Chapter-5

Discussion

Iron and zinc are the primary element of the plants and they are essential for their development as well as metabolic processes. The most prevalent micronutrient shortage in agricultural plants globally is a deficiency in Fe and Zn, which significantly lowers crop production. The use of inorganic fertilisers to supplement the plant's Zn/Fe requirement might not be economical in overcoming iron and zinc paucity and intensifying crop yield [150]. Further, the consumption of produce insufficient in Zn/Fe may result in micronutrients deficiency in human beings, which leads to diverse health related issues [151]. The current study highlighted the influence of Fe and Zn solubilizing bacterial endophytes that encourage plant development, their individual and consortium application on finger millet micronutrients (Fe and Zn) biofortification in shoot, root, and grains, as well as effect on plant growth parameters. Endophytic microorganisms have the potential to solubilize iron and zinc and are reported to stimulate development and growth of plants, making them a viable alternative to commercial fertilisers [19,107]. Endophytes microorganisms are of agricultural significance since they have been proposed as agricultural inoculants [152]. In this investigation, endophytic bacteria were evaluated for their capability to solubilize insoluble iron/zinc salts in growth medium. Results demonstrated that the solubilization potential of bacterial endophytes varied with different insoluble iron/zinc compounds. The occurrence of a clearing zone surrounding a bacterial colony demonstrated their capacity to solubilize metals. (Fe/Zn) [113], with The SI (solubilization index) for $ZnCO_3$ ranges from 1.9-4.8 mm, for ZnO from 2.1-5.6 mm, for $ZnPO_4$ from 2.1-3.6 mm, and for $FePO_4$ from 2.8 to 4.2 mm, respectively. According to the findings, the selected isolates had varying levels of solubilization with distinct insoluble iron and Zn salts. Several investigations have been conducted in which endophytic bacterial isolates solubilized iron and zinc utilising various Fe/Zn sources, with varying results [22,107,153]. When different microorganisms were studied for Zn solubilization, it is usual for the microbial isolates to differ from one another, the solubilization efficacy data showed that no isolate consistently performed on the three Zn salts (ZnO, $ZnCO_3$, and $Zn(PO)_4$, and similar findings were observed in case of iron solubilization. After zinc carbonate and zinc phosphate, zinc oxide had the most soluble Zn salt. These outcomes coincide with that of [96,98], but not with [154], who demonstrated the maximum zinc phosphate dissolution by bacteria. All the isolates tested, including *Priestia megaterium* NBRC 15308^T, and *Pseudomonas bijieensis* L22-9^T solely and in consortium were able to solubilize iron phosphate with varied solubilization index which is similar with [155] findings. To substantiate that the halo zone production by Zn/Fe solubilizing bacterial endophytes might be due to H^+, protons and organic acid production. In broth, a reduction in pH was observed when the Zn, Fe and phosphate solubilizing broth was inoculated with Zn/Fe solubilizing bacterial isolates. After 48 h, a reduction in pH up to 4.84 was observed as compared to uninoculated flasks, which showed neutral (7.00) pH. This shows that a reduction in pH could be one of the factors in halo zone formation. The organic acids production by endophytes, like gluconic, oxalic, citric acid, etc. may be responsible for the pH decrease [113].

The isolates were also assessed for a variety of PGP features, including phosphate solubilization, synthesis of IAA, HCN, ammonia, siderophore, organic acid, and extracellular enzyme production, in order to select best Zn/Fe solubilizing bioinoculants for further research work. All the isolates exhibited a variety of PGP features that might be advantageous for the finger millet plant's overall growth parameters and Zn/Fe mobilization in plant part and grains. Endophytes including *Pseudomonas putida*, *Bacillus* sp., and *Bacillus subtilis* are well recognised for producing indole acetic acid (IAA) [156]. In fact, according to our research selected bacterial endophytes synthesized IAA (indole acetic acid) phytohormone with concentrations varying from 65.4 - 117.2 µg/ml. EC3B-22 synthesized the maximum level of indole acetic acid (117.2 µg/ml) followed by isolate EC3B-23 with the range of 105.5 µg/ml in broth supplemented with tryptophan, which can be valuable for the plant growth [157] Additionally, our findings are supported by earlier studies that endophytic bacterial species can promote plant development by producing IAA [158,159], and were consistent with a prior investigation in which seven out of ten endophytic isolates produced IAA [160]. Phytohormone-producing endophytes have been suggested to be important for enhancing plant growth [99,161], as well as for a variety of stress management techniques [162,163]. IAA is the most significant phytohormone, and bacteria with IAA-producing capacity can promote plant root growth and root length, which leads in the production of a larger surface area of roots and aids the host plant in absorbing more minerals and nutrients from the soil [164–166].

Another significant characteristic exhibited by bacterial endophytes was the ability to produce ammonia, which the host can exploit as its primary source of nitrogen for growth and productivity [167]. In the current investigation, none of the isolates produced ammonia except EC-3B-23 (*Priestia megaterium*) and EC-2B-21 synthesized ammonia. Synthesis of IAA, hydrogen cyanide and ammonia could be correlated with the root elongation, accumulation of nitrogen, and accumulation of phosphorus, biomass production, and shoot elongation along with biocontrol potential [168]. Finger millet endophytic bacteria EC-3B-22 (*Pseudomonas bijieensis*) produced HCN in maximum amount (0.043), while EC-3B-23 (*Priestia megaterium*) (0.029) and other endophytic isolates exhibited HCN production in significant amount (between 0.003 to 0.041) except EC-2B-21, and EC-3B-1 which produced HCN in minimum amount (0.003, and 0.016 respectively). [169], demonstrated the capacity of endophytes to promote plant development isolated from *Teucrium polium,* they describe their efficiency for IAA, ammonia synthesis and P solubilization. Furthermore, [122] demonstrated the capability of bacterial endophytes of *Cassia tora* L. that were *Pseudomonas putida, Bacillus sp.*, and *Agrobacterium tumefaciens*. They described the salt tolerance of the endophytic isolates and along with this the isolates were also tested for characteristics that promoted plant development. The large number of endophytes synthesized IAA, ammonia, also displayed phosphate solubilization. The synthesis of siderophore and HCN by *A. tumefaciens, Pseudomonas* sp., and *P. putida* has been demonstrated.

Although there are multiple ways in which this bacterial plant growth endorsing attributes can affect plant growth, it is likely the combination of all-plant development stimulating traits exhibited by bacterial endophytes are liable for the augmentation in the progression parameters in finger millet.

Another advantageous effect that bacterial endophytes have on crop development by chelating iron is the siderophores synthesis, which are tiny, highly-affinity metal-chelating compounds that, when released adhere with the insoluble ions of iron and create Fe-siderophore compounds that are absorbed either by microbes or by plant [170]. Additionally, the capability to synthesize siderophores is crucial for providing plants with iron in iron-deficient situations and for preventing phytopathogen attack [171]. Previously [171] demonstrated about the siderophore synthesizing abilities of *P. fluorescens* which further led to the augmentation of dry and fresh biomass. We believe that the siderophore-producing capacities of EC-3B-22 (*Pseudomonas bijieensis* L22-

9^{T}), and EC-3B-23 (*Priestia megaterium* NBRC 15308^{T}) that have contributed in the plant growth parameters and micronutrient (Fe and Zn) enhancement.

Agricultural microbiologists have been intrigued by bacteria's capacity to solubilize mineral phosphates because it can increase the accessibility of phosphorus for microbial and/or crop growth [172,173]. Selected bacterial endophytes were also evaluated for their P solubilizing potential, this was checked by spotting the bacterial isolates on P solubilizing medium. On the basis of the solubilization zone, the isolate EC-2B-21 showed greater mineral phosphate solubilization index (3.6 mm) as compared to other isolates. Additionally, the isolates EC-3B-22 (*Pseudomonas bijieensis*) and EC-3B-23 (*Priestia megaterium*) showed significant solubilization activity (2.4 mm, and 2.5 mm respectively). The solubilization of inorganic phosphorus may result from the pH being lowered by low molecular weight organic acids [174] such gluconate, citrate, along with protons released during bacterial growth in medium. The solubilized phosphate can be easily up taken by the plant roots, resulting in fulfilling plant's phosphate requirement [175]. Other mechanism of P solubilization could be the hydrolysis of phosphoric esters catalysed by a variety of phosphatases, produced by microbes during the mineralization of organic phosphorus. The same bacterial strain has the ability to solubilize and mineralize phosphate. In our study among all selected iron and zinc solubilizers, five endophytic isolates showed organic acid production. Both the selected isolates EC-3B-22, and EC-3B-23 showed the synthesis of organic acids and phosphate solubilization activity, which are significant parameters for encouraging plant growth and they have previously stated in a number of endophytic microbes' studies on varied host plants [172,176].

Endophytic bacteria act as an additional resource for plants, owing to their capacity to generate advantageous secondary metabolic compounds, enzymes, and nutrients which aid the host in overcoming a diversity of abiotic and biotic challenges [177]. Extracellular enzymes are byproducts of microbial cell growth that function in a range of biological or environmental processes outside of the cell. In the present work, endophytic isolates produced extracellular enzymes, not all isolates produced all enzymes but produced some or all of the enzymes. EC-3B-22 (*Pseudomonas bijieensis*) produced gelatinase, protease, and phytase, while EC-3B-23 (*Priestia megaterium*) synthesised amylase, and lipase. Similarly other isolates also produced same enzymes, but none of the isolates produced urease and tannase. [178] investigated the ability of endophyte bacterial isolates *Bacillus, Pseudomonas*, *Curtobacterium*, *Paenibacillus*, *Streptococcus*, *Micrococcus*, and *Chryseobacterium*, sp. from Poaceae plants to produce extracellular hydrolytic enzymes. These isolates produced lipases, proteases, amylases, cellulases, pectinases, and xylanases. Similar to this, [179] showed that bacterial endophytes derived from *V. radiata* and *Cajanus cajan* produce hydrolytic enzymes (amylase, protease, cellulase, and pectinase) all endophytes belonged to *Bacillus* genera. reported endophytic bacteria *Priestia aryabhattai* from *Triticum aestivum* and demonstrated the potential of endophytic bacteria to synthesize extra cellular enzymes like pectinase, protease, cellulase, amylase and lipase.

Endophytic bacteria have evolved to be able to adapt in various types of environmental stresses such as salinity, temperature and pH. Salinity is a serious issue in many contemporary agricultural systems, as it can negatively impact a wide range of commercially significant crops' growth, biological features, and production [180]. In order to overcome these challenges, we searched for an endophytic bacterium that could withstand stress and be employed as an inoculant to accelerate the development of as well as the productivity in finger millet crop under diverse abiotic stress conditions. The stability of every endophytic isolate was evaluated at various pH values, NaCl concentrations, and temperatures. All endophytic isolates were found to grow effectively in the pH range between 5.0 - 9.0, although limited growth was seen even at a pH 10.0. The ideal temperature range was determined to be between 15°C to 35°C, while EC3B-23 could also grow between 40°C to 50°C. NaCl content ranging from 0.5% to 3% allowed all bacterial isolates to grow adequately. Isolate EC2B-21 showed good growth on NaCl concentration 8.0% and restricted growth was recorded on concentration 10.0%. Our findings were in accordance with the investigation done by [181], where stress tolerant wheat endophytic isolate *Priestia aryabhattai* displayed salt resistance and tolerate NaCl concentration up to 12%. [182] conducted an experimental study on the microbial reduction of heat stress in wheat crops. [183] used a different bacterial species *Pseudomonas aeruginosa* in an experiment to study the impact of helpful microorganisms on *T. aestivum* cultivar (HUW-234) under heat stress. [122] also NaCl tolerance of *Pseudomonas* sp. isolated from *Cassia tora*. Zinc and iron are the important plant's constituents and it is also vital for plant development and growth. Deficiency of zinc and iron are one of the common deficiencies of micronutrient in crop, globally resulting in huge losses in crop production. Application of chemical fertilizers containing zinc and iron may not be a good choice in overcoming Zn/Fe deficiency and enhancing crop yield because the chemical fertilizers are costly and moreover may not be environmentally friendly [15]. Further application of zinc and iron fertilizers in fields in India is underutilized, despite the wide occurrence of soils insufficient in zinc and iron. Therefor soils insufficient in zinc does not yield healthy crops, and the produce (grains/seeds) are also insufficient in Zn and Fe content. People consuming crops produce insufficient in essential micronutrients develop a chance to remain deficient in that particular micronutrient, that may result in health-related issues. Moreover, the application of chemical fertilizers to overcome the micronutrient deficiency threatens environment and public health, and the marginal farmers does not afford to procure the costly chemicals putting his livelihood at risk. Consequently, the use of artificial fertilizers has been discouraged in many countries and the farmers are coming back to the organic farming, which is becoming quite popular nowadays as consumer also prefer organic products.

Endophytes plays a significant part in bio-geo chemical cycle process such as metal mineralization and solubilization which are present in insoluble form [99,173]. Plant may absorb the metal in its soluble form, which greatly improves plant nutrition and development. The primary objective of the investigation was, to isolate, distinguish and categorize the finger millet endophytic bacterial isolates having potential to be employed as zinc and iron bioinoculants in the finger millet crop. Two of the most notable bacterial endophytes (*Pseudomonas bijieensis* and *Priestia megaterium*) were chosen based on the efficacy of solubilizing zinc and iron.

Finger millet seed inoculation with endophytes resulted in early germination as compared to the uninoculated hydro primed seeds. The reason for the early germination of seeds could be the good amount of phytohormone production by bacterial endophytes). Our findings were also corroborated by the results of [184], who also reported that biogermination of bacterial endophytes *Pantoea stewartia, Pseudomonas aeruginosa, and Kosakonia cowanii* resulted in early germination of pearl millet.

Inoculation of these potential endophytic bacteria was tested in three finger millet cultivars viz PRM-1, VL-348, and VL- 352 under a pot experiment. After harvesting the crop (90 days after sowing) the metrics for measuring plant growth (root/shoot dry biomass, plant height, and root length), zinc, iron, and NPK content in grains as well as in shoot and root were recorded. It was observed that the bioprimed plants resulted in maximum plant height (66.7 cm) in treatment B1 (EC3B-22; *Pseudomonas bijieensis*) and 68.3 cm by B2 (EC3B-23; *Priestia megaterium*) in variety VL-352, while the maximum root length (22.3 cm) was observed in B2 (EC3B-23; *Priestia megaterium*) in variety VL-348 and 22.1 cm again by B2 in VL-352. The highest plant dry weight 20.9 g and 21.3 g was observed in variety VL-352 by B1 (EC3B-22; *Pseudomonas bijieensis*) and B2 (EC3B-23; *Priestia megaterium*) respectively. While the maximum root dry weight was observed 6.31g and 6.30g by B2 (EC3B-23; *Priestia megaterium*) in variety PRM-1, VL-352 respectively. Our findings are in accordance with [113], who also demonstrated that bacterial endophytes *Rhizobium sp., E. cloacae, Pantoea dispersa, p. agglomerans and Pseudomonas fragi* contributed in enhanced plant development characteristics such as plant/root length and shoot/root dry weight in wheat. Similar to this, enhanced plant-growth parameters and harvest in the wheat was described by [107]. [185] also demonstrated capability of bacterial endophyte *Pseudomonas* sp. and *Klebsiella* sp. in soybean and mungbean growth and yield.

With reference to zinc uptake, the maximum zinc content in grains (2.78 mg) was observed in VL-348 by B2, followed by (2.75 mg) by B1 (EC3B-22; *Pseudomonas bijieensis*) in variety VL-352. Our findings concur with those of [185], where endophytes *Klebsiella* sp. and *Pseudomonas* sp. solubilized inorganic sources of zinc [ZnO and $Zn_3(PO_4)$] and made them available to the plant and proved as promising bacterial endophytes. Our research is consistent with previously published literature for the zinc-solubilizing bacteria *Priestia aryabhattai* by [186] and *Pseudomonas olevorans* by [187], and demonstrated progressive results. Additionally, [107], demonstrated that the absorption of zinc was enhanced by several folds as compared to control by bacterial endophytes *Arthrobacter* sp. and *Bacillus subtilis* and. Furthermore, in another research work by [188], isolates belonged to genus *Serratia*, *Bacillus*, *Pseudomonas* showed 7 to 12% improved zinc accumulation towards grains of wheat in comparison to zinc fertilizer supplementation to the plant, which is in agreement with our research. On the other hand, zinc content in plant part i.e., shoot and root the maximum zinc content in shoot was 2.54 mg by B2 (EC3B-23; *Priestia megaterium*) in variety VL-348 followed by 2.51 mg by B2 in variety PRM-1 and B1 in VL-352. [113] reported similar outcomes, after the inoculation of *E. cloacae* in shoot and by *P. agglomerans* in roots of wheat for Zn uptake. Previous research has shown that applying PGPR or PGPE to rice and wheat grains has increased zinc translocation in grains [118,189].

Numerous endophytic isolates have been developed as biofertilizers since plant inoculation with endophytes improved and enhanced plant growth as well as nutritional content [19,190]. The zinc and iron-solubilizing endophytic isolates utilised in this study not merely enhanced plant-growth parameters but additionally increased the content of zinc in grains, shoot and root as compared to uninoculated plants, which was phenomenally observed and has been reviewed in other research publications. Several researches have showed the efficiency of plant growth enhancing endophytes for zinc solubilization and translocation in crops such as *Triticum aestivum* [191], maize[192], *Oryza sativa* [102], *Cicer arietinum* [193], *Capcicum annum* [194]. Endophytes that promote plant development have this capability due to their ability to modulate plant physiological processes, mineral salt solubilization, and their translocation in the edible part. Studies have demonstrated that plants respond physiologically to chemical fertilisers or biofertilization to help them absorb soil nutrients [195,196].

Endophytic bacteria used in the present investigation significantly increased the iron content of grains, shoots, and roots when compared with un-inoculated plants, in addition to increasing biomass and solubilizing zinc, which is an acknowledged effect that has been discussed in numerous earlier research investigations. [107,155]. In our studies, endophytic bacteria B2 (*Priestia megaterium*) treatment showed maximum grain iron content in variety VL-348 (3.65 mg), followed by PRM-1 (3.63 mg). Endophytic bacteria B1 (*Pseudomonas bijieensis*) enhanced the iron content in grain in variety VL-348 (3.56 mg), variety PRM-1, and VL-352 showed similar iron enhancement i.e., 3.59 mg. Our outcomes are in line with [107], they described the enhancement in iron content in wheat after the inoculation of endophytic bacteria *Enterococcus hirae* and *Arthrobacter sulfonivorans.* In cereals, like rice and wheat, researchers have long focused on increasing the bioavailability of grain's Fe/Zn content [22,23,197]. Our study supports the findings of [198,199], who described the solubilization and mobilisation of Zn, Fe, and other micronutrients in plants. In addition, they stated that microbes might be used to saturate the complex form of these salts in soil, so providing plants with the iron and zinc they require. Crop quality and mineral nutrient absorption are typically negatively impacted by iron, zinc, and NPK deficiency or accessibility concerns in the soil. According to this study, inoculating endophytic bacteria enhanced Zn and Fe absorption as well as NPK concentration when compared to the uninoculated control. According to several findings, when plants are inoculated with endophytes, they accumulate more micro and macronutrients [109,147]. The ability to improve plant development and nutrient absorption is a result of their tendency to colonise finger millet plants and govern plant physiology processes for improved Solubilization and mineralization of salts. [19] Both of the bacterial endophytes used in this plant development experiment were capable of mobilising zinc and iron in addition to producing ammonia, IAA, HCN, a variety of enzymes, and solubilizing phosphate, which could be a step towards acting as antagonists as well as biopriming agents. This research illustrates the capability of these endophytic bacteria (*Priestia megaterium* NBRC 15308^{T}, and *Pseudomonas bijieensis* L22-9^{T}) to be used as potential biopriming agents to alleviate the severe zinc and iron deficiency in regions like India and Africa where Zn/Fe fertilisers are expensive and underutilised to overcome to the Zn/Fe deficiency among consumers.