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Investigating the Interaction of Endophytic Bacteria and Biochemical Compounds in Pearl Millet (*Pennisetum glaucum*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pearl millet (*Pennisetum glaucum*) is an important cereal crop in Asia and Africa. This study focuses on quantifying endophytic bacteria in several parts of the pearl millet plant—roots, stems, and leaves. Total phenolic content, orthodihydroxy phenols, total soluble sugars, protein levels, and enzymatic activity (peroxidase, polyphenol oxidase, and catalase) were all measured and linked with the amount of endophytic bacteria. The leaves have the highest total phenolic content, followed

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by the stem. Orthodihydroxy phenols were also significantly greater in leaves (1.82 mg catechol equi./g) than in stems and roots. Total soluble sugars showed higher levels in leaves (9.97 mg glucose equi./g), in stems (9.21 mg glucose equi./g) and in roots (6.83 mg glucose equi./g). Protein content exhibited significant variation among the plant parts, with leaves (10.63%) containing the highest protein content followed by stems and roots (8.47% and 6.67% respectively). Enzymatic activities, crucial for plant defence and growth, varied across the plant sections. Peroxidase activity was highest in roots (359.98 OD/g), followed by stems and leaves. Polyphenol oxidase activity was lowest in the roots (1.63 OD/g) and highest in the leaves with 2.19 OD/g, while catalase activity was the highest activity in the roots with 17.79 OD/g versus 14.79 OD/g and 14.63 OD/gin the leaves and Roots respectively. The number of Pearl millet endophytic bacteria in the roots is 16, whereas the amount in the stem is 12 and 10 in the leaves. This in-depth analysis sheds light on future research targeted at better understanding plant-microbe interactions and developing ways to improve plant health and productivity.

Keywords: Pearl millet; orthodihydroxy phenols; total soluble sugars; peroxidase; polyphenol oxidase; catalase.

1. INTRODUCTION

Plants can be thought of complex ecosystems in which a wide variety of microorganisms inhabit various niches. Such niches are made up of internal tissues where endophytic bacteria can thrive without harming the host [1]. Plant-associated bacteria serve a crucial role in nutrient cycling and preventing phytopathogens from causing harm. Plant microbe interactions occur mostly in the rhizosphere and within plant tissues. Microbial endophytes are microorganisms that complete their one phase of life cycle within plant tissues without generating visible symptoms [2]. Endophytes can be defined, in a generalist manner, as a group of microorganisms that infect internal tissues of plant without causing any immediate symptom of infection and/ or visible manifestation of disease and live in mutualistic association with plants for at least a part of their life cycle [3]. Pearl millet was developed as a fodder and cereal crop in Africa around 3000 years ago and subsequently introduced into India. Appa and De Wet [4] identified India as the world's second largest hub of pearl millet diversity. Pearl millet has a better level of heat tolerance and is more adept at using soil moisture than maize and sorghum. It is commonly used as a pasture crop in the United States, Australia, Southern Africa, and South America. It is mostly grown in India during the Kharif season, with modest production in Andhra Pradesh, Karnataka, and Tamil Nadu during the Rabi season.

Endophytic bacteria are microorganism that live within plant tissues without inflicting noticeable harm. They've been identified as important contributors to plant health and growth. Endophytic bacteria specifically alter the quantities and activities of phenols, flavanols, total soluble sugars, proteins, peroxidase enzyme, orthodihydroxy phenols, and catalase enzyme. These chemicals, which include phenols and flavanols, have been linked to a variety of plant physiological activities, including growth, development, and defence against biotic and abiotic challenges [5]. Understanding these interactions can provide valuable insights into the potential applications of endophytic bacteria in agriculture, biocontrol, and sustainable plant production systems.

Endophytic bacteria are important for the synthesis and accumulation of phenolic chemicals in plant tissues. Endophytic bacteria can create particular enzymes that increase phenolic compound synthesis in plants [6]. Sugar availability in plants can also influence endophytic bacteria colonisation and activity. Endophytic bacteria use sugars as a carbon source from plants, which can alter their growth, metabolism, and interactions with the plant host [7]. Peroxidases are a class of enzymes involved in various physiological processes in plants, including the metabolism of reactive oxygen species (ROS), lignin synthesis, and defense against pathogens. The peroxidase enzyme activity can vary among different plant parts depending on various factors such as the plant species, developmental stage, and environmental conditions. Rizwan *et al.* [8] investigated the peroxidase activity in different plant parts of *Brassica juncea* (Indian mustard) under heavy metal stress. They found that the roots exhibited significantly higher peroxidase.activity compared to stems and leaves, suggesting a greater role of peroxidases

in root tissues under stress conditions. This study investigates the complex link between endophytic bacteria and biochemical substances found in plant parts such as roots, stems, and leaves. Keeping in view, the importance of endophytic bacteria, the present study was undertaken entitled "Exploring the Relationship Between Endophytic Bacteria and Biochemical Compounds in Pearl Millet".

2. MATERIALS AND METHODS

The present investigation was carried out in the laboratory and field area of Department of Plant Pathology, CCS Haryana Agricultural University, Hisar. Plant samples were collected from pearl millet grown at Plant Pathology experimental area of CCS Haryana Agricultural University, Hisar of the *kharif* 2021. The Root, stem and leaves of pearl millet were collected with latitude 29°14'N and longitude of 75°70'E, having sandy loam soil texture. Roots, leaves and stems were washed with running tap water and then surface sterilized sequentially in 75% (v/v) ethanol for 2 min, 2.6% (w/v) sodium hypochlorite solution for 5 min and 75% (v/v) ethanol for 1 min. For isolation of bacterial endophytes one g of plant tissue was crushed in pestle and mortar with 10 ml sterile distilled water to get homogenous paste and allowed to settle down for 20 min. The supernatant was diluted and approximately 10µl was placed on NA plates and incubated at 28±2°C for 3 days [9]. Bacterial colonies appearing on the plates were considered to be endophytes. Colonies were characterized according to different visual observations and finally purified using streak plate technique. The bacterial colonies were maintained at 4 ±1°C for further studies.

2.1 Prevalence of Endophytic Bacteria in Relation to Biochemical Compounds of Pearl Millet

Endophytic bacteria were isolated from different plant parts and their colony number were counted. One gram sample of roots, leaves and stems were washed with running tap water and then surface sterilized sequentially in 75% (v/v) ethanol for 2 min, 2.6% (w/v) sodium hypochlorite solution for 5 min and 75% (v/v) ethanol for 1 min. Finally, the plant parts were thoroughly washed six times with sterile distilled water. For isolation of bacterial endophytes one g of plant tissue was crushed in pestle and mortar with 10 ml sterile distilled water to get homogenous paste and allowed to settle down for 20 min. The supernatant was diluted and approximately 10µl was placed on Nutrient Agar plates and incubated at 28±2°C for 3 days. Fifteen petri plates from 5 different samples of each roots, leaves and stem respectively were taken. Bacterial colonies appearing on the plates were considered to be endophytes. Similarly, biochemical compounds were recorded from different plant parts of pearl millet and their correlation were measured with the number of endophytic bacteria.

2.1.1 Extraction and estimation of total phenols

Extraction: Total phenols and different phenols were extracted from leaves using 80% hot alcohol. Dry powdered sample (0.1 g) of susceptible and resistant genotype was homogenized with 80% ethanol (total 10 ml) and centrifuged at 5000 rpm for 15 minutes, supernatant was taken and final volume was made upto 10 ml with 80% ethanol.

Estimation of phenols: Total phenolic content was estimated by the method of Swain and Hillis [10] using Folin- Ciocalteau reagent.

Reagents

i. Folin- Ciocalteau reagent: Folin-Ciocalteau reagent was diluted 1:1(v/v) before use
ii. Saturated sodium carbonate solution: Dissolved anhydrous sodium carbonate (35 g) in 500 ml of distilled water by heating on a water bath at 70-80°C and then cooled the contents overnight and used the supernatant.

Method : The alcohol extract (0.5 ml) was taken in test tube and diluted with 3.5 ml of distilled water. To this added 0.5 ml Folin- Ciocalteau reagent and 2 ml saturated sodium carbonate solution and then test tubes were heated in boiling water for 2-3 minutes. Cooled the test tubes and read the absorbance at 650 nm using spectrophotometer. The blank was prepared by taking ethanol in place of extract and rest of procedure was same.

Calculations: The amount of phenol in the sample was determined from the standard curve prepared simultaneously by taking Catechol as the standard phenol and the data was expressed as mg/g dry weight.

2.2.2 Estimation of Orthodihydroxy phenols

The Orthodihydroxy phenols were determined by the method of Johnson and Schaal [11].

Reagent

i. Arrow's reagent was prepared by dissolving sodium nitrite 10 g and sodium molybdate 10 g in distilled water and made volume to 100 ml with distilled water
ii. 0.5 N HCl
iii. 0.5 N NaOH

Method : Two ml of extract was taken in test tube and added 2 ml of 0.5 N HCl, 1 ml of Arrow's reagent and 4 ml of distilled water in succession. After this 2 ml of 0.5 N NaOH was added and the solution was shaken till, pink colour appeared. Blank was prepared without the addition of Arrow's reagent

Calculation: The amount of Orthodihydroxy phenol in the sample was determined from the standard curve prepared simultaneously by taking Catechol as the standard phenol and the data was expressed as mg/g dry weight.

2.2.3 Estimation of flavanol

The Flavanols were determined by the method of Balbaa et al. [12] with slight modification.

Reagent: Aluminium chloride (0.1M): 24.143 g of aluminium chloride was dissolved in one litre of distilled water.

Procedure: One ml of the extract was taken in a test tube and then 5ml of 0.1 M Aluminium chloride solution was added to it and thereafter, the absorbance of the solution was read at 420 nm using spectrophotometer. A blank was prepared simultaneously and calculate the amount of flavanols by taking Catechol as standard and the data was expressed as mg/g dry weight.

2.2.4 Estimation of total soluble sugars

Reagent

1. Concentrated H_2SO_4.
2. Anthrone reagent: 0.2 g in 100 ml conc. H_2SO_4.

Procedure: 0.5 ml of the extract and 1.5 ml of distilled water was taken in a test tube. Then 4 ml of anthrone reagent was added. The tubes were shaken and allowed to cool for 30 minutes and read the absorbance at 625 nm on spectrophotometer. The concentration of total sugars was calculated from the standard curve of glucose prepared simultaneously and the data was expressed as mg glucose equi.g .

2.2.5 Estimation of protein content

Total nitrogen in the samples was estimated by conventional Micro-Kjeldahl's method. Multiplied nitrogen content with suitable factor (3.25) to calculate crude protein content.

Reagent

i. Concentrated sulphuric acid
ii. Mixed indicator :-Bromocresol green (0.1% in 95% alcohol) and Methyl red (0.1% in 95% alcohol)
iii. Boric acid solution (2%)
iv. Sodium hydroxide solution(40%)
v. Digestion mixture: Mix $CuSO_4$ and K_2SO_4 in 1:10 ratio
vi. Standard hydrochloric acid solution (0.01 N)

Procedure

Digestion: 250 mg dried leaves sample was weighed and taken into clean and dry digestion flask. In this 0.5g of digestion mixture and 10.0 ml of concentrated sulphuric acid was added. The flask was heated on a hot plate until the solution became colorless/very light green. Cooled it and after cooling made the final volume up to 100 ml with distilled water.

Distillation and titration

1. Ten milliliter of 4% boric acid was taken in a 100 ml conical flask. Added 2-3 drops of mixed indicator and then the flask was placed under the condenser and end of the condenser was dipped in boric acid solution.
2. Pipetted out 10 ml of digested material into the distilling portion (vacuum jacket) of the set followed by 10 ml 40% NaOH.
3. Steam distilled the mixture by opening the steam inlet and keeping the other outlets closed except the receiving end of the condensor. The liberated ammonia was collected in the boric acid which resulted.in

the colour change of the solution from pink to blue. Distilled for 5-6 min til the volume in the conical flask increased by 2 to 2.5 folds (30-40 ml).

4. Closed the steam inlet, washed down the tip with a few ml of distilled water, removed the conical flask containing the distillate and titrated against standard 0.01N HCl until the colour changed to light pink. Noted the titre value.

Run a blank preparation which was identically prepared except that it does not contain the sample.

Calculation: Crude protein content was calculated by multiplying the nitrogen content with a suitable factor (3.25).

2.3 Enzymatic Study

Preparation of extract: Plant material (roots and leaves separately) were taken and washed with cold distilled water and dabbed dry with several folds of filter paper. Extraction conditions were standardized with respect to molarity and pH of buffer to achieve maximum extraction of enzymes in leaves. All the steps of extraction were carried out at 0-4°C. 200 mg tissue was macerated in chilled pestle and mortar in the presence of 10 ml 0.1 M phosphate buffer (pH 7.5). The homogenate was centrifuged at 10,000 g for 20 minutes at 4°C. The supernatant was carefully decanted and used as the crude enzyme preparation.

2.3.1 Peroxidase

The enzyme activity was estimated by the method of Shannon et al. [13]. The reaction mixture contained 2.7 ml of 50 mM phosphate buffer (pH 6.5), 0.1 ml of 0.5% hydrogen peroxide, and 0.1ml of 0.2% O- dianisidine and 0.01 ml of enzyme extract. The reaction was initiated by the addition of 0.1 ml of H_2O_2. The assay mixture without H_2O_2 served as blank. Change in absorbance was followed at 430 nm for 3 min. One unit of peroxidise was defined as amount of enzyme required to cause change in O.D. of 1 unit per minute.

2.3.2 Catalase

Catalase activity was determined by the procedure of Sinha [14]. The reaction mixture (1 ml) consisted of 0.5 ml of 0.2 M phosphate buffer (pH 7.0), 0.4 ml of 0.2 M hydrogen peroxide and 0.1 ml of properly diluted enzyme extract. After incubating at 37°C for 2 min, the reaction was terminated by adding 3 ml mixture of 5% (w/v) potassium dichromate and glacial acetic acid (1:3 v/v) to the reaction mixture. The tubes were heated in boiling water bath for 10 min. A control was run under similar conditions where enzyme extract was added after stopping the reaction. After cooling the tubes, absorbance of test and control was measured at 570 nm. The amount of residual H_2O_2 in the reaction mixture was determined by subtracting the absorbance of test samples from that of control. One unit of enzyme activity is defined as the amount of enzyme which catalyzed the oxidation of 100 µmole H_2O_2 per minute under assay conditions.

2.3.3 Polyphenol oxidase

The enzyme activity was estimated by the method of Taneja and Sachar [15].

Reagents:

1. Assay buffer : 0.1 M phosphate buffer (pH 6.2)
2. Catechol solution: 0.11g Catechol dissolved in 100 ml phosphate buffer.

Procedure: The reaction mixture consisted of 0.1 ml enzyme extract and 2.9 ml catechol solution. The reaction mixture was incubated at 37°C for 1 h. Run a blank. Simultaneously consisting of catechol solution. The absorbance was measured at 410 nm on a spectrophotometer. The enzyme activity was expressed as change in 0.01 absorbance min g protein.

2.4 Statistical Analysis

All experimental data were statistically analyzed by the method of analysis of variance (ANOVA) as described by Panse and Sukhatme (1967). The significance of treatment effects was tested with the help of “F” (variance ratio) test. Appropriate standard errors along with critical differences (CD at 5%) were worked out for differentiating the treatment effects from those of change effects.

$$CD = \sqrt{\frac{2 \text{ Error Variance}}{n}} \times t \text{ at 5\% probability level}$$

where,

CD = is the critical difference
n = t0.05% = number of observations

3. RESULTS

Bacterial endophytes provide various benefits to the host plant, including growth stimulation, protection from pathogens, and the ability to live in a variety of environmental conditions. Bacterial endophytes are better capable of communicating and interacting with the plant than rhizospheric bacteria. Samples from root, stem and leaves of pearl millet were collected from Plant A total of 38 pearl millet bacterial endophytes (PMEB) were obtained. Out of which, 16 bacterial endophytes were obtained from Root (PMREB 1, PMREB 2, PMREB 3, PMREB 4, PMREB 5, PMREB 6, PMREB 7, PMREB 8, PMREB 9, PMREB 10, PMREB 11, PMREB 12, PMREB 13, PMREB 14, PMREB 15 and PMREB 16), 12 obtained from stem (PMSEB 1, PMSEB 2, PMSEB 3, PMSEB 4, PMSEB 5, PMSEB 6, PMSEB 7, PMSEB 8, PMSEB 9, PMSEB 10, PMSEB 11 and PMSEB 12) and 10 from the leaves (PMLEB 1, PMLEB 2, PMLEB 3, PMLEB 4, PMLEB 5, PMLEB 6, PMLEB 7, PMLEB 8, PMLEB 9 and PMLEB 10) (Table 1).

These endophytic bacteria of pearl millet were streaked on nutrient agar plates retrieved during *kharif* crop 2021. The bacterial colonies were maintained at 4 ±1°C for further studies.

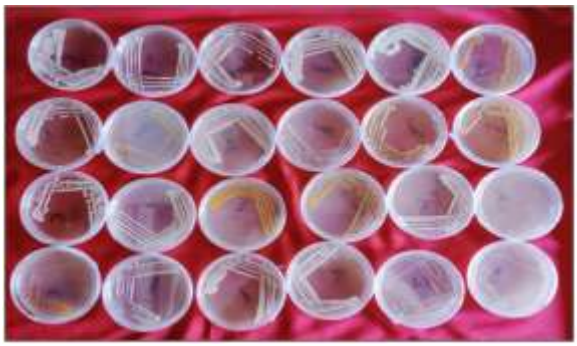

Plate 1. Pearl millet bacterial endophytes (*kharif* 2021)

Prevalence of endophytic bacteria in relation to biochemical compounds of pearl millet: Endophytic bacteria were isolated and enumerated from different plant sections. Samples of roots, stems, and leaves were collected from pearl millet. The plant material was processed and analysed for total phenolic content, Orthodihydroxy phenols, flavanols, total soluble sugars, protein levels Peroxidase, Polyphenol oxidase and Catalase. The analyses were performed using established methods, including spectrophotometry and biochemical assays. Similarly, biochemical substances were extracted from several plant portions of pearl millet and their correlation with the quantity of endophytic bacteria was determined. Statistical analysis was conducted to determine significant differences among the plant parts, using CD (critical difference) values at a significance level of 0.05.

3.1 Total Phenols

The leaves exhibited the highest total phenolic content (8.3 mg catechol equi./g), followed by the stem (6.2 mg catechol equi./g) and the root (3.1 mg catechol equi./g). Minimum number of Pearl millet endophytic bacteria in the leaves is 10 followed by stem (12) and maximum found in roots (16) (Table 2).

3.2 Ortho-dihydroxy Phenol and Flavanol

Orthodihydroxy phenols were also found in varying amounts, with the leaves (1.82 mg catechol equi./g) containing higher levels compared to the stem (1.59 mg catechol equi./g) and root (1.02 mg catechol equi./g) but minimum number of Pearl millet endophytic bacteria in the leaves is 10 followed by stem and maximum found in roots. Regarding flavanols, the stem had the highest concentration (11.9 mg catechol equi./g), followed by the leaves (10.10 mg catechol equi./g) and the root (8.2 mg catechol equi./g) (Table 2).

3.3 Total Soluble Sugars

The analysis of total soluble sugars indicated a similar trend, with the stem (9.21 mg glucose equi./g) and leaves (9.97 mg glucose equi./g) having higher levels compared to the root (6.83 mg glucose equi./g) (Table 2).

3.4 Protein Content

Protein levels were also found to vary significantly among the plant parts. The leaves exhibited the highest protein content (10.63%), followed by the stem (8.47%) and the root (6.65%). but minimum number of Pearl millet endophytic bacteria in the leaves is 10 followed by stem and maximum found in roots.

Table 1. List of endophytic bacterial isolates retrieved from root, stem and leaf of pearl millet

Sr. No.	Source	Endophytic bacteria
1	Pearl millet root endophytic bacteria	PMREB 1, PMREB 2, PMREB 3, PMREB 4, PMREB 5, PMREB 6, PMREB 7, PMREB 8, PMREB 9, PMREB 10, PMREB 11, PMREB 12, PMREB 13, PMREB 14, PMREB 15 and PMREB 16.
2	Pearl millet stem endophytic bacteria	PMSEB 1, PMSEB 2, PMSEB 3, PMSEB 4, PMSEB 5, PMSEB 6, PMSEB 7, PMSEB 8, PMSEB 9, PMSEB 10, PMSEB 11and PMSEB 12.
3.	Pearl millet leaves endophytic bacteria	PMLEB 1, PMLEB 2, PMLEB 3, PMLEB 4, PMLEB 5, PMLEB 6, PMLEB 7, PMLEB 8, PMLEB 9 and PMLEB 10.

Table 2. Estimation of total phenol, orthodihydroxy phenol, flavanol, total soluble sugar and protein (%) from different parts in Pearl millet

Sr. No.	Source	No. of Pearl millet endophytic bacteria	Total phenolic content (mg catechol equi./g)	Orthodihydroxy phenol (mg catechol equi./g)	Flavanol (mg catechol equi./g)	Total soluble sugars (mg glucose equi./g)	Protein (%)
1.	Root	16	3.1	1.02	8.2	6.83	6.65
2.	Stem	12	6.2	1.59	11.9	9.21	8.47
3.	Leaves	10	8.3	1.82	10.10	9.97	10.63
CD (P=0.05)			0.578	0.139	0.955	0.819	1.521
SE(m)			0.164	0.039	0.271	0.232	0.431

Table 3. Estimation of peroxidase, polyphenol oxidase and catalase from different parts in pearl millet

Sr. No.	Source	Peroxidase (Change in OD/g)	Polyphenol oxidase (Change in OD/g)	Catalase (Change in OD/g)
1.	**Root**	359.98	1.63	17.79
2.	**Stem**	349.22	1.87	14.63
3.	**Leaves**	327.05	2.19	14.33
CD (P=0.05)		9.077	0.178	1.463
SE(m)		8.161	0.05	0.415

3.5 Enzymatic Activities

Enzymes play a crucial role in various physiological processes in plants, including defense against pathogens, oxidative stress management, and regulation of plant growth and development. In this study, we investigated the activities of three important enzymes, peroxidase, polyphenol oxidase, and catalase, in different plant parts. The root, stem, and leaves of a specific plant species were analyzed, and the changes in enzyme activity were measured. The results provide valuable insights into the distribution and potential functions of these enzymes in different plant organs. The results of the enzyme activity assays are presented in Table 3. The peroxidase activity was highest in the root (359.98 OD/g), followed by the stem (349.22 OD/g) and leaves (327.05 OD/g). Polyphenol oxidase activity showed highest activity in the leaves (2.19 OD/g) and the lowest in the roots (1.63 OD/g), whereas the lowest activity was in Roots with 1,63 OD/g. Catalase activity was significantly highest in the root (17.79 OD/g), followed by the stem (14.63 OD/g) and leaves (14.33 OD/g). The CD values indicate significant differences among the plant parts for all three enzymes (Table 3); No; there were no significant differences between Stems and leaves in Catalase activity, and no significant differences between Root and Stem in Polyphenol oxidase activity also no significant differences between Root and Stem in the Peroxidase activity.

4. DISCUSSION

Endophytic bacteria were isolated from different plant tissues *viz.* roots, stems and leaves. The plant material was processed and analysed for total phenolic content, Orthodihydroxy phenols, flavanols, total soluble sugars, protein levels, Peroxidase, Polyphenol oxidase and Catalase. Phenolic compounds play various roles in plants, including defense against pathogens, UV radiation, and herbivores. Similarly, the leaves exhibited the highest total phenolic content (8.3 mg catechol equi./g), followed by the stem (6.2 mg catechol equi./g) and the root (3.1 mg catechol equi./g). According to Strobel et al. [16], the leaves have the highest phenol concentration. Endophytic bacteria use sugars as a carbon source from plants, which can alter their growth, metabolism, and interactions with the plant host [7]. The analysis of total soluble sugars indicated that stem (9.21 mg glucose equi./g) and leaves (9.97 mg glucose equi./g) having higher levels compared to the root (6.83 mg glucose equi./g). In comparison to roots, stems, and leaves have higher TSS content. Rather than storing sugar, these plant parts provide different purposes such as nutrient intake, structural support, and photosynthesis. Some plants, however, may store starch or other carbs in their storage roots or stems, resulting in somewhat higher TSS content than ordinary roots or stems [17].

Enzymes play a crucial role in various physiological processes in plants, including defense against pathogens, oxidative stress management, and regulation of plant growth and development. In this study, we investigated the activities of three important enzymes, peroxidase, polyphenol oxidase, and catalase, in different plant parts. The root, stem, and leaves of a specific plant species were analyzed, and the changes in enzyme activity were measured. The results provide valuable insights into the distribution and potential functions of these enzymes in different plant organs. Peroxidase activity was highest in the root (359.98 OD/g), followed by the stem (349.22 OD/g) and leaves (327.05 OD/g). Rizwan et al*.* [8] investigated the peroxidase activity in different plant parts of *Brassica juncea* (Indian mustard) under heavy metal stress. They found that the roots exhibited significantly higher peroxidase activity compared to stems and leaves, suggesting a greater role of peroxidases in root tissues under stress conditions. Rennenberg et al. [18], found that the roots showed the highest peroxidase activity,

followed by stems and leaves. The study suggested that peroxidases in roots might play a crucial role in the defense mechanisms of trees against environmental stresses in different parts of *Quercus ilex* (holm oak) trees.

Catalase activity was highest in the root (17.79 OD/g), followed by the stem (14.63 OD/g) and leaves (14.33 OD/g). Polyphenol oxidase activity showed a similar trend, with the highest activity in the root (1.63 OD/g) and the lowest in the leaves (2.19 OD/g). However, the specific plant part with the maximum PPO enzyme activity can vary depending on the plant species and the physiological state of the plant. Murata and Galis [19], emphasizes that leaves often exhibit higher PPO activity compared to other plant parts. This is because PPO plays a significant role in plant defense mechanisms against herbivory and pathogen attack, and leaves are the primary site of interaction with these threats.

5. CONCLUSION

The study of endophytic bacteria in pearl millet sheds light on their presence within the plant's tissues, emphasizing their role in influencing biochemical compounds and enzymes. These microorganisms, without causing visible symptoms, impact the plant's total phenolic content, soluble sugars, and enzymatic activities. The investigation reveals significant variations in biochemical compounds and enzyme activities among plant parts, indicating the crucial influence of endophytic bacteria. Understanding these complex interactions between endophytes and biochemical substances within pearl millet presents potential insights for agricultural practices, offering prospects to enhance plant health and productivity in sustainable crop production systems.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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