment had a significantly higher average number of fruits, with an average of 10.36 fruits and seedlings. In comparison, the comparison treatment had the lowest average of 6.62 fruits and seedlings.

تأثير خليط من مستخلصات األحياء الدقيقة)بكتيريا وفطريات(والطحالب البحرية في بعض صفات النمو للفراولة(Gem Roby .cv(

احمد يوسف لفته هزاع ، عال حمزة ، على سعيد الجنابي قسم البستنة وهندسة الحدائق – كلية الزراعة - جامعة البصرة - العراق قسم البستنة وهندسة الحدائق – كلية الزراعة - جامعة كربالء – العراق قسم البستنة وهندسة الحدائق – كلية الزراعة - جامعة الكوفة – العراق

الخالصة :

 أجريت التجربة خالل موسم 2021 لمالحظة تأثير الخلطات المختلفة على نمو الفراولة. تتكون المخاليط من مستويات متساوية من الكائنات الحية الدقيقة، وتحديدا Arbuscular of -1 of Rhizobium combined with 20mlL -1 (20mlL mycorrhizal, and 20 ml.L⁻¹ of Bacillus subtilis combined with 20 ml.L⁻¹ of Arbuscular mycorrhizal. 20mlL⁻¹ of Rhizobium combined with 20mlL⁻¹ of *Trichoderma harzianum*, 20ml.L⁻¹ of Bacillus of *Trichoderma harzianum*). -1 mlL20 with combined subtilis. باإلضافة إلى ذلك، تم إضافة مستخلصات الطحالب البحرية (MAE) بتركيزين 0.5 و 1 مل لتر-'، بالإضافة إلى معاملة المقارنة بدون أي رش. أظهرت النتائج أن معاملة الشتلات بـ 1- من بكتيريا Bacillus و 20 مل لتر 1- 20 مل.لتر من فطر المايكورايزا المفصلي أدى إلى ارتفاع 11.02 سم. وبالمثل، أظهرت معاملة الشتلات بـ 20 مل لتر ¹ من بكتيريا الرايزوبيوم و 20 مل لتر ¹ من فطر الترايكو ارتفاعاً أعلّمى بكثير بلغ 11.13 سم مقارنة بالمجموعة غير المعالجة التي وصلت إلى 2.20 سم فقط. أظهرت جميع مجموعات الكائنات الحية الدقيقة زيادة كبيرة في قطر الشتالت. وكانت قياسات القطر ،2.73 ،2.50 ،3.13 و2.01 ملم للمخاليط المختلفة، على التوالي. عالوة على ذلك، أظهرت المعاملة ببكتريا الريزوبيوم وفطر الترايكو تفوقا معنويا لمساحة ورقية بلغت 94.333 سم2، في حين بلغت المساحة الورقية للمعاملة ببكتريا Bacillus والفطري الشجري 88.866 سم². في المقابل بلغت مساحة الورقة في معاملة المقارنة 68.666 سم². أدت إضافة 2 مستخلصات الطحالب البحرية بتركيز 0.5 مل إلى الحصول على مساحة ورقية قدرها 66.4 سم . تفوقت معنويا معاملة MAE في متوسط وزن الثمرة واعطت اعلى متوسط بلغ 71.37 غرام، في المقابل أعطت معاملة المقارنة أقل متوسط لوزن للثمرة بلغ 22.873 غرام. من ناحية أخرى، كانت معاملة الكائنات الحية الدقيقة أعلى بكثير في متوسط عدد الثمار، بمتوسط بلغ 10.36 ثمرة وشتلة مقارنتا بمعاملة المقارنة التي أعطت أقل متوسط بلغ 6.62 ثمرة وشتلة.

الكلمات المفتاحية: الفراولة، األحياء الدقيقة، البكتيريا والفطريات، مستخلص الطحالب البحرية.

INTROUCTION

The domesticated strawberry. *Fragaria ananassa* Duch, is an octaploid species belonging to the Rosaceae family. It holds great commercial significance due to its fruit production. The cultivated species (Hancock *et al*., 1991) is a result of a cross between *Fragaria virginiana* Duch. and *Fragaria chiloensis* (L.) Duch. It has been successfully grown in various habitats, including temperate, Mediterranean, subtropical, and taiga locations. A recent global production of strawberries reached 9.2 million tonnes (United Nations Food and Agricultural Organization (UN-FAO) statistics 2022. Due to the detrimental impact of chemical fertilizers on the ecosystem, the utilization of such fertilizers has been widely criticized. Consequently, scientists are actively seeking more appropriate approaches to managing water and fertilizer. In this regard, researchers have shifted their focus towards modifying the composition of fertilizers and exploring their application methods, as advancements in technology have shown promising outcomes (Mukherjee and Patel 2020).

The increasing importance of food, combined with environmental concerns, as well as the growing demand for energy conservation through eco-friendly "green" technologies and organic products, calls for the development of a new, efficient, environmentally conscious, and safe method to reduce postharvest food losses. In the treatment of postharvest diseases, biological substances derived from beneficial strains, such as plant growthpromoting bacteria (PGPB), could potentially serve as a research-driven alternative to synthetic fungicides and even food preservatives. These products induce various changes in the metabolism of the host plant, resulting in systemic resistance and a longer shelf life, while ensuring the safety of humans, plants, and the environment (Sarma *et al*, 2012; Arroyave-Toroa *et al*, 2017; Baez-Rogelio *et al*, 2017).

PGPB, or non-pathogenic beneficial organisms, have the ability to enhance plant growth, increase resistance to diseases, and improve tolerance to abiotic stress. These organisms can be found naturally in the soil or can colonize various parts of the plant, such as the phyllosphere, rhizosphere, and even the internal tissues as endophytes. Several studies have highlighted the positive effects of PGPB, including those conducted by Lastochkina *et al*. (2017), and Seifikalhor *et al*. (2018).

Bacillus subtilis, a noteworthy plant growth-promoting bacteria (PGPB) from the Bacillus spp. genus, is highly regarded for its potential in producing natural medicinal products, as acknowledged by the US Food and Drug Administration. Bacillus spp. are widely recognized as safe bacteria for application in the food industry. They coexist in the same environment as many pathogens and possess the ability to generate a wide range of bioactive compounds with antibiotic properties. These compounds induce various beneficial changes in the host plant's metabolism, without causing any harm to the environment or human health. (Maksimov *et al*, 2015 and Sarma *et al*, 2012). Moreover, Bacillus spp., including B. subtilis, have the ability to form endospores that exhibit resilience against different chemical and physical treatments, such as heat, desiccation, UV irradiation, and organic solvents. This unique characteristic allows them to consistently stimulate defense mechanisms in host plants, even under unfavorable conditions. (Nagórska *et al*, 2007; Ongena *et al*, 2008 and Gao *et al*, 2016).

Bacillus is known for producing a wide range of biologically active substances, as extensively documented in the literature. These substances include antibiotics, siderophores, lipopeptides (LPs), enzymes, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and exopolysaccharides. Moreover, Bacillus spp. have been observed to have an impact on phytohormone biosynthesis pathways, ethylene levels in plants, and the release of volatile organic compounds (VOCs). They also have the ability to induce systemic resistance or tolerance in plant hosts. (García-Gutiérrez *et al*, 2013, Shafi *et al*, 2017; Pandey *et al*, 2017). The scientific evidence supports the use of biofertilizers, which consist of beneficial microorganisms, as a substitute for synthetic chemicals. This substitution has been shown to improve plant growth by increasing nutrient availability. According to Egamberdieva in 2008, this approach has the potential to make a substantial contribution to the preservation of environmental well-being and soil fertility. Numerous research have been undertaken on T. harzianum's potential to be utilized as a peanut biocontrol agent to reduce the generation of aflatoxin by the fungus Aspergillus flavus (Rodriguez & Redman, 2008),

Horace *et al*., (1986) documented the identification, biological activity, and isolation of 6-pentyl-apyrone, a secondary metabolite produced by T. harzianum. This metabolite has been linked to plant development regulators. According to (Brussaard *et al*., 2007), Trichoderma's secondary metabolites can function as auxin-like substances, exhibiting optimal activity between 105 and 106 M. However, at higher concentrations, these metabolites may have inhibitory effects. Several studies have investigated the impact of competitors on A. mellea infections (AL-hadrawi and Al-janabi.2020; Al-Janabi and Alhasnaw,2021b) across various species. However, there is limited knowledge regarding the interaction between these competitors and the root shape and dynamics.

Arbuscular mycorrhizal fungus (AMF), actinobacteria, and Trichoderma species are examples of competitors in the field (Brussaard *et al*., 2007). The majority of plant species have the ability to form symbiotic relationships with Arbuscular mycorrhizal fungi (AMF), which can provide various benefits to the host plant including increased nutrient uptake, improved drought tolerance, and enhanced disease resistance. Agricultural practices play a significant role in influencing mycorrhizal symbioses in agro-ecosystems. Techniques such as soil tillage, fertilizer application, and plant protection measures can have an impact on these symbiotic relationships. In order to ensure sustainable food production and minimize the spread of harmful organisms, it is essential to consider and integrate all possible agronomic and plant protection measures within an integrated pest management (IPM) approach. Arbuscular mycorrhizal fungi (AMF) form a symbiotic association with plant roots and contribute to nutrient absorption, plant growth, and resilience to stress (Prisa, 2023).

MATERIAL AND METHODS

The research was conducted from September 2021 to April 2022 at the plastic house of the Horticulture and Forestry Division in the Najaf Agriculture Directorate. The variety's seedlings were acquired from the Rice Research Center in Al-Mishkhab, Najaf Governorate. These seedlings were grown from August to September, which marked the beginning of the experiment. The parameters of the soil are shown in Table 1.

Properties	Value
Sand %	41.2
Clay $%$	38.9
Silt %	19.9
Texture	Sandy Clay
$F.C$ %	18.44
pH	7.88
EC (dS.m ⁻¹)	9.78

Table (1): Characteristics of the soil site, in terms of its physical and chemical properties.

Plant growth measurements: The measurement was documented on August 1st, 2021.

Plant Length (cm): The measurement of plant length was conducted using a measuring tape after the study was concluded. **Stem Diameter (mm):** The Vernier was utilized to measure the stem diameter at a height of 5cm above the surface of the earth. **Leaves Number Increase Mean per Transplants:** The calculation of the number of leaves was performed on three randomly selected transplants from each treatment. **Leaf Area (cm²):** The average leaf area of the 6-10th leaves from the apex of transplants under treatment was evaluated using a gravimetric approach, as stated by Drovnic *et al* (1965). The weight and quantity of strawberries were measured biweekly from February to March by counting the fruits, weighing them, and then dividing the total by the number of fruits. **Plant yield (g/plant):** The mean yield per plant was determined for 10 plants in each replicate, as indicated by the formula: Plant yield (g/plant) equals total weight yield divided by 10 plants.

The experiment was designed according to the completely randomized block design (R.C.B.D.), as a factorial experiment, with three sectors for each treatment, 8 seedlings (7 treatments * 8 seedlings), resulting in a total of 56 seedlings per sector and 168 experimental units in total. Additionally, 20 seedlings were placed as guard lines along the sides of the greenhouse. The obtained data was analyzed using the analysis of variance table in GeneStat v.12 software. To determine the statistical differences between the treatments, the least significant difference test (L.S.D.) was employed at a probability level of 0.05, (Al-Sahuki and Wahib in 1990).

RESULTS AND DISSCUSION

The results indicate that the greatest height in strawberry seedlings occurred when treated with 20 ml of Bacillus bacteria and 20 ml of Arbuscular mycorrhizal fungus, reaching 11.02 cm. Additionally, the seedlings treated with 20 ml of Rhizobium bacteria and 20 ml of tricho fungus also exhibited a significantly higher growth of 11.13 cm compared to the untreated control, which only grew by 2.20 cm. The data from Table (2) Showed that the combined effect of adding microorganisms had a greater impact on seedling length, resulting in an increase of 8.99 cm compared to the control treatment. Furthermore, the treatment involving the addition of MAE showed intermediate growth, reaching 6.62 cm.

	Treatments	Plant Length (cm)	Mean of Plant Length (cm)
Control		2.2c	2.20c
	$20ml.L^{-1}$ Rhizobium+	9.41 _b	
	20 ml.L ⁻¹ Arbuscular mycorrhizal		
	20 ml.L ⁻¹ Bacillus subtilis +	11.02a	
	$20ml.L^{-1}$ Arbuscular mycorrhizal		8.99 a
Microorganism	$20ml.L^{-1} Rhizobium +$	11.13a	
	20 ml.L ⁻¹ Trichoderma harzianum		
	20 ml.L ⁻¹ Bacillus subtilis +	4.43c	
	20 ml.L ⁻¹ Trichoderma harzianum		
MAE	0.5 ml.L ⁻¹	6.13 bc	
	1 ml.L^{-1}	7.12 _b	6.62 _b

Table 2: The impact of microorganism and marine alga extracts on the length of strawberry

transplants(cm)

Table (3) indicates that the various combinations of microorganisms significantly enhanced the growth of seedlings, resulting in an increase in Diameter to 2.73, 2.50, 3.13, and 2.01 mm, respectively. Conversely, the application of Marine Alga Extracts at a concentration of 0.5 ml did not show any significant difference, while the control group without any additional treatment exhibited a growth of 1.52 mm. Notably, the combined treatment of microorganisms outperformed all other treatments, with a Diameter increase of 2.59 mm, surpassing both the control group and the average growth observed in seedlings treated with Marine Alga Extracts at a concentration of 55 mm.

Table (4) indicates that the number of leaves increased to 15.7 leaves per transplant when strawberry were treated with Bacillus 20 ml and Arbuscular mycorrhizal fungus. Conversely, treated with Bacillus bacteria and Arbuscular mycorrhizal fungus had 10 leaves each. The treatments involving bacteria, Rhizobium, Bacillus, and other fungi showed moderate significance, with a noticeable range between the highest and lowest values. Table (4) also demonstrates that the addition of combined microorganisms resulted in a lower rate of 10.3 leaves per transplant compared to the treatment with no addition. Additionally, the treatment involving Marine Alga Extracts fell between the highest and lowest rates, with 12.6 leaves per seedling.

Table 3: Effects Microorganism and Marine Alga Extracts, on Diameter (cm) of strawberry transplants

Table 4: Effects Microorganism and Marine Alga Extracts, on Leaves Number of

	Treatments	Leaves Number per transplants	Mean of Leaves Number per transplants
Control		10.3 _d	10.3c
	$20mL^{-1}$ $Rhizobium+$	14.5a	
	$20ml.L^{-1}$ Arbuscular mycorrhizal		
	20 ml.L ⁻¹ Bacillus subtilis +	15.7a	
	20 ml. L^{-1} Arbuscular mycorrhizal		14.3a
Microorganism	20 ml.L ⁻¹ Rhizobium +	13.4 _b	
	20 ml.L ⁻¹ Trichoderma harzianum		
	20 ml.L ⁻¹ Bacillus subtilis +	13.6 _b	
	20 ml.L ⁻¹ Trichoderma harzianum		
MAE	0.5 ml.L ⁻¹	12.4c	12.6 _b
	$1 \text{ ml} . L^{-1}$	12.8c	

strawberry transplants

Table (5) illustrates the enhanced leaf area resulting from the application of Bacillus bacteria in combination with Arbuscular mycorrhizal fungus, with measurements of 88.866 and 94.333 cm², respectively. This is in stark contrast to the smaller leaf area of strawberry transplants without any additives, which measured 68.666 cm2. Additionally, the inclusion of Marine Alga Extracts at a concentration of 0.5 ml resulted in a leaf area of 66.4 cm². The remaining treatments fell within the range between the highest and lowest values. In terms of the impact of experimental factors, the addition of Microorganisms as a group demonstrated a superior mean leaf area of 85.049 cm2 for strawberry seedlings. Conversely, treatments without any additives or with only Arbuscular mycorrhizal showed no significant difference, with leaf areas of 68.666 cm2 and 70.843 cm2, respectively.

	Treatments	Leaves area	Mean of Leaves area
Control		68.666 c	68.666 b
	$20ml.L^{-1}$ Rhizobium+	88.866 a	
	20 ml.L ⁻¹ Arbuscular mycorrhizal		
	20 ml.L ⁻¹ Bacillus subtilis +	94.333 a	
	20 ml.L ⁻¹ Arbuscular mycorrhizal		85.049 a
Microorganism	20 ml.L ⁻¹ Rhizobium +	77.346 b	
	20 ml.L ⁻¹ Trichoderma harzianum		
	20 ml.L ⁻¹ Bacillus subtilis +	79.651 b	
	20 ml. L^{-1} Trichoderma harzianum		
MAE	0.5 ml.L ⁻¹	66.426c	
	1 ml.I $^{-1}$	75.261 h	70.843 b

Table 5: Effects Microorganism and Marine Alga Extracts, on leave area (cm²) of strawberry transplants

Table (6) indicates that the experimental variables had a notable impact on the weight characteristic of the fruits. Specifically, the treatment involving Microorganism at a concentration of 0.5 displayed a significant effect, resulting in fruit weights of 70.300 and 72.440 g, as opposed to the untreated group which had the lowest fruit weight at 22.873 g. The combination of bacteria and fungus treatments fell in between the highest and lowest values. In terms of the average fruit weight across the experimental factors, the treatment involving Marine Alga Extracts stood out with the highest average weight of 71.37 g, showcasing a significant effect. In contrast, the comparison treatment had the lowest average fruit weight at 22.873 g.

The mean quantity of fruits was notably influenced by the variables in the study, resulting in the highest number of fruits when combining the bacteria Rhizobium and Bacillus with Arbuscular mycorrhizal fungus, with 11.12 and 11.25 fruits and seedlings, respectively. The treatments involving microorganisms surpassed both the treatment with no additives and the treatment with Marine Alga Extracts in terms of the average number of fruits, with 10.36 fruits and seedlings (Table 7).

Table (6): Effects Microorganism and Marine Alga Extracts on weight of the fruits of strawberry transplants

Table (7): Effects Microorganism and Marine Alga Extracts on number of the fruits of strawberry transplants

It appears from Table (8) that the yield of strawberries, measured by weight, was significantly superior when adding the treatments for adding bacteria and fungi, as well as adding Marine Alga Extracts, as the average yield of added microorganisms reached 644.005 gm seedlings, while the average yield when adding Marine Alga Extracts was 30.496 gm.

	Treatments	Total yield	Mean of Total yield
Control		151.419 d	151.419 b
	$20ml.L^{-1}$ $Rhizobium+$	495.807 a	
	20 ml.L ⁻¹ Arbuscular mycorrhizal		
	20 ml.L ⁻¹ Bacillus subtilis +	964.248 a	
	20 ml.L ⁻¹ Arbuscular mycorrhizal		
Microorganism	20 ml.L ⁻¹ <i>Rhizobium</i> +	497.697 c	644,055 a
	20 ml.L ⁻¹ Trichoderma harzianum		
	20 ml.L ⁻¹ Bacillus subtilis +	618.471 b	
	20 ml. L^{-1} Trichoderma harzianum		
MAE	0.5 ml.L ⁻¹	601.065 b	
	1 ml. L^{-1}	659.928 b	630.496 a

Table (8): Effects Microorganism and Marine Alga Extracts on yield of strawberry transplants

When compared to other treatments, The outcomes of this investigation clearly demonstrated that the treatments incorporating Marine Alga Extracts showcased exceptional qualities, surpassing those of other treatments. The findings correspond with those of Abd El Moniem and Abd-Allah (2008), Mac and Archer (2010), Turan *et al* (2014) and Zarraonaindia, *et al*., (2023). Their role in facilitating cell division and augmenting the synthesis of organic products leads to the accumulation of glucose and protein in the leaves. Mousavi *et al*., (2023) studied the effects of algal extract application on thirty 12-year-old Anna Apple trees. Seaweeds are recognized for their composition of plant growth regulators, organic osmolites like betaines, amino acids, mineral nutrients, vitamins, and vitamin precursors (Jameson, 1993). For example, seaweed consists of kahydrin, alginic acid, and betaines, which collaborate to enhance the effectiveness of the formulation (Vernieri et al., 2006).

The scientists discovered that 18 weeks after inoculation, the Trichoderma population had the highest soil density and vertical and horizontal mobility. Because of its long persistence in soil, Trichoderma spp. is a viable choice for reducing A. mellea infections (Das, 2014 and Yadav and Solanki,2015). Some strains of Trichoderma harzianum can attach to plant roots, form colonies, and flourish, thereby improving resistance against rhizomorphic A. mellea. Our research suggests that using Trichoderma harzianum has a similar effect on root structure and survival as Arbuscular mycorrhizal biofertilizer. Previous studies have shown that Arbuscular mycorrhizal fungi stimulate the growth of secondary root branches and increase root length in plants. (Subramanian *et al.*, 2015; Mosanna and Khalilvand, 2015 ; Al-janabi and Alhasnawi,2021a). The research showed that the Arbuscular mycorrhizal fasciculatum had a positive impact on the growth of Vitis vinifera roots, specifically on the development of higher order laterals, leading to an increase in length. Furthermore, it has been established that T. harzianum and several Bacillus species possess the capability to dissolve unattainable phosphorus (P) and convert it into accessible forms, thereby enhancing the ability of arbuscular mycorrhizal fungi (AMF) to uptake P. (Syvertsen & Graham, 1999; Rodrigues et al*.* ,2021 and Marschner, 2012). Based on the study and the aforementioned tables, it can be observed that both Trichoderma harzianum fungi and Marine Alga Extracts exerted a significant impact on the majority of the examined characteristics, with each according to its role. Trichoderma harzianum's primary function was to enhance the process of atmospheric nitrogen fixation within the soil, while also facilitating the systemic absorption of essential minerals like phosphate and microelements. Consequently, this led to an overall improvement in the nutritional value of the plant. This enhancement was achieved through the assistance of the organisms present in the soil or through the application of fungal vaccines. (Kareem et al, 2022; Mohammed and Al-Janabi, 2022a,b). One possible reason for this phenomenon could be attributed to the fungus's ability to stimulate the heightened secretion of growth hormones, specifically auxin, thereby augmenting the growth and development of plants. (Al-Qawami *et al*., 2002; Metep and Hasan, 2020, Al-janabi, *etal*.2021). (Gobara, 1998 and Hasan *et al*., 2019).

CONCLUSION

Numerous microorganisms possess the ability to enhance the growth of plants, and there are currently microbial products accessible in the market that facilitate this process. Bacteria originating from the rhizospheres of plants have been discovered to be advantageous for the roots of plants. These bacteria, known as plant growth-promoting rhizobacteria (PGPR), are present in significant quantities on the roots of plants and have been proven to exert positive influences on the direct and indirect aspects of plant development, such as mitigating biotic stress.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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