Chapter -2

Review of literature

Micronutrient deficiencies, particularly Fe and Zn deficiencies, continue to be a major global well-being concern, impacting a significant proportion of the population, especially in developing nation. Insufficient intake of iron and zinc can lead to severe health consequences, including anemia, impaired cognitive function, and compromised immune responses [4]. FM (*Eleusine coracana L.*) is an extensively cultivated cereal crop with significant agricultural importance has been recognized for its high nutritional value and adaptability to diverse agro-climatic conditions [27]. Nonetheless, the bioavailability of iron and zinc in finger millet is restricted because of the presence of antinutritional elements for example phytates, which inhibit the absorption of finger millet in the digestive system of human [28]. Bacterial endophytes, a diverse group of microorganisms residing within the internal tissues of plants, have emerged as promising candidates for improving nutrient availability in crops. These endophytes have been found to possess unique mechanisms to solubilize minerals, chelate metal ions, and produce enzymes that enhance the bioavailability and transport of vital micronutrients from the soil [19].

Multiple studies have demonstrated the successful isolation and characterization of bacterial endophytes from various plant species, including cereals like rice, corn, and wheat [22,23]. One of the key characteristics of endophytic bacteria is their capability to solubilize insoluble forms of iron and zinc in the soil, thereby increasing their bioavailability for plants. Several studies have investigated the iron and zinc solubilization efficiency of bacterial endophytes isolated from diverse plant varieties [29,30]. These studies have employed various screening assays, including the synthesis of organic acids, siderophores, and enzymes involved in mineral solubilization, to identify endophytes with high nutrient solubilization potential [19]. Several findings have examined the effects of selected bacterial endophytes on the development, nutrient content, and harvest of numerous crops [23,31]. In context of FM, researchers have evaluated the impact of specific endophytic isolates on plant-growth traits, for example root/shoot length, plant dry/fresh weight, and nutrient accumulation [19]. These studies have also investigated changes in the concentration of iron and zinc in the plant tissues following endophyte inoculation, aiming to assess the effectiveness of the selected strains in enhancing nutrient content. Understanding the mechanisms underlying the enhanced Zn/Fe bioavailability in finger millet mediated by bacterial endophytes is crucial for developing targeted approaches to improve nutrient uptake [19]. Several researches have explored the possible mechanisms involved, including organic acids synthesis, enzymes, and siderophores by endophytes, which facilitate the solubilization and chelation of iron and zinc, making them more accessible to the plant roots. Additionally, researchers have investigated the effect of endophytes on gene regulation responsible in nutrient uptake [12,32].

2.1 Food for nutritional security: Finger millet (*Eleusine coracana*)

The name "finger millet" denotes to the "finger-like" branching of the panicles, while the name "*Eleusine*" is taken after the Greek name for the goddess of grains. It come from Ethiopia, and is a member of the grain family Poaceae [33], Asia and Africa are the two continents where it is most commonly cultivated. It is a self-pollinating crop species with 20 species and chromosomal number 36 (department of economics and statistics tetraploid) spread across around 10 genera. One of the most extensively cultivated grain species worldwide, the crop was domesticated for the first time around 5000 BC. It may be one of the earliest indigenously grown tropical cereals in Africa. Considering that it contains high amount of protein, lipids, and carbohydrates, higher than rice and comparable to wheat, finger millet is a particularly valuable cereal. Compared to other millet species, it is highly nutritious and climate-adaptable [34]. FM is an excellent crop to utilise as secure nutrition, and an emergency backup since it can be stowed for a longer period without experiencing damage by insects. Owing to its superior nutrient content and storing potential, it serves as an important crop for food safety. Due to its abundance of minerals, micronutrients, and macronutrients, carbohydrates, fiber, and calcium, it fulfils many rural households' nutritional requirements and serves as a resource of income in developing nations as well as for sub-Saharan African nations [35]. This crop may be grown in soils with low-fertility and doesn't require too much of synthetic fertilizers and water, which is good for the large dry areas of the world [36]. *Eleusine coracana*, occasionally referred to as "Ragi," is the most significant cereal crops in tropical and semiarid areas [35]. The crop is more resilient to diseases and pests and have excellent environmental adaptation. Finger millets are a significant yet underused crop in semi-arid and tropical locations across the world. In relation to other crops, it has a short growing season and an excellent production yield. It can also endure significant saline levels, and droughts and resists water logging [36]. As the world's population continues to rise and water supplies are depleting, it represents a significant crop for future human consumption [27].

With its ability to be stored safely and without insect damage for many years, finger millet is a well-cultivated plant in several Indian states and is regarded as a traditional element of farmers' risk-avoidance tactics in drought-prone areas [35]. It requires low input during growth and supplies food and fodder to the population. In India, it ranked at sixth position after main cereals like rice, wheat, corn, bajra (pearl millet), and sorghum [37]. India is the leading millet producer in the world, according to FAO, followed by Nigeria and Niger. In 2021, India produced approximately 11.5 million metric tons of millet, which accounted for about 37% of the total millet production worldwide. Notwithstanding the fact that Karnataka contributes as much as 58 percent of the worldwide supply, only a tiny fraction of Indians is aware of the finger millet's nutritional contents and health benefits [38].

In addition to being important for the unpredictable environment, the crop also provides nutritional and fodder security and is able to address the problem of nutrition insufficiency by continuing to work toward sustainable food security and retaining its socioeconomic significance. In impoverished and developing countries, there are several opportunities to transform finger millet grains into beverages and goods with additional value. Additionally, finger millet is recommended for stomach (abdominal) sufferers because it is gluten-free [35].

The finger millet grains are 1.0 to 1.5 mm in diameter and globular in form.

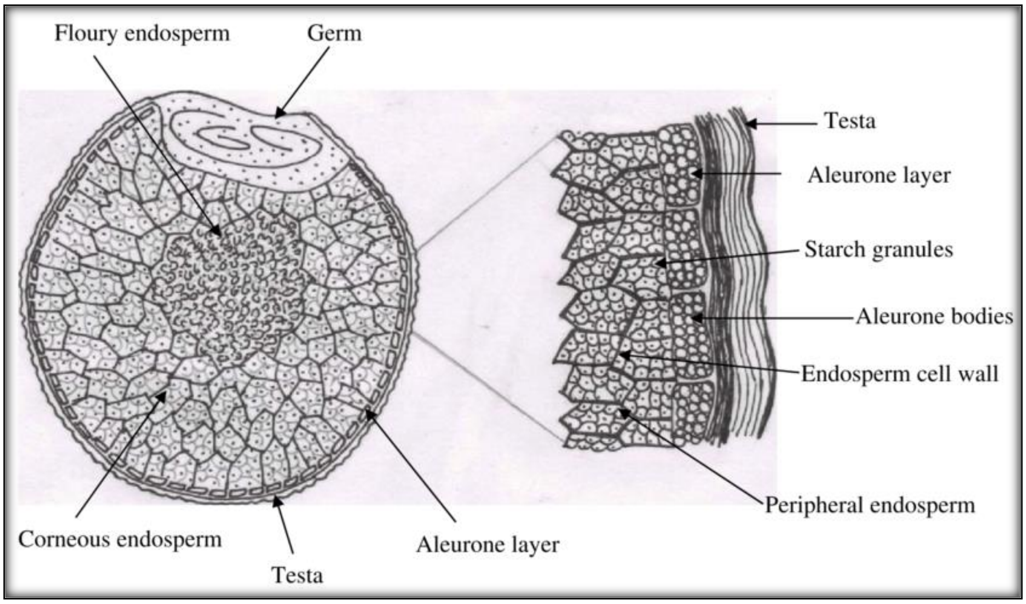

Figure 2.1: Detailed internal morphology of finger millet grain. Source: [39].

The finger millet kernel is consisted of three primary parts: the seed coating, the endosperm, and the embryo. Pericarp, also known as the glume, is the millet's outermost layer and has low nutritional value [40]. Contrasted with other millet species, *Eleusine coracana* (finger millet) stands out due to its multilayered seed coat, or testa (Figure 2.1). The millet is abundant in phytochemicals due to the presence of polyphenols content in seed coat, germ, and endosperm cell walls [41]. The crop's edible part contains various nutrients and it is an excellent source of dietary fibers, polyphenols phytochemicals, and minerals among which calcium contributes the highest. Following points consider the finger millet crop as an important crop; i. tolerant to biotic and abiotic stress; ii. can be grown in Kharif and Rabi season; iii. longer storage capacity; and iv. The grains offer several health-beneficial properties to its consumers, which are making it a “Wonder grain” [42].

Finger millet harbors a wide variety of nutraceutical properties such as its regular intake protects against diabetes type II, cardiovascular complications, gastrointestinal diseases, cancer obesity, and many other disorders [43–45]. Polyphenols, tannins, and phytates in the edible part of finger millet show antioxidant properties that are helpful in metabolic diseases and aging [46]. Phenolic compounds also show antimicrobial activity and the essential amino acids in ragi endorse health [47]. This "wonder-cereal" is vital in eradicating the malnutrition issue due to its high nutritional content. Due to its abundance of vitamins, macronutrients, and nutraceutical benefits, finger millet is currently gaining attention [48].

2.2. Global production of finger millet

Worldwide, around 97% of the millet is yielded and consumed in developing nations, whereas, only little portion coming from the remainder of the globe. According to be an average of data on production of millet across the mainland between 2011 and 2021, Asia produced most of the millets i.e., 13.2 metric ton, followed by Africa, Europe, America, and Oceania with 6.9, 2.3, 0.32, 0.03 metric ton respectively [49](www.fao.org) (Figure 2.2). In Asia, India, Niger, China, and Mali produce the majority of the world's millet. The greatest producer of millet is India, accounting for over 37.5% of the world's output, followed by Nigeria and Sudan [50](Figure 2.3).

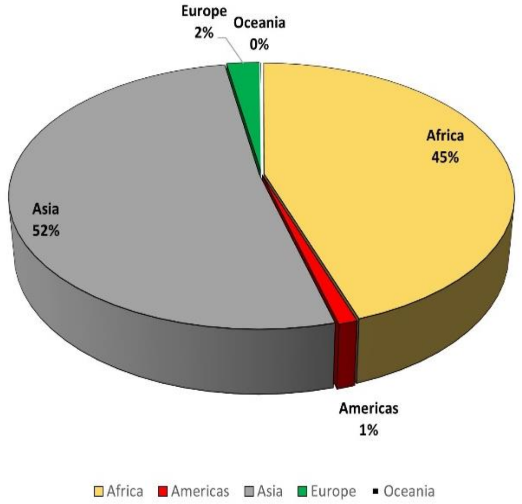

Figure 2.2: Average production percentage of millet by region from year 2011 to 2021

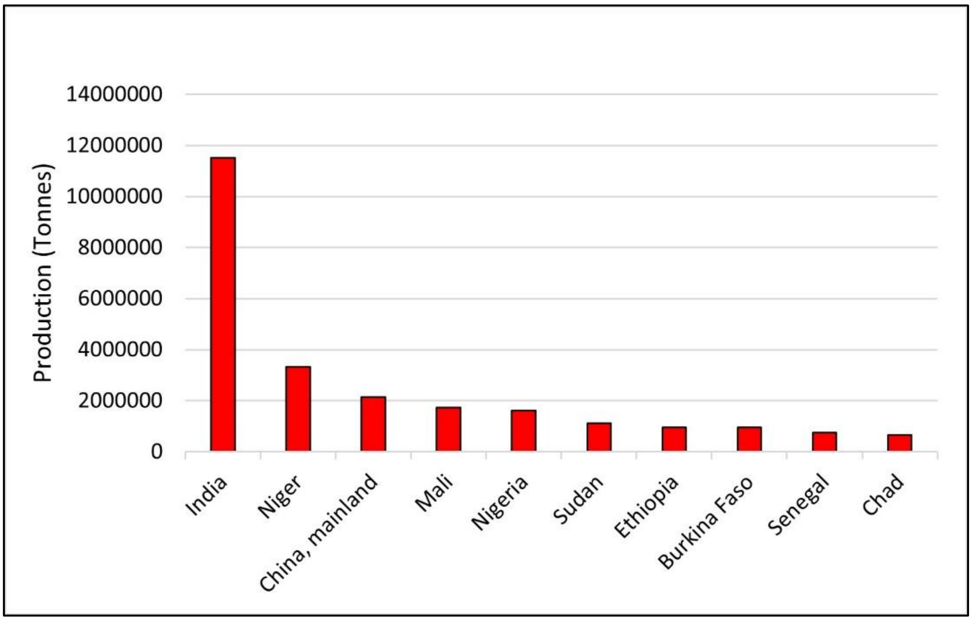

Figure 2.3: Top ten nations in the world for millet production

(Source: https://www.fao.org/faostat/en/#data/QCL/visualize)

Finger millet, is a small-scale tropical millet crop grown in India, Nepal, Taiwan, USA, China, and Japan that is adapted to various agro-climatic conditions and easily cultivated in little rainfall. Worldwide, Africa produces 55-60% of the world's finger millet, primarily in Burundi, Kenya, Tanzania, Malawi, Rwanda, Ethiopia, Zimbabwe, Uganda, and Zambia [40]. In terms of all millets produced globally, finger millet accounts for 11%. The majority of it is cultivated in sub-humid regions of eastern Africa. The most common millet in India is finger millet, which is produced over a 1.17 million hectares total area [51]The primary states that produce finger millet are Andhra Pradesh, Tamil Nadu, Odisha, Maharashtra, and Uttarakhand, accounting for more than 90% of the national production [50]. Figure 2.4 depicts the share of major millets production in India.

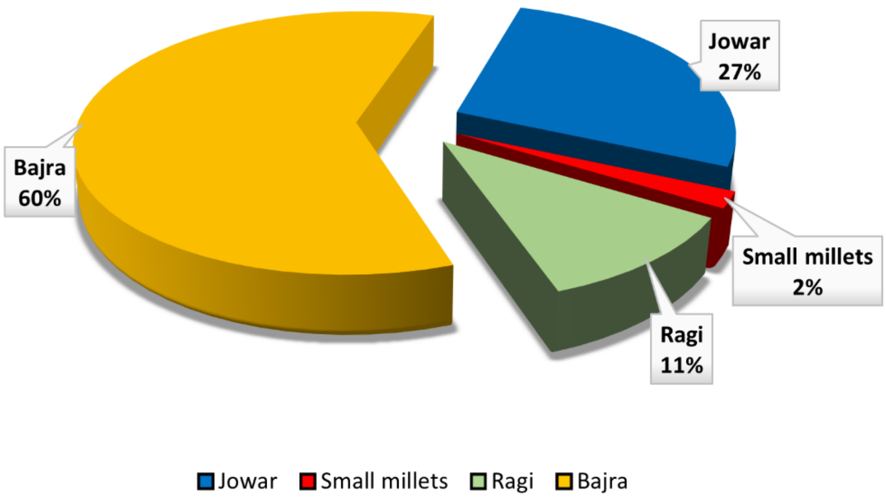

Figure 2.4: Major millet production in India: year 2021-2022

2.2.1. Finger millet production in India

Over the last decade, there has been a certain level of variability in finger millet production in India, including changes in the area that is being cultivated, the overall output, and the yield per hectare. However, finger millet continues to be a significant crop in India, especially in areas with poor soil fertility and scant water supplies [52]. According to milletstats [53], the highest production in the previous ten years occurred in 2010-2011. In India, finger millet was grown on 1,286.19 thousand hectares of land, yielding a total of 2,193.45 thousand tonnes. 1,705.38 kg were produced on average per hectare, and in 2018-19, the least production was carried out. This year, there were only 890,940 hectares of finger millet planted, yielding 1,238.70 thousand tonnes overall. According to the Ministry of Agriculture, the area under finger millet cultivation in India was 1,004.46 thousand hectares in 2019-20, producing a total of 1,755.06 thousand tonnes of finger millet, with an average yield of 1,747.27 kg per hectare (Figure 2.5).

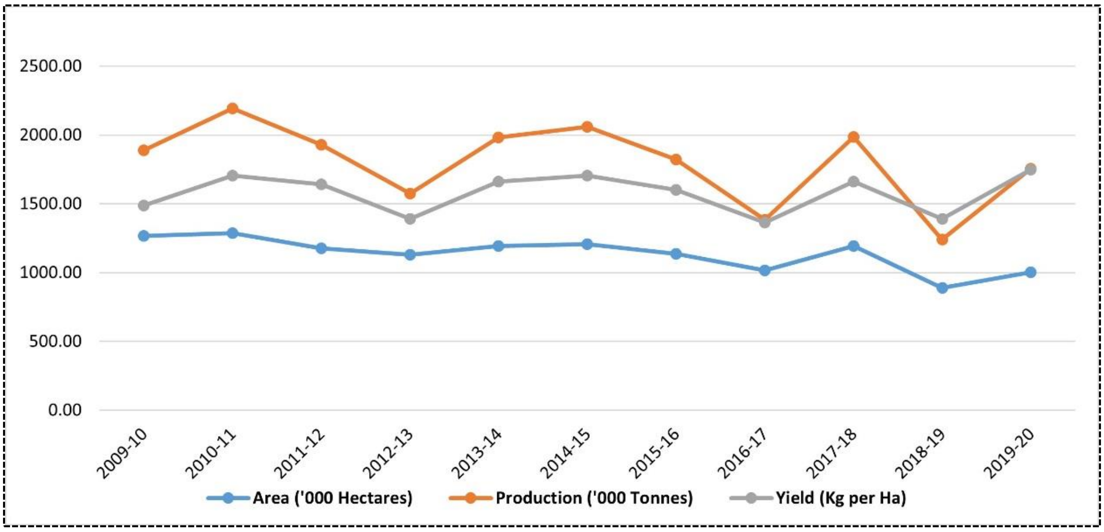

Figure 2.5: Finger millet area, production and yield in India (https://www.milletstats.com/finger-millet-ragi/).

According to www.milletstats.com[53], in 2017-18 growing season, finger millet was produced in quantities totaling 19.85 lakh metric tonnes on an area of 11.94 lakh hectares, and 64.8% of the finger millet produced in India comes from Karnataka. With a production of 1286.034 thousand tonnes, or 64.78% of the total production, Karnataka is the state that produces the most finger millet in India. With an output of 321.29814 thousand tonnes, or 16.18% of the overall production, Tamilnadu is the second-largest producer. The following three highest producers of finger millet are Uttarakhand, Maharashtra, and Andhra Pradesh, each of which contributes 7.09%, 5.36%, and 2.25% of the total production. Finger millet production percentages in Orissa, Jharkhand, West Bengal, and Gujarat range from 0.54% to 1.65%, making these states very small producers of the grain, Figure 2.6.

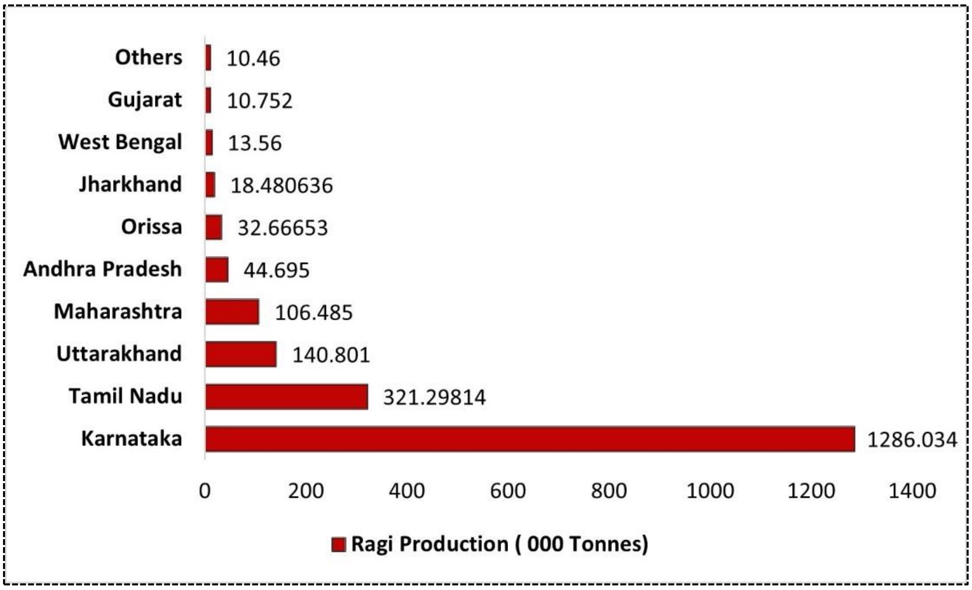

Figure 2.6: Finger Millet (Ragi) production in top ten states on India (2017-2018)

(https://www.milletstats.com/finger-millet-ragi/)

In Uttarakhand, finger millet is grown in nearly all of the districts and contributes significantly to agricultural output. The Garhwal Himalayan Districts of Chamoli, Tehri, Uttarkashi, Dehradun, Pauri, Haridwar, and Rudrapryag cultivate ragi in the 53769 ha^{-1} area, producing 81052 Mt in 2016-17. In the Kumaun region's Almora, Champawat, Bageshwar, Udham Singh Nagar, Nainital, and Pithoragarth districts, ragi is produced in an area of 54406 ha, with a yield of 78554 Mt in the 2016-17 year [54]. According to department of economics and statistics' directorate of agriculture statistics in the year 2017-2018, the finger millet production in Uttarakhand was 103 thousand metric tonnes, which increased to 140.8 thousand metric tonnes in 2018-2019. However, there was a decline in production in 2019-2020, where it was 84 thousand metric tonnes. The production increased again in 2020-2021 with 89 thousand metric tonnes and remained stable in the current year 2021-2022 with 86 thousand metric tonnes. In terms of yield, the maximum yield was documented in the year 2019-2020, with 120.12 quintals per hectare, while the lowest was in 2018-2019 with 109.85 quintals per hectare. The highest yield in terms of production was recorded in 2021-2022 with 1478 thousand metric tonnes [55] (Figure 2.7).

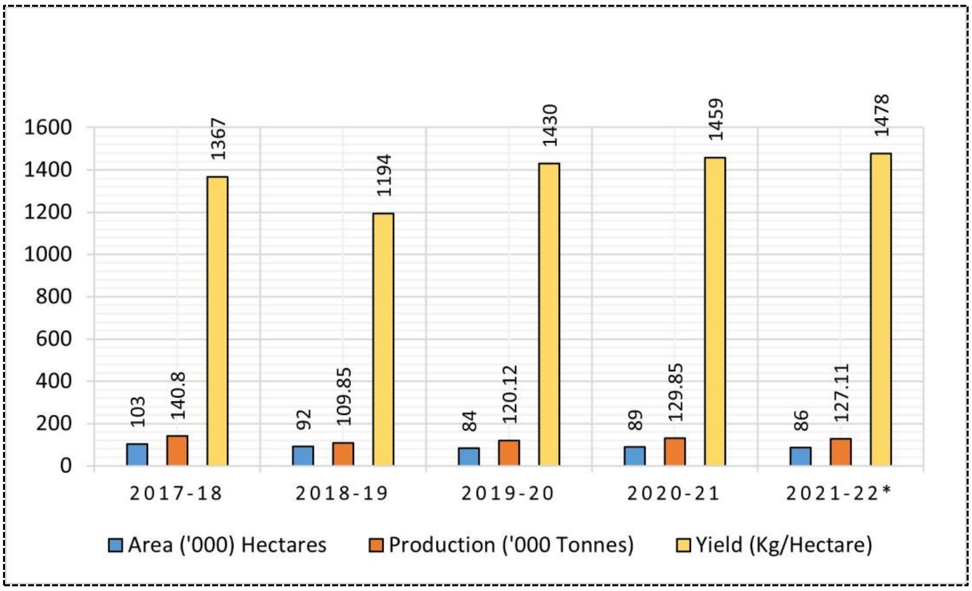

Figure 2.7: Finger millets production in Uttarakhand, area, production, and yield. (*As per 4th Advance estimate (Source: department of economics and statistics' directorate of agriculture statistics.)

2.3. Finger millet grain's nutritional profile

The USA, National Academies consider finger millet to be a promising "super cereal," as amid all main cereals it is the most nutrient-dense. In terms of nutrition, finger millet has a greater micronutrient density than the two most common cereals wheat, and rice, additionally, it is significantly richer in minerals [38]. On average, finger millet has 65-75% carbohydrates, 15-20% fiber (dietary), 5-8% protein, 1-2% ether extract and 2.5-3.5% minerals [56]. Among all grains, it has the highest quantity of calcium. However, it also contains dietary fiber, polyphenols, tannins, inhibitory factors trypsin, and phytates, that were formerly regarded as "anti-nutrients" due to their ability to chelate metals and inhibit enzymes nevertheless they are now referred as nutraceuticals. [57] The Table 2.1 provides a summary of finger millet's nutrient composition.

Table 2.1: Nutrient constituents of finger millet (*Eleusine coracana*)

Proximate composition (per 100g)		Trace elements and minerals (mg/100g)		Vitamins profile		Amino acids (g/100 g protein)	
Protein (%)	7.3	Calcium	344	Thiamine (mg)	0.42	Phe	6.2
Fat (%)	1.3	Phosphorus	283	Riboflavin (mg)	0.19	His	2.36
Starch (%)	59	Iron	3.9	Niacin (mg)	1.1	Ile	5.1
Ash (%)	3	Magnesium	137	Ascorbic acid (mg)	1.0	Leu	13.5
Crude fiber (%)	3.6	Sodium	11	Vitamin E (mg)	22	Lys	3.7
Total Dietary Fiber (%)	19.1	Potassium	408	Retinol (mg)	6.0	Met	2.6
Total Phenol (mg/100g)	102	Copper	0.47	Total Folic acid(µg)	18.3	Thr	5.1
Carbohydrates (g)	72.6	Zinc	2.3			Val	7.9
Moisture (g)	13.1	Manganese	5.94				
Energy (kcal)	336	Molybdenum	0.102				

2.3.1. Carbohydrate

The carbohydrate in the grains of finger millet incorporated free starch, sugars, and dietary fiber or non-starchy polysaccharides. 80-85% amylopectin along with 15% amylose constitute the starch content, which varies between 59.4 to 70.2% of dry matter [58]. [59] estimated that approximately 20–30% of the carbs in finger millet come from non-starchy polysaccharides. Other studies found carbohydrate levels between 65.00 and 83.3% [40]. Various reports state, *Eleusine coracana* contains a total of 0.59 to 0.69 g of sugar per 100 g, with sucrose accounting for the majority of that amount i.e., 0.20 to 0.24g per 100g [60]. Depending upon their water-soluble capability, dietary fiber can be categorized into two groups. Each category provides different therapeutic benefits. The water-soluble category comprises non-starchy polysaccharides, like arabinoxylan and glucan, and water-insoluble fiber comprises of hemicelluloses, cellulose, lignin, and non-starchy polysaccharides arabinoxylan. Non-starchy polysaccharides are the most significant source of soluble and insoluble dietary fibers in millets [24].

2.3.2. Protein content

Protein is the second essential millet constituent, with the majority of finger millets having crude protein contents between 5.6 and 12.70%. [61] demonstrated the highest content of protein (about 11%) in FM, while [62] reported 6.32% protein content. According to many authors, protein levels ranged from 5 to 13% [38,40]. The protein quality is typically determined in most plant crops by the types of needed amino acids and the protein digestibility in finger millet, 44.7% of the total amino acids are necessary amino acids [58]. Table 1 lists the amounts of the essential amino acids. Unlike other plant-based diets, finger millet is an excellent supplier of lysine and methionine, two important amino acids [33]. As finger millet varieties change, so does their protein content.

In finger millet, prolamins make up the majority of the protein. The globulin and albumin fractions contain several essential amino acids required by the body, while the prolamin fraction has relatively higher quantities of certain amino acids such as valine, phenylalanine, isoleucine, glutamic acid and proline and lower amounts of glycine, arginine, and lysine. The content of sulfur-containing amino acids in finger millet is relatively high [62].

2.3.3. Fat content

Finger millet fat content ranges between 1.3% and 1.8%, which is lower than the other minor cereals such as barnyard, foxtail, and pearl millet. The lipid profile of finger millet is made up of 70 to 72% neutral lipids, mostly triglycerides and traces of sterols, and 10-12% glycolipids, and 5-6% phospholipids. Oleic, linoleic, palmitic, and linolenic acids make up 46–62, 8–27, 20–35, and 20–35%, respectively of the lipids. Free lipids make up 2.2% of the total lipid in finger millet, whereas bound lipids make up 2.4% and structural lipids make up 0.6% [58]. With higher proportion of poly unsaturated fatty acids, it was found that finger millet had a total crude fat content of 2.1%. The substantial quantity of unsaturated fats in the grain further increases its capacity to be healthy. The abundance amount of linoleic and nolenic acids, that are necessary for the healthy operation of the CNS (central nervous system), is found in the seeds [34].

2.3.4. Micronutrients

Finger millet ‘Ragi’ has a greater ash content than the other major cereal grains [58]. In 100 g of finger millet, there are 130-283 mg of phosphorus, 430-490 mg of potassium, 78-201 mg of magnesium, 162-398 mg of calcium, 49 mg of sodium, and, 2.3, 3.3-14.39, 17.61-48.43 and 0.47mg of Zn, Fe, Mn, and Cu, respectively. In comparison to staple cereal wheat and rice, "Ragi" is more nutrient dense, and has a remarkable amount of calcium i.e., 344 mg relative to other cereals and millets (eight times more than pearl millet), as well as 283mg, and 3.9mg phosphorus, and Fe respectively, it also includes a number of additional trace minerals and vitamins [40,57].

2.3.5. Phytochemicals

In ‘Ragi’, the phenolic content varies from cultivar to cultivar. Brown variety is described of having more phenolic compounds as compared to the white variant. Several phytochemicals have been reported in ‘Ragi’, and in particular the seed coat, might have beneficial effects on well-being and a good source of numerous phenolic components. In ‘Ragi’, phenolic components have been reported in both unbound and bound form [57]. It also contains Caffeic acid and catechin. Few research has reported regarding the biological availability of phenolics found in ‘Ragi’ [39]. Ragi also contains other phytochemicals, such as flavonoids, sterols, and alkaloids. These phytochemicals are considered good for health, including as antioxidants, anti-inflammatory, and antimicrobial properties [57].

2.4. Finger millets health benefits

There are few research reports regarding the bioavailability of phenolics found in ‘Ragi’. Finger millet is a repository of health benefits due to its abundance in dietary fibres, proteins, phytochemicals, and micro and macro nutrients, and essential for human health [58]. However, it is necessary to extract these components and develop them as functional meals and nutraceuticals while sparingly preserving their inherent functional and nutritional qualities. The following sections discuss more information about the finger millet's nutraceuticals attributes.

2.4.1. Anti-oxidant properties

Antioxidant chemicals are becoming more and more important because of their primary functions as inhibitors of excessive oxidation, and as lipid stabilizers and as, which leads to malignance and ageing. The stable radical intermediates of a number of food ingredients, particularly fatty acids and oils, prevent oxidation from occurring [63]. The millet seed coat contains tannins, flavonoids and phenolic acids, and their derivatives. The body needs these antioxidants to protect itself from oxidative stress, which has been linked to the onset of a number of chronic ailments, such as cancer, diabetes, and cardiovascular disease. Polyphenol content varies between white finger millet and brown finger millet, ranging from 0.04 to 0.09 percent to 0.08 to 3.47 percent respectively. These compounds perform many kinds of functions, including those of chelators, quenchers of singlet oxygen, and reducing agents. The ability of phenolic compounds to provide H (hydrogen) atoms to e^- deficient free radicals through OH (hydroxyl group) groups on benzene rings results in the creation of a resonance-stabilized and less reactive phenoxyl radical, a factor responsible for their effectiveness as antioxidants [64]. With reference to 'Ragi' total phenolic compounds, it was observed that benzoic acid derivatives made up 85% of the mixture, while flavonoids and cinnamic acid derivatives [65]. A trial on rats found that feeding them a feed containing 55% of 'Ragi' improved the activity of anti-oxidant enzymes including glutathione peroxidase, catalase, and glutathione reductase, demonstrating the protective value of this grain. Moreover, the rigidity of elastic tissues like skin, tendons, and blood vessels can also be minimized by finger millet., which helps to delay the ageing process [46].

According to studies, finger millet's antioxidant qualities may help reduce inflammation, enhance blood sugar regulation, and minimize the chance of developing several types of cancer. Furthermore, finger millet has been demonstrated to possess antibacterial qualities, which may aid in preventing specific bacterial illnesses [57].

2.4.2. Antidiabetic Characteristics

Finger millet's carbohydrates are more slowly digested and absorbed than those found in other grains [57]. The jeopardy of type II diabetes and gastrointestinal disorders has been demonstrated to decrease with regular finger millet consumption [43].These effects were linked to the high polyphenol content and dietary fibre of the grain. The beneficial effects of phenolics occur from the partial suppression of glucosidase and amylase during the enzyme-mediated breakdown of dense carbohydrates and a delay in glucose absorption, which in turn controls the levels of postprandial blood sugar [39]. These two characteristics may result in a delay in the absorption of carbs and a decrease in the total amount absorbed. Ragi-based food preparations and formulations possess a lower GI and result in a reduced glycemic reaction [47]. Some anti-nutritional elements present in complete fractions of Ragi, like phytates, phenolics, and tannins may facilitate to reduce the digestion and absorption of starch and hence help to lower glycemic response.

2.4.3. Anti-hyperlipidemic and cardio-protective properties

Cardiovascular illnesses rank among the most serious health issues people face worldwide. There are risk factors associated with the problem, including elevated cholesterol levels, high blood pressure, hypertension, depressive disorders, obesity, and diabetes. A diet high in finger millet lowers the lipid peroxidation reaction, which lowers the risk of arteriosclerosis and offers significant defense against heart attacks and strokes [66].

2.4.4. Combating gastrointestinal diseases

Among the most prevalent autoimmune genetic disorders that affect people their entire lives are celiac disease. The consumption of a class of proteins known as gluten, which is frequently present in cereals including wheat, rye, and barley, specifically causes this enteropathic condition. Consuming gluten-free flour is a necessary component of the treatment, and finger millet, which has a non-glutinous nature and a similar protein structure to wheat, can be used in place of wheat in this situation [38]. The soluble and insoluble dietary fibres found in finger millet are abundant, resistant to digestion, and helpful in lowering the jeopardy of diabetes, colon cancer, cardiovascular disease, and gastrointestinal issues. The polyphenols found in the outer layer of the skin can also aid in reducing ulcers and peptic inflammation [43]. When food products manufactured from finger millet are consumed, gratification levels rise, calorie consumption decreases, and weight reduction is facilitated [44].

2.4.5. Anti-carcinogenic properties

In recent years, it has become very alluring to protect against cancer by moving to healthier eating options. Including anticarcinogenic foods into the diet can lower the occurrence or degree of impulsive or caused cancers [67]. Phytochemicals and antioxidants are two particular nutraceutical ingredients with considerable anti-carcinogenic properties because they function as removers of harmful free radicals and singlet oxygen species. These compounds are found in finger millet, and they may prevent excessive cellular oxidation thus shield against a variety of cancers that are frequent in humans [47]. [68] demonstrated the ‘anti-cancer’ advantages of FM and pearl millet’s phenol compounds using in silico studies and cyto-toxicity testing on HepG2 (hepatic cancer cell lines). The prevention of breast cancer by dietary fibre from whole cereals has been shown in numerous case-control and cohort studies [69].

2.4.6. Anti-microbial properties

Plant phenolics have been linked to reducing the severity of a number of diseases as well as to inhibit the growth of a number of diverse fungus taxa *in vitro*. Tannins in the phenolics of Ragi grain may contribute to the grain's resistance against fungus. Acidic extracts of methanol from the coating of the seed showed more antifungal and antibacterial properties than the whole-wheat extract because the seed coat contains a lot of polyphenols [36].

2.4.7. Osteoporosis prevention

The "silent" condition of osteoporosis results in bone loss and is linked to porous bones. High dietary calcium intakes of naturally occurring calcium are advantageous for preventing osteoporosis. In comparison to other cereals, finger millet contains up to 350 to 400 mg Ca/100 g of seed, making it a worthy source of calcium [38,57,70].Consequently, the products made by finger millet can be used to increase growing children bone mineral density as well as to protect adults and the elderly population from osteoporosis as well as other bone problems [47].

2.5. Micronutrients in soil: Global status

The status of micronutrients in soil varies greatly depending on the region and specific location. However, there are several general factors that can be observed globally. Soil nutrients have a big impact on the crop's health, growth, and development. There Nutrients in soil can be divided into two categories: macronutrients and micronutrients. Although each type of nutrients is necessary for a plant's overall health and development, macronutrients are required in greater amounts. Similarly, micronutrients are essential elements that plants require in min amounts for their development and growth, their deficiency can significantly impact crop yield and quality. They consist of substances like chlorine, boron, molybdenum, zinc, copper, manganese, and iron. They are involved in metabolic pathways that control how plants respond to and perceive stress, which makes them essential for resilience to stress and innate immunity in addition to their role in the development and growth of plants. [71]. Using various analytical methods, the deficiency of micronutrients in soil have been evaluated in diverse area of the world. In general, soil micronutrient accessibility is persuaded by diverse reasons like type of the soil, pH, organic matter, and management practices. Micronutrient deficiencies in soil can be addressed through various management practices, comprising the usage of fertilizers and soil alterations, rotation of crop, and assimilation of organic matter into the soil. However, it is essential to address these deficiencies in a sustainable and environmentally responsible manner to ensure the long-term health and productivity of soils. According to [72], in Indian soil the availability of zinc and iron might be low around 47 and 13%, respectively. Additionally, it is thought that zinc and iron are not readily available in low pH arable soil. The maximum levels of Zn deficiencies were found to be 49% [6].

The majority of Indian soil has adequate levels of micronutrients, although several Indian regions' soils are insufficient due to their inadequate bioavailability for plant absorption. Despite the higher levels of iron in the earth's crust, the alkali soils prevalent in the Indo-Gangetic areas contain low levels of iron that is readily available to plants. More than 12 percent of Indian soils, according to research on soil studies, are iron deficient [6].

2.5.1. Micronutrient deficiency in India

India is going through a transformation in nutrition. In spite of the fact that being overweight or underweight is a prevalent problem, micronutrient deficiency has become its most severe, and the main cause may be India's cereal-based eating habits. In 2016, 0.5% of all deaths in India were attributable to nutritional inadequacies [73]. India has the highest global anemia burden, according to the 'National Family Health Survey-4' (2017) (http://rchiips.org/nfhs/pdf/NFHS4/India.pdf). The Comprehensive National Nutrition Survey of Children (CNNSC 2019) found that, in India conducted in 2019 among children aged 0 to 19 years, zinc insufficiency was found in 19% of preschoolers and 32% of adolescents, while folate deficiency was found in 23% of preschoolers and 37% of adolescents (Comprehensive National Nutrition Survey).

2.5.2. Causes of soil micronutrient (Zn/Fe) deficiencies

Micronutrient deficiency in Indian soil poses a significant challenge to agricultural productivity and crop health. The prevalence of this issue can be attributed to a combination of natural factors and anthropogenic activities [14]. Understanding the scientific reasons behind micronutrient deficiency in Indian soil is crucial for formulating effective remediation strategies. Indian soils exhibit inherent geological variations and weathering processes, which contribute to the natural scarcity of certain micronutrients. Soil composition plays a vital role, as specific regions may lack essential elements such as zinc, iron, manganese, copper, boron, and molybdenum due to the characteristics of their parent materials. Additionally, factors like high rainfall can lead to leaching, causing the depletion of micronutrients from the soil. The bioavailability of micronutrients is influenced by a variety of environmental and edaphic parameters [32,74].Soil micronutrient deficiencies, specifically in Zn and Fe, are prevalent issues with significant implications for plant growth and human nutrition. Several scientific factors contribute to the occurrence of these deficiencies in soils, the causes of soil micronutrient deficiencies, specifically Zn and Fe, are multifaceted and involve geological factors, soil pH and redox conditions, organic matter content, agricultural practices, and microbial activity [75]. Understanding these scientific factors is crucial for implementing effective strategies to address and mitigate micronutrient deficiencies in soils, thereby promoting optimal plant growth and human nutrition.

A significant portion of the Fe in the soil is found as Fe^{3+}, that is inaccessible to plants. Fe^{2+} is the form of iron that is more soluble, but it is easily converted to the ferric form (Fe^{3+}), which precipitates in the soil as hydroxide/oxide, carbonate, phosphate, and other inaccessible complex forms. Fe is abundant in soils, although it has a very low bioavailability. According to [76], Zn bioavailability in soil reduced with rising pH levels as a result of precipitation or Zn adsorption on $CaCO_3$ and Fe oxide surfaces. The cation exchange capacity of soil and the amount of accessible Zn or Fe are inversely correlated. According to [77], the availability of Zn decreased as soil clay content increases. Electrical conductivity was inversely associated with the amount of Zn available [22]. In addition, according to [78], soil phosphorus content and zinc availability are inversely associated.

2.5.3. Iron deficiency in soil: Effects on plants and human health

The fourth most common element on Earth is iron and it may be found in minerals such as silicates, hydroxide compounds, and ferric oxides, that are difficult for plants to access as iron sources. Depending on the nature of soil and depth, the amount of iron (Fe) in a kilogram can range from 20,000 to 550,000 mg/kg, or 0.2% to 55%, with 2–15 cm having the greatest concentration [72,79]. In soil, Fe can exist as divalent (Fe^{2+}) or trivalent (Fe^{3+}) ions. Due to the production of soluble oxides or hydroxides, Fe^{3+} is not readily utilized by plants or microorganisms [80]. According to estimates, nearly one third of the soil on earth is Fe deficient. Fe solubility and availability to plants are affected by a number of variables, including soil aeration (dry, aerobic soil), organic matter concentration, pH level, redox potential of soil, microorganisms, etc. Soil pH is the utmost significant factor affecting Fe availability, this apparently makes Fe less accessible by 95% for each unit of soil pH above neutral pH [81]. Furthermore, given the importance of plant-derived micronutrients for human health, biofortification of crop end products with micronutrients is a crucial component of crop nutrition. This practice could aid in the fight against the mineral deficiencies that affect a large portion of the world's population.

Moreover, iron is among the crucial micronutrients that has a significant impact on all living organisms and plant development, although it is essential in small quantity it has the potential to reverse chlorosis in the plants. It takes involvement in a variety of physiological functions and the synthesis of chlorophyll. Iron-containing protein such as cytochrome, participates in the ETC in chloroplast and mitochondria in the plant. Certain non-iron-containing proteins also contain iron such as ferredoxin (Kroh & Pilon 2020). Fe is crucial for the development of chloroplast structure and functionality, respiration, nitrogen fixation, DNA synthesis, protection from ROS, repair and control of cell cycle, and also participates as a co-factor for many enzymatic reactions in phytohormones production [83]. Iron insufficiency in plants is caused by an unbalanced level of iron in the soil, which causes the yellow coloration of budding leaves and the veins of the leaves remain green. It affects mainly the younger leaves as there is low mobility of iron. The bioavailability of iron is low because of its insoluble form (ferric) present in the soil. The deficiency and excess both are harmful to plant cell. It is a major component of chloroplast structure as it constitutes the porphyrin ring of chlorophyll. Iron deficiency in the process of chlorosis is a major concern in plant development, it affects crop quality and production. Additionally, because it participates in a variety of metabolic activities, it is a necessary micronutrient for practically all living cells [84].

In mammalian cells iron participate with many enzymatic and non-enzymatic proteins (myoglobin and haemoglobin) and helps in transport of oxygen and storage. It also plays a vital role in neuron signalling during myelination Iron is a necessary mineral that required for the production of red blood cells and redox reactions [85]. The deficiency of iron leads to poor health, shortness of breath, tiredness, anaemia, impaired physical activity and learning disorders in children and also in adults. Women and children are more affected by iron deficiency due to malnutrition. Nowadays, iron deficiency anaemia has become a major health issue [86].

2.5.4. Zinc deficiency in soil: Effects on plants and human health

Zinc is an additional essential component for plants' maximum growth and development. Zinc occurs naturally in soil as a trace element, and its concentration can vary depending on factors such as soil type, climate, and geological history [87]. Wetland soils that are calcareous, saline, sandalloid, and rich in organic matter as well as being compacted and having high amounts of nitrogen and phosphate, are soil types that are susceptible to zinc shortage. Zn concentration in unfertilized and uncontaminated soil varies between 10-300 mg/kg (averaging between 50 and 55 mg/kg). Around 80 mg kg^{-1} of total zinc is present in the lithosphere, while between 7 and 1000 ppm of zinc are found in soil. It averages 117.35 mg/kg in agricultural soil, with a range of 4.65 to 427.8 mg/kg. Insufficiency of Zn is recognized as the 5th most significant risk element for diseases in underdeveloped nations. The deficiency of zinc in soil has been stated in several region of the world, such as Europe, Asia North, South and America Africa. The study found that around 50% of soils in Asia, 30% of soils in Africa, 25% of soils in Europe, and 20% of soils in the Americas are deficient in zinc [15].

The degree of zinc inadequacy in soil can be assessed through soil testing. Soil analysis can provide information on the available zinc content in soil and other factors that affect zinc availability. Zn deficiency affects 50% of paddy soils, 35% of which are in Asia. Almost 49% of the major agricultural areas in India have Zn-deficient soils [88].

Zn is a crucial trace element (micronutrient), that is required by plants, humans as well as for animals in small but important amounts. It is necessary for a vast variety of macromolecules, comprising numerous enzymes, to be structurally as well as functionally intact. Over 3,000 proteins, including Zn prosthetic groups, are thought to exist in higher animals including humans. It is the merely metal that contributes to all of the six groups of enzymes, and it is estimated that near about three thousand proteins contain zinc prosthetic groups in higher animals and humans [89]. Furthermore, Zinc ion plays a crucial part in the reproductive health, and neurological and sensory performance. Deficiency of zinc leads to oligospermia, neurological disorders, immune system disfunctioning, and hyperammonaemia. It is necessary for numerous metabolic activities in plants such as activation of enzymes (RNA polymerase, carbonic anhydrase, and dehydrogenase), metabolism of carbohydrates, nucleic acid and lipid, protein synthesis [90]. Zn is also helpful in the process of cell proliferation, differentiation and chloroplast development. The recommended daily intakes for Zn are 3 to 5 mg for infants, 10 mg for kids aged 1 to 10, 15 mg for adults, 12 mg for females, and 16 to 19 mg for nursing mothers [91]. However, in daily practise, these intake constraints are rarely observed. There is a risk of inadequate zinc consumption for more than 25% of India's population. 2.8 million DALYs (disability-adjusted life years) have been lost as a consequence of Zn deficiency-related abnormality, of which 2.7 million were lost to mortality and 1,40,000 to morbidity, 70% of which affect babies [92].

Zinc deficiency affects the plant growth, chloroplast synthesis and tolerance to various stresses also causes physiological stress, resulting in aberrant growth in plants [16]. Impeded growth, tiny leaves, spikelet sterility, and chlorosis of foliage are all obvious signs of severe "acute" Zn deficiency. The quality of plant products is also negatively impacted, and they are more vulnerable to damage from high light intensity, high temperatures, and fungal disease infection [93].

2.6. Biofortification of staple crop to alleviate human malnutrition: microbial approach

As the world prepares to feed the expected 9.6 billion people by the year 2050, new methods for growing food effectively are being investigated, but the struggle against micronutrient deficiency continues to rank among the most pressing global problems. In regions where cereal grains make up a substantial component of the diet, dietary diversification is poor, and/or supplementation/fortification programmes are weak, the prevalence and destructive effects of micronutrient deficiencies are aggravated [8]. Micronutrient deficiency has become a global concern now days which is also the major reason behind hidden hunger. For these micronutrients agriculture is a primary reservoir. The essential micronutrients in soil have a very poor bioavailability, and even when they are present in the soil, plants are unable to absorb them [14].

By adding micronutrients like Fe and Zn to the grains of basic crops that humans consume, the malnutrition issue can be solved. Finding solutions to this problem may be attained by enhancing the availability of soil micronutrients and by enhancing the uptake and retention of these vital nutrients in crop plants' edible parts in bioavailable form [23].

Although many approaches have been applied to combat the issue of micronutrient deficiency, like fortification, genetic modification etc. but these approaches are not as much cost effective. In this regard, microbes could play a crucial role and they are helpful in the solubilization and accumulation of the essential micronutrients to the crop. In the process of biofortification, microorganisms use their machinery in the delivering of the micronutrient. This process is eco-friendly as well as economically friendly [94]. Microbes are the promising agent in biofortification process, they increase the micronutrient uptake via many processes like phytohormones production, production of siderophore, mineral solubilization (zinc, iron etc.), phosphate solubilization, fixation of nitrogen, regulation of ethylene concentration, ACC-deaminase production etc. [26]. By these mechanisms, microbes increase the yield, quality and production of crop.

In a study, solubilization of Fe in host plant is performed by bacterial strain- *Pseudomonas putida, P. fluorescens,* and *Azospirillum lipoferum* [95]*. Pseudomonas* sp., also reported as zinc solubilizers and their inoculation improved the production and trait of the crop [96]. Another study carried out for the augmentation of micronutrient (iron and zinc) in wheat using endophytic bacteria *Arthrobacter* sp., and *Arthrobacter sulfonivorans* shows a higher concentration of Zn and Fe micronutrients [23]. Similar to this, the content of zinc in the host (wheat) plant was increased by *Arthrobacter* sp. and *Bacillus subtilis* [97]. Bacterial endophyte (*Enterobacter* sp. MN17) elevated zinc content and grain quality in chickpea plant [31]. In another work *Pseudomonas* sp. MN12 improves the Zn concentration in the wheat plant, the inoculation also increased the production of organic acid in root exudates, photosynthesis, and grain quality [98].

2.7. Endophytes: emerging component of microbe-mediated biofortification of Fe and Zn

The plant rhizosphere and endosphere harbours a variety of microorganisms and the plant-microbes relationship is being studied from decades for their beneficial effect on plant. In this regard endophytic microbes make considerable involvement in plant development and health. They are the microorganisms, live inside the plant tissue during their whole or once in their life cycle [29]. Endophyte microorganisms are usually non-pathogenic and colonize inside the host plant tissue (root, shoot, seed fruit, flower, etc.). They contribute to plant well-being and overall development by producing phytohormones, enzymes, antagonistic compounds, and nitrogen fixation [19,99]. These endosymbionts were also reported for their involvement in mitigating biotic as well as abiotic stress on plant via direct or indirect mechanisms. Endophytic microbes mainly include a wide variety of fungal and bacterial endophytes, and both of the community promotes plant growth and potentially promotes plant development by multiple mechanisms [81]. Bacterial endophytes reduce the usage of chemically synthesized fertilizers in sustainable agriculture and they potentially reduce the harmful impact of chemically synthesized fertilizer in environment. They convert the free nitrogen and ammonium in the plant to usable form which helps in plant growth and sustainable agriculture. This bacterial strain improves the root exudation and accumulation of nutrient from the soil by generating phytohormones (IAA, gibberellins, and cytokinins) [19,29]. Endophytic bacteria perform varied mechanisms to improve plant health and development such as solubilization of nutrients (phosphorus, micronutrients, etc.), plant hormone production, siderophore synthesis, nitrogen fixation, accumulation and transport of essential nutrients and micronutrients to the plant, (Figure 2.8). Along with plant development these bacterial endophytes also eliminate abiotic stresses by the production of ACCD [100]. Bacterial endophytes facilitate overall plant development and become important tool for sustainable agriculture, along with the growth of the pants, they are also playing important roles in sustaining the environment [101]. These endophytic microorganisms have been discovered to be essential for plants' biofortification with Fe and Zn.

Iron and zinc are crucial micronutrients required for development and growth of plants. However, the soil often lacks these essential nutrients, which can lead to low nutrient uptake by plants. Bacterial endophytes can improve the bioavailability of Fe and Zn to the plants by using various mechanisms. Firstly, bacterial endophytes can solubilize iron and zinc, making them more accessible to plants. It is accomplished through the production of siderophores, which are small molecules that bind to iron and zinc, making them more soluble in water and available for uptake by plants [102,103]. Secondly, bacterial endophytes can increase the accumulation of Zn and Fe by plants. This is accomplished by producing phytohormones such auxins and cytokinins, that encourage the development of root hairs, enhancing the surface circumference of the roots and improving nutrient absorption [104]. Thirdly, bacterial endophytes can induce alterations in the expression of gene participated in iron, and zinc absorption and transport into the plants. This might contribute to increased plant growth and increased nutritional content by increasing the effectiveness of nutrient absorption by plants [105]. Overall, the bacterial endophyte's role in iron and zinc biofortification is essential. By enhancing the bioavailability and uptake of these micronutrients, bacterial endophytes can ameliorate the nutrition quality of crop, thus providing an enduring solution to malnutrition and food insecurity. The significance of endophytic bacteria in the biofortification of Fe and Zn in different crop plants has been thoroughly investigated. [106] described the potential of endophytic strain *Bacillus altitudinis* WR10 in iron solubilization and iron homeostasis in wheat grain. Similarly, in the pot experiment carried by [107], Zn and Fe absorption increased by several folds over the NPK (RDF) after the inoculation of *Bacillus subtilis*, *Arthrobacter sp*. (Zn uptake), and *Arthrobacter sulfonivorans*, *Enterococcus hirae* (Fe uptake). In a study, endophytic bacterial isolates (*Pantoea dispersa* MPJ9 and *Pseudomonas putida* MPJ6) reported to solubilize and transport iron metal to the plant, these isolates were also reported as potential plant growth promoter [108].

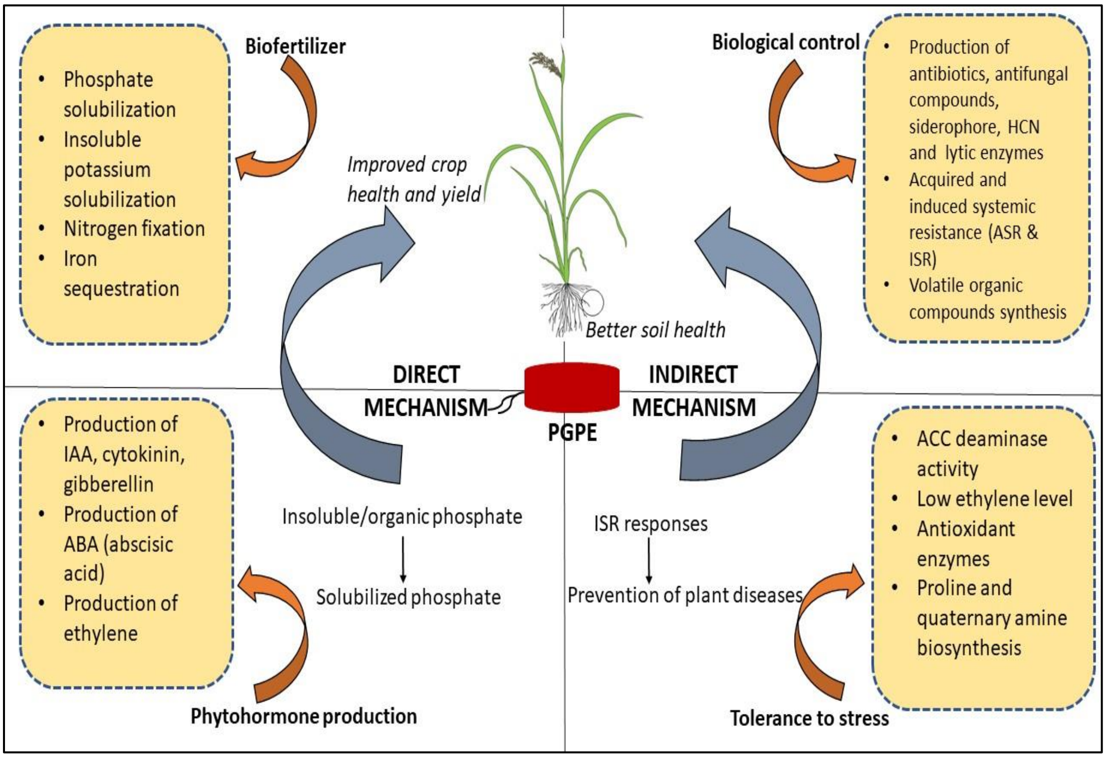

Figure 2.8: Mechanism of plant growth promoting bacterial endophytes for plant growth promotion

In an experiment, [96] found that *Pseudomonas protegens* (RY2, MF351762) is the most promising isolate with zinc-solubilizing capacity in Chickpea plant. Also, [109] demonstrated the effectiveness of two bacterial endophytes, *Microbacterium* *hydrothermale* M10 and *M. proteolyticum* B2, in solubilizing zinc in maize. In another report, bacterial endophyte also solubilizes zinc which is another important micronutrient next to iron. The significant amount of inorganic zinc is converted to usable form by the isolates. Bacterial endophytes are an effective alternative for micronutrient nutrition mobilizer [108].

In summary, these researches provide strong evidence for the beneficial role of bacterial endophyte for zinc and iron biofortification and uptake in crop plants. Endophytic bacteria can modify the regulation of genes involved in iron, and zinc absorption and transport, solubilize them in soil, and improve the efficiency of plants' nutritional intake. These findings possess significant ramifications for enhancing the nutritional value of agricultural products and addressing the worldwide malnutrition problem.

2.7.1. Sequestration and mobilization of iron and zinc for plant uptake via bacterial endophytes

Microbes and plants are known to have coevolved, and the connections between the above-ground and below-ground biota are thought to be the primary factors in sustaining soil health and crop yield. Inoculation of particular microbes has been used in agriculture for decades to increase yields. Researchers are becoming more interested in the "core microbiome" concept in order to create relevant inoculants for nutrient and disease management.

Iron and zinc are important micronutrients for human health, and their deficiency can lead to serious health problems. Staple crops such as wheat, maize, and rice are principal sources of calories, and nutrient ions for millions of people worldwide, but these crops are often deficient in iron and zinc due to the low bioavailability of these micronutrients in soil [110]. Endophytes can contribute significantly in improving the accumulation and transportation of iron and zinc in staple crops. Uptake mechanisms of iron and zinc by endophytes in these crops are similar and involve the production of chelating compounds that solubilize the micronutrients and make them available for plant uptake. One common mechanism used by endophytes to improve the uptake of these two micronutrients in staple crops is the synthesis of phytosiderophores (PS) and organic acids [111]. Phytosiderophores are Fe-chelating complexes that are synthesized and secreted by the roots of some plants in response to iron deficiency. Some endophytes can produce PS and secrete them into the rhizosphere to chelate iron and make it available for plant uptake. Organic acids produced by endophytes can also solubilize iron and zinc and improve their availability for plant uptake. Endophytes can also improve the uptake of iron and zinc by enhancing root growth and modifying architecture. Some endophytes produce phytohormones that endorse root growth and branching, enhancing the absorption of minerals like iron and zinc and expanding the root system's surface area, (Figure 2.9) [112]. Given their ability to subtly support the regulation of metal transporters, endophytes are considered a more powerful factor in improved zinc and iron absorption and transportation [12]. According to [113] bacterial endophytes isolated from wheat and sugarcane effectively enhanced the zinc content along with shoot and root biomass in wheat. [114] demonstrated that siderophore-producing *Xanthomonas melonis, Sphingobium amiense, Caulobacter vibroides, Pseudomonas jessenii*, and *Agrobacterium tumefaciens*, enhanced the iron content in *Sorghum halepense*. [115] demonstrated the role of siderophore-producing *Bacillus cereus, Paenibacillus mucilaginosus*, and *Lactobacillus* sp. in finger millet for iron uptake. Likewise, endophyte was also reported in zinc solubilization and biofortification in cereals like *Triticum aestivum* (wheat), and *Oryza sativa* (rice) [23,116]. Investigations have been conducted into the remarkable ability of such ZSB for the enhancement of Zn content in the eatable portion of the crops [117]. Moreover, Zn-solubilizing endophytes enhance Zn localisation in the edible part of the chickpea [31,96], rice [118], wheat [98], and maize by *Microbacterium* sp. [109] and may be a useful biofortifying agent.

Endophytic microbes are crucial for the metals solubilization in soil and their redistribution inside plant tissues. Endophytic microbes can improve iron and zinc mobilisation in the plant parts, upsurge their availability in the soil, they also boost their bioavailability in grains through a variety of processes such as: production of chelating compounds and siderophore, proton extrusion and organic acid production, improvement in root anatomy and morphology, increased Fe and Zn transporters activity, phytohormone and signaling molecules secretion, Phenolics and associated reducing moieties secretion [32].

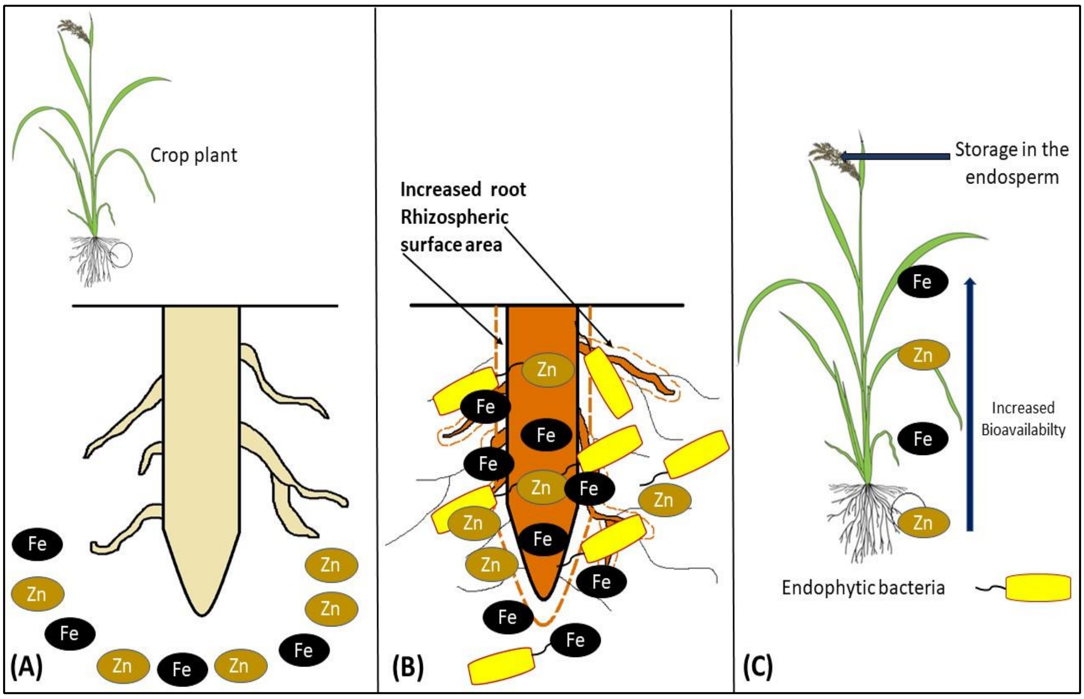

Figure 2.9: Bacterial endophytes aids in enhance the nutrient content in crop. (A). micronutrient (Fe/Zn) bioavailable in the soil but unable to reach to the plant, (B).

bacterial endophytes increased the root rhizospheric surface area, solubilize and translocate the available micronutrient (Fe/Zn) to the plant, (C). endophytic bacteria increased the bioavailability of (Fe/Zn) to the gains.