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INSIGHT OF MICROBIAL BIO FERTILIZERS FOR INTEGRATED NUTRIENT MANAGENTS IN FODDER CROPS – A REVIEW

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SUMMARY

The world population is increasing at a disquieting rate. However, livestock, being significant contributors to the Indian economy, are unexpectedly declining due to fodder scarcity. Therefore, to gratify the escalating demands of food, feed, and fodder, there's a growing inclination towards using agrochemicals comprising inorganic fertilizers and pesticides. Consequently, their rampant utilization has resulted in detrimental effects on soil, ecology, and human and animal health. Moreover, the current scenario of unscrupulous exploitation of natural resources threatens the ability of future generations to access clean air and nutritious food, thus necessitating the judicious use of fertilizers. Integrated nutrient management (INM) offers a multifaceted capacity for ameliorating plant performance while promoting environmental protection. INM involves the efficient, judicious, and integrated use of organic, inorganic, and biological components to blend conventional and contemporary techniques of nutrient management into an environmentally sound and economically optimal agricultural system. Bio-fertilizers are integral components of INM and are available in a myriad of formulations in the commercial market as a solid carrier or liquid-based formulations (plant growth-promoting bacteria or PGPB). The PGPB can augment plant growth by multifarious direct and indirect methods such as siderophore production, biological nitrogen fixation, phosphate solubilisation, production of 1-aminocyclopropane-1- carboxylate (ACC) deaminase, phytohormone production, etc. The present review focuses on various components of INM, the mechanism of action of bio-fertilizers, along with their vital role in sustainable crop production and INM.

Keywords: Bio-fertilizers, fodder crops, INM and PGP

Integrated nutrient management (INM) is a technique that involves the use of a minimum effective dose of sufficient quantities of organic and inorganic fertilizers, combined with specific microorganisms, to make nutrients more available and highly effective, thereby perpetuating the yield and quality of crops (Selim, 2020). The application of this combination leads to better nutrient absorption by crop plants and soil compared to the use of chemical fertilizers alone (Bilam *et al.*, 2022).

The major objectives of INM are as follows –

(1) Maintenance of soil fertility
(2) Balanced use of synthetic (Chemical) fertilizers and organic fertilizers
(3) Optimization of yield
(4) Maximization of profitability
(5) Reduced environmental pollution

Biofertilizers (microbial inoculants) have proven to be one of the most important assets of modern agricultural practices, including INM.

Furthermore, the land plants are inhabited by varied interactive and complex microbial communities, which could be either endophytic, rhizospheric, or epiphytic. The rhizosphere region is termed a hotspot of microbial biodiversity, including bacteria as one of the predominant groups. The rhizospheric bacteria are known as plant growth-promoting rhizobacteria (PGPR) or plant growth-promoting bacteria (PGPB) or as plant beneficial bacteria (PBP). There are myriad PGPR formulations available in the market as microbial inoculants, either as liquid or solid-based carriers (Kaur and Pandove, 2023). However, the liquid formulations with the addition of some cell protectants are more advantageous than the solid carrier-based ones (Ramya *et al.*, 2021). The PGPR are the widely used bacteria in producing biofertilizers because it enhances plant

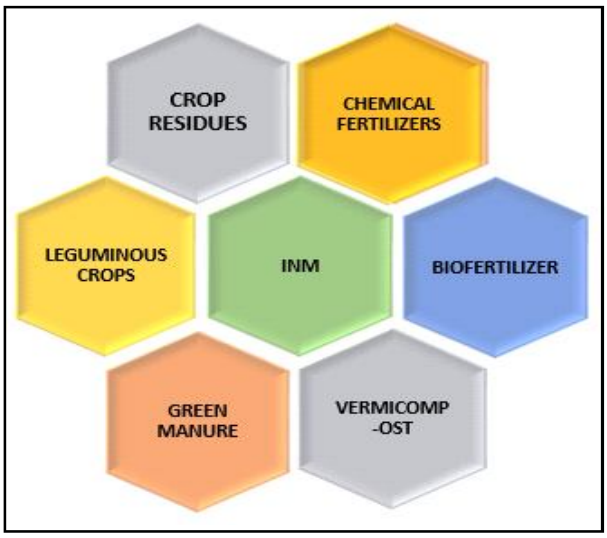

Fig. 1. Components of the INM.

growth (Liu *et al.,* 2020). These PGPR/PGPB act via a variety of direct and indirect mechanisms that enhance plant growth, suppress disease-causing microorganisms, and boost the availability of nutrients, along with increased nutrient uptake and accumulation (Elnahal *et al.,* 2022). The direct mechanisms include biological nitrogen fixation, nutrient (Zn)solubilisation, siderophore production, phosphorus solubilisation, phytohormones production, and ACC deaminase activity (Kenneth *et al.,* 2019). The indirect mechanisms consist of quorum quenching, prevention of attack from pathogens, induced systemic resistance, antibiotics production, expulsion of cell wall-degrading enzymes, hydrogen cyanide (HCN) production, and modulation of plant stress markers such as saline stress, drought stress, and heat stress (Kumar *et al.,* 2020). Apart from the aforementioned points, PGPR can also act as biocontrol agents.

The utilization of biofertilizers has the potential to ameliorate crop yields by about 25% for different types of crops. Additionally, it can lead to a reduction of approximately 25–50% in the usage of inorganic nitrogen and phosphorus fertilizers. This highlights the significant capacity of bio-fertilizers in the promotion of sustainable agricultural practices, especially in the context of changing climatic conditions.

The imminent challenge in the coming decades would be to fulfil the future demands of food, feed, and fodder without harming the environment. Techniques like INM could be seen as a potent and sustainable alternative to the conventional agricultural practices that have already caused enormous harm to our environment. Therefore, in the present review, considering the benefits of biofertilizers in INM, the following aspects would be divulged: -

Direct Mechanism of action of biofertilizers

(1) Biological nitrogen fixation
(2) Phosphorus solubilisation and mobilization
(3) Potassium solubilisation
(4) Zn solubilisation
(5) Sulphur oxidation
(6) Siderophore production
(7) ACC deaminase activity
(8) Plant growth regulators production, such as Auxins and Gibberellic acid

Role of bio-fertilizers in INM and Sustainable fodder crop production

Mechanism of action of biofertilizers

The Plant Growth-Promoting Microorganisms (PGPM) have been extensively researched as biofertilizers that possess the potential to improve the presence of macro and micronutrients, stimulate plant growth, and decrease dependence on synthetic fertilizers (Lopes *et al.,* 2021).

The PGPM acts via a plethora of direct and indirect mechanisms for the growth of plants and soil health.

Biological Nitrogen fixation

Nitrogen is an indispensable and a limiting element for plant growth and development. Despite being abundantly present in the atmosphere (approximately 78%), it is not readily accessible for plant uptake. The term "*Biological Nitrogen Fixation" (BNF)* is a microbial-mediated procedure that transforms atmospheric nitrogen (N_2) into ammonia that can be easily absorbed by the roots. Nitrogen-fixing biofertilizers comprise bacteria that carry out biological N_2 fixation (BNF) and boost soil N supply to crops (Aloo *et al.,* 2022). Fig. 3 represents different types of nitrogen fixers, comprising of free living, associative, and symbiotic nitrogen fixers.

Phosphate Solubilisation

Phosphorus is the second most important element for plant growth and development after nitrogen. Phosphorus facilitates a range of metabolic

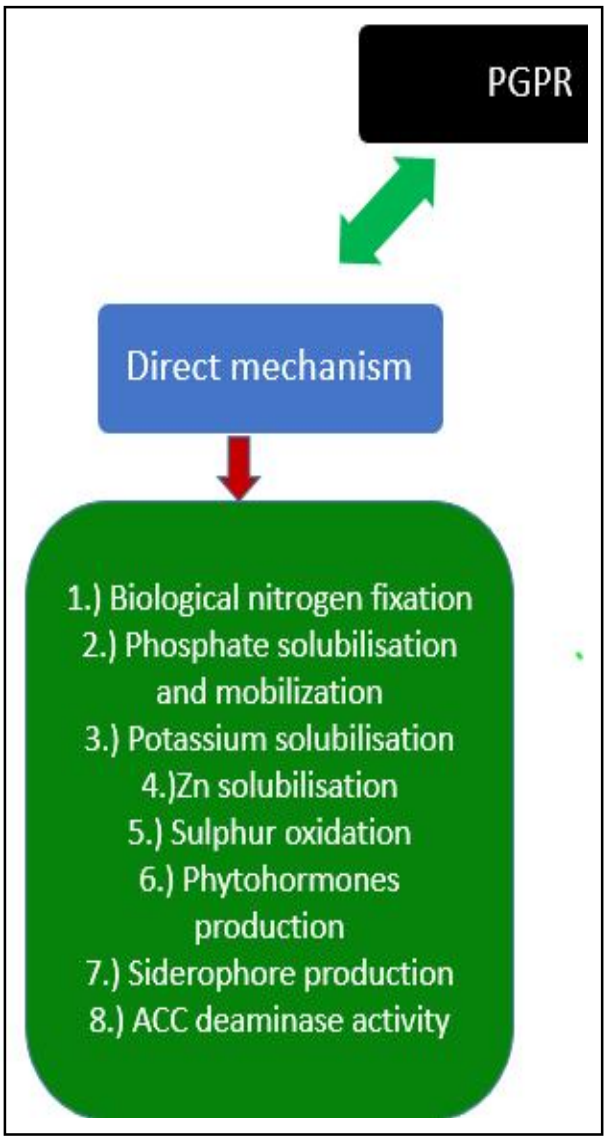

Fig. 2. Direct mode of action of PGPR/PGPB.

activities, including photosynthesis, signal transduction, energy transfer, respiration, and the synthesis of essential molecules. The soil contains a significant amount of phosphorus in both inorganic and organic forms (Raghothama, 2015). However, due to its insoluble nature, phosphorus is not readily available to plants, with only 0.1% existing in a plant usable form. Therefore, chemical phosphate fertilizers are added to crops in recommended doses to ensure continuous and sustainable availability of phosphate.

Moreover, Organic phosphate is the largest pool of soil phosphate; however; their complexity necessitates the transformation of complex compounds with the help of microorganisms to be able to get absorbed by plants. Therefore, mineralization process with the help of production phosphatases and phytases is carried out in the soil.

Prominent examples of Phosphorus-solubilising microbes are- *Corynebacterium, Xanthomonas sp., Nostoc, Rhodococcussp, Bacillus, Pseudomonas, Achromobacter,Brevibacterium, Erwinia sp., Flavobacterium sp, Serratia phosphaticum, Acytonema, Calothrix brauna, Tolypothrix ceylonica, Micrococcus sp., Escherichia freundii, Burkholderia, Sarcina sp. and Scytonema* (Santoyo *et al.,* 2021).

Phosphate mobilization

Phosphorus-mobilizing biofertilizers consist of beneficial bacteria that play a crucial role in efficiently mobilizing soluble phosphorus and facilitating the mineralization of organic phosphorus compounds, which are otherwise typically unavailable to plants. Representative phosphorus-mobilizing microorganisms (PMB) include Bacillus, Pseudomonas, and Rhizobium (Kirui *et al.,* 2022). In addition, endomycorrhizal fungi like Glomus, Gigaspora, and Acaulospora are other prominent phosphate-mobilizing microbes (Fasusi *et al.,* 2021).

Potassium solubilisation

Potassium plays a crucial role in cell constitution and functioning, protein synthesis, photosynthesis, and other metabolic functions. However, despite being abundant, only a minor portion of approximately 1-2% is accessible for plants to absorb (Nosheen *et al.,* 2021). To address the insufficient levels of potassium (K), K fertilizers are commonly utilized. However, there is an increasing need to explore alternative sources of potassium to reduce dependence on expensive K fertilizers.

Potassium-solubilizing microorganisms play a crucial role in enhancing K solubilization *via the* synthesis and discharge of organic acids like tartaric, oxalic, citric, gluconic, lactic, and malic acids, leading to acidification of the surrounding environment, thereby causing the discharge of Potassium from minerals. Other important mechanisms include the generation of siderophores, exchange reaction, and complex formation (Sattar *et al.,* 2019). Prominent K solubilising bacteria are-e.g., *Acidithiobacillus, Bacillus, Pseudomonas, Burkholderia, Rhizobium, Paenibacillus,* and fungi like *Aspergillus, Cladosporium, Macrophomina, Sclerotinia, Trichoderma, Glomus,* and *Penicillium* (Kour *et al.,* 2020).

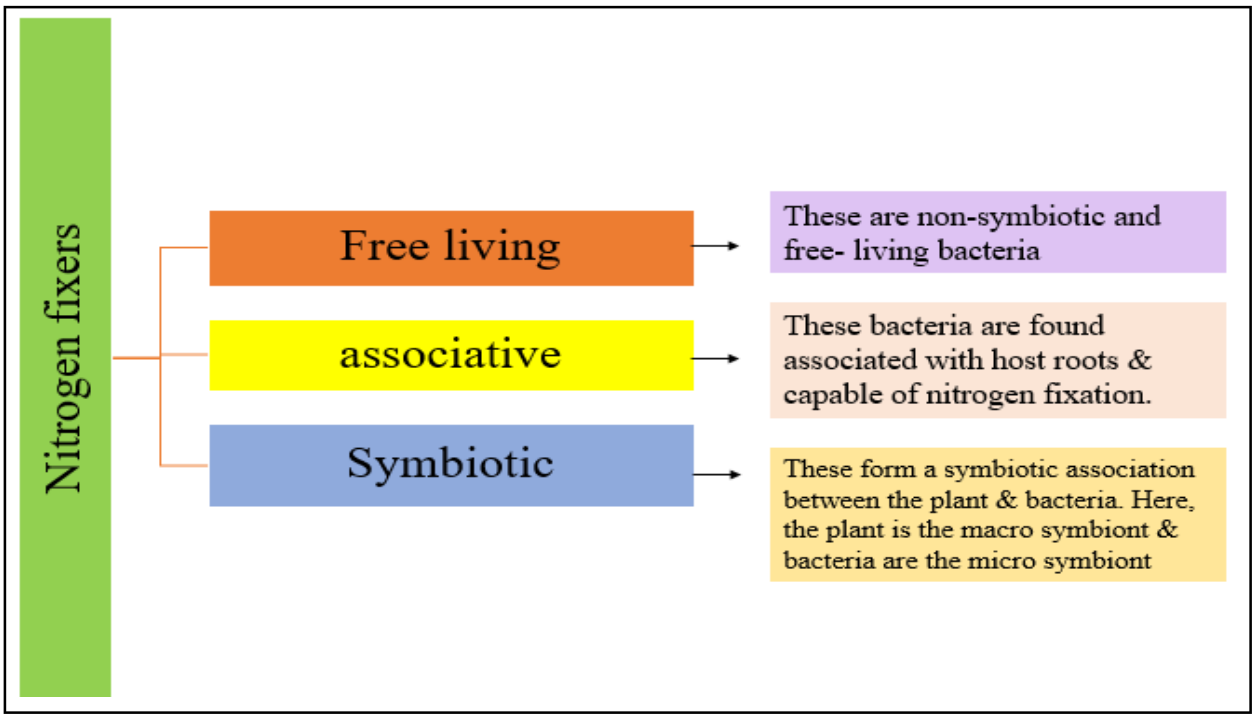

Fig. 3. Types of nitrogen fixers.

Zn Solubilisation

Zinc is an imperative micronutrient among various other micronutrients for both plants and humans. Plants depicting Zn deficiency manifest symptoms such as chlorosis, stunted/retarded shoot growth, low membrane integrity, reduced leaf size, decreased pollen formation, grain yield, and water uptake and root development, along with enhanced susceptibility towards light, heat, and fungal infections (Kamran *et al.*, 2017; Tavallali *et al.*, 2010). The agrochemicals can successfully meet the deficit levels of Zn, nevertheless the repercussions of extensive use of agrochemicals on the environment cannot be overlooked. Therefore, the more sustainable approach is to incorporate Zn-solubilizing microorganisms as inoculants in the crops. These microorganisms provide the major portion of Zn in available forms to plants *via* various mechanisms, such as secretion of organic acids, chelation, alteration in the root architecture, and lowering of pH of the rhizosphere (Sindhu *et al.*, 2019).

PGPR is reported to produce organic acids, *viz.*, acids and 2-ketogluconic acids, release protons, which leads to lowering of pH of rhizosphere that improves Zn solubilization and uptake by plants. Zn chelation by soil or rhizospheric microorganisms is also known to enhance Zn bioavailability and absorption by plant roots. Zn is typically present in lesser quantities, and with the incorporation of Zn chelating compounds, the bioavailability of Zn is augmented (Obrador *et al.*, 2003, Sindhu *et al.*, 2019). Some examples of the prominent Zn solubilising microbes (bacteria) are Rhizobium *spp.*, *Pseudomonas sp.*, *Azospirillum sp.*, *Bacillus thiooxidans* etc.

Sulphur oxidation

Sulphur is a macronutrient required in high quantities by plants as it is involved in vital processes like the generation of chlorophyll, triggering specific enzyme activity, synthesis of vitamins, creation of amino acids, and facilitation of nodulation. The deficiency of sulphur in soils causes stunted plant growth with the yellowing of young leaves. Large-scale deficiency of available Sulphur in soils necessitates the management of this nutrient with chemical or biological means to support optimum growth and yield of plants (Rakesh *et al.*, 2020).

Furthermore, Sulphur exists in two forms, organic and inorganic (SO_4^{2-}), with the inorganic form being readily absorbed by plants (Fasusi *et al.*, 2021). Sulphur solubilizers, also called sulphur-oxidizing bacteria, are capable of transforming the highly insoluble form of sulphur i.e hydrogen sulfide (H_2S), into an accessible form of sulphur known as sulfate. Sulphur transformation in the soil occurs mainly

because of the microbial activity via immobilization, oxidation, mineralization, and reduction (Malik *et al.*, 2021).

(1) Aerobic sulphur-oxidizing bacteria include *Bacillus, Beggiatoa, Aquifer, Sulfolobus, Thiobacillus, Paracoccus, Thermithiobacillus, and Xanthobacter*.
(2) Phototropic anaerobic sulphur -oxidizing bacteria include *Allochromatium, Chlorobium, Rhodobacter, and Rhodopseudomonas*.
(3) Non-phototrophic obligate anaerobes include *Wolinella succinogenes*, and Aerobic Sulphur-oxidizing archaea include *Sulfolobales* members (Kusale *et al.*, 2021).

Siderophore production

Iron is a pivotal element required by all living forms. Microorganisms, except certain lactobacilli, require iron (Nguyen *et al.*, 2020). Iron mainly exists as Fe^{3+} with the tendency to form insoluble hydroxides and oxyhydroxides under aerobic conditions, thus making it largely unavailable to microorganisms. Therefore, bacteria have developed strategies for solubilisation and efficient metal uptake. Siderophilic bacteria is an important type of PGPR that can secrete siderophores to chelate unusable Fe^{3+} in the soil for plant growth (Wang *et al.*, 2022).

Siderophores are specialized iron-binding molecules of low molecular weight (~ 400-1000 Da) that can scavenge iron (DalCorso *et al.*, 2013; Saha *et al.*, 2013). Notably, the siderophores produced by PGPB exhibit an exceptional affinity for binding to Fe3+. Following binding, the resulting soluble iron-siderophore complex is taken up by specific receptors on the surfaces of bacteria or plants. Subsequently, through either reduction to the ferrous state (Fe^{2+}) or cleavage of the siderophore molecule, the bound iron is released from the siderophore (Saha *et al.*, 2013). Plants can directly absorb microbial siderophores in their iron-bound forms. Microbial siderophores in their Fe-bound forms can be directly absorbed by plants. In addition, acidification improves Fe^{3+} mobility; thus, rhizosphere acidification contributed by microbes facilitates plant iron acquisition.

Furthermore, siderophores produced by PGPB exhibit a significantly higher affinity for iron compared to those produced by plants or fungi. This heightened affinity allows PGPB-produced siderophores to capture or sequester even trace amounts of iron. Microbial siderophores fall into three primary categories: carboxylate, hydroxamates, and catecholates (Timofeeva *et al.*, 2022).

Plants utilize siderophores to absorb iron by releasing the chelating agent through interactions with bacteria. Instances of this phenomenon include *Pseudomonas fluorescens C7, Pseudomonas aeruginosa RSP5, and Pseudomonas aeruginosa RSP8*. Additionally, when introduced into maize plants, *Pseudomonas koreensis* restrained the growth of plant pathogens while simultaneously generating antioxidant enzymes and siderophores (Ghazy and El-Nahrawy 2021).

ACC Deaminase activity

Ethylene is a gaseous hormone produced by all higher plants and some bacteria, fungi, and other organisms. Being a gaseous plant hormone, it can be passively diffused through plant tissues quickly and without the aid of specific transporters. Ethylene is produced in varying concentrations depending on the environmental conditions, with the ability to trigger the germination of seeds, the lengthening of plant roots, the development of leaf and root primordia in stems and roots, and the start of flowering at low concentrations (Orozco-Mosqueda *et al.*, 2020).

However, stress conditions that occur frequently and for extended periods of time can cause plants to produce ethylene levels higher than their thresholds, which reduces plant growth and crop productivity. Various plant species are increasingly using plant growth-promoting bacteria (PGPB), including Plant growth promoting rhizobacteria (PGPR). The PGPR possesses 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can equilibrate the ethylene levels to an optimum level by cleaving the plant-derived ACC to ammonia and á-ketobutyrate in plants under drought stress (Glick 2014, Chandra *et al.*, 2020). The reduced ethylene levels allow the plant to be more tolerant to a variety of abiotic stresses, including drought stress (Murali *et al.*, 2021).

Plant growth regulators

Phytohormones, which are not naturally synthesized by the plants but are produced exogenously via natural and synthetic means, are known as plant growth regulators (Vejan *et al.*, 2016). These plant growth regulators play a substantial role in plant metabolism and are secreted by bacteria and plants in minute quantities, affecting cell division, development,

gene expression, stress response, and root and shoot growth, shape, flowering, senescence, and seed growth.

Additionally, phytohormones are essential for controlling nearly every aspect of plant biology, including signalling networks that help plants respond to a variety of biotic and abiotic stresses and developmental processes. Since microorganisms synthesize these plant growth-promoting regulators extrinsically, they are also termed as exogenous hormones (Vejan *et al.,* 2016).

Auxins: Auxin is a crucial signalling molecule synthesised in organisms like bacteria, land plants, and green algae. Indole-3-acetic acid (IAA) is one of the chief auxins found in nature and is a substance that 'influences almost anything' (Paque and Weijers 2016). Moreover, it controls specific phenotypic effects in plants such as the reaction of shoots and roots towards light and gravity, vascular tissues' differentiation, apical dominance, beginning of lateral and adventitious roots, induction of cell division, and elongation of stems and roots. Though plant is able to synthesize IAA, the levels of phytohormones produced by plants need to be complemented with an appropriate quantity of phytohormones produced by bacteria.

The quantity of IAA produced is significantly affected by the production of tryptophan, which is the main IAA precursor and plays a pivotal role in modifying its biosynthesis level.

A minimum of 5 different processes, for the synthesis of IAA, beginning with tryptophan, have been extrapolated. The first pathway involves the generation of IAA from indole-3-pyruvic acid and indole-3-acetic aldehyde. The vast majority of bacteria, including *Klebsiella, Erwinia herbicola, Rhizobium*, and *Azospirillum*, use this pathway. The second process involves converting tryptophan into indole-3-acetic aldehyde and may involve a different pathway for the production of tryptamine. This path is used by *Pseudomonas* and *Azospirilla.* In the third pathway, indole-3-acetamide is used to synthesise IAA. This pathway is utilised by phytopathogenic bacteria like *Pseudomonas syringae, Agrobacterium tumefaciens*, and others. The fourth step in the biosynthesis of IAA is the transformation of tryptophan into indole-3-acetonitrile and *Cyanobacteria* use this mechanism. The final mechanism involves the production of IAA through a tryptophan-independent pathway, which is more commonly seen in "plants, *Cyanobacteria*" and *Azospirilla* (Daniel *et al.,* 2022).

Gibberellic acid: Gibberellins are responsible for regulating and influencing various growth and developmental processes like flowering, stem elongation, seed germination, and enzyme induction. Further, gibberellic acid is termed a stress protectant as they mitigate the harmful repercussions of abiotic and biotic stress conditions via modulating antioxidant levels by decreasing superoxide dismutase, flavonoids, and scavenging reactive oxygen species (ROS) responsible for the release of antioxidants.

Prominent gibberellin-producing bacteria include - *Acinetobacter sp., Bacillus sp., Agrobacterium sp., Arthrobacter sp., Brevibacterium sp., Chromobacterium sp., Nocardia sp., Clostridium sp., Flavobacterium, and Pseudomonas sp.*

Role of biofertilizers in INM and sustainable fodder crop production

The importance of livestock in the farmer's life is indispensable. Most of the income of marginal and especially small farmers comes from the rearing of livestock, particularly the milch animals such as buffaloes or cows. However, there is a total deficit of 61.1% green fodder and 21.9% dry fodder supply that results in livestock malnutrition and, therefore, the quality and quantity of the produce is also hampered. Amongst the upgraded agro techniques and strategies, INM has proven to be the best alternative for the augmentation of good-quality forage production per unit area. Further, the combined application of inorganic and organic sources of nutrients in integrated nutrient management (INM) proves to be better in comparison to the individual application of each component (Ramya and Pandove 2019).

Ramya *et al.,* (2021) carried out a field experiment at Bathinda and Ludhiana with a total of 11 treatment combinations of microbial inoculants (*Bradyrhizobium, B.seminalis and Burkholdera sp).* with the application of 75% and 100%RDF. The results concluded that treatment T_{10} (75% RDF + *Burkholderia* sp. + *B.seminalis*) significantly enhanced vine length, leaf length, leaf breadth, and dry matter yield at both locations.

Oberoi *et al.,* (2020) carried out a field experiment during the *kharif* seasons of 2016 and 2017 at two locations of Punjab *i.e.* Ludhiana and Bathinda, to evaluate the influence of *Azotobacter* sp., *Streptomyces badius, Burkholderia* sp., and *Sphingobacterium* sp. inoculation and their interactions on SL 44single-cut forage sorghum [*Sorghum bicolor* (L.) Moench]. It was discovered that the control was

outperformed by the dual inoculation of *Azotobacter* with *Burkholderia sp.* in terms of crude protein levels and in-vitro dry-matter digestibility (IVDMD).

Pandove *et al.*, (2022) extrapolated the effects of liquid microbial inoculants – *Azotobacter* sp. and *Stenotrophomonas maltophilia* on the productivity of *Cyamopsis tetragonoloba* (L.) *Taub.* (Cluster bean). They found that LMIs with 75% of the recommended fertilizer dose (treatment T_7) caused notable improvements in the height of the plant, dry fodder yield, and yield of seeds. Additionally, there was a 10% increase in the quantity of branches, pods, length of pod, grains per pod, and 1000-grain weight, among other growth and yield characteristics. The increase in dry fodder yield and seed yield produced over produced by T_7 over the control T_1 (RDF) was 16% and 19%, respectively.

Arora *et al.*, (2022) experimented to analyse the improvement in the traits with the application of liquid microbial inoculants in forage pearl millet (*Pennisetum glaucum*). The results indicated that the application of the liquid microbial inoculants portrayed improved quality traits as compared to the untreated control. The application of RDF + *B. seminalis* + *S. Maltophilia* (treatment T_9) resulted in significant improvement in the *in vitro* dry matter digestibility (IVDMD), crude protein (CP), total sugar and ash content. However, a significant reduction in the acid detergent fibre (ADF) and neutral detergent fibre (NDF) was recorded.

According to Verma *et al.*, (2023), the application of synthetic fertilizers in the recommended amounts improved the quality of oats at both Bathinda and Ludhiana. The consortia of *B. seminalis* and *S. maltophilia* used in treatment T_{11} produced the best results, with improved IVDMD (71.66 and 70.20%), ADF content (33.10 and 33.50%), and crude protein (9.23 and 8.92%) at both locations**.**

CONCLUSION

With rapid escalation in human population, the agriculture sector has been under constant pressure to meet the consequential increasing demands of food and feed. Hence, chemical fertilizers and pesticides have been used in an unsustainable way, leading to the deterioration of soil health and ecological imbalance. There is an immediate need for sustainable alternatives via a cost-effective approach. The plant-bacterial relationships remain an area of active research in the midst of such a crisis. Biofertilizers comprising beneficial bacteria have the potential to enhance plant growth sustainably owing to their ease of application and economical aspects. The usage of microbial inoculants ameliorates growth, productivity, and yield of fodder crops with positive impacts on the quality as well.

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