

REVIEW

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# Microbial formulation and growth of cereals, pulses, oilseeds and vegetable crops

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## Abstract

Effective microbes (EM) are the coexisting naturally occurring useful microbes applied as inoculant to enhance the beneficial microflora of the soil ecosystem to facilitate agricultural production. The participating microbial consortium includes lactic acid and photosynthetic bacteria, actinomycetes, fermenting fungi, and yeast, among others. These microbes are physiologically well-matched and coexist in a provided medium. EM formulation could be applied to a target crop in the most appropriate manner and form, and is easy to handle. It could be applied in several manners, as soil application, foliar application and as seed treatment. Microbial formulation in agricultural practices for enhancing productivity is sustainable and eco-friendly approach. When applied, EM formulations reportedly have positive effect on several crop growth parameters. It enhances the productivity, biomass accumulation, photosynthetic efficiency, and antioxidative response to abiotic stress in rice. EM formulations reportedly augment the trace elements contents, root and shoot weight, nodulation and pod yield in rajmah, while it boosts the root and shoot weight, nodulation and seed yields in bean, and drought and virus tolerance, shoot weight, pod number and biomass in soybean. Reportedly, formulated EM perks up the chlorophyll, N, P, carbohydrate and protein contents in sunflower, whereas it stimulates the root and shoot growth, leaf number, fungal disease resistance in groundnut. It could lead to an improved root growth, plant height, chlorophyll content, pod yield, fungal disease resistance, Cr-resistance and pest resistance in okra. This review compiles and provides critical insight to the effects of EM formulations on various crops, particularly the cereals (rice), pulses (rajmah, bean and soybean), oilseeds (sunflower and groundnut) and vegetable (okra).

**Keywords:** Agriculture, Effective microbe, EM formulation, Environmental probiotics, Microbe-microbe interaction, Plant-microbe interaction

## Introduction

Healthy soil ecology entails the ability of soil to save the flora against soil-borne pathogenic microbes and parasites. Soil ecosystem balances the relationship between the pathogenic and numerous useful microbes working together in synergy [1]. The obliging saprobic microflora ferments and decomposes the soil organic material and supplement to the nutrient pool for the plants, while additionally augmenting the soil particles helping in its

moisture and nutrient holding capacities [1]. Applying animal manures and liquid compost (composed of plant growth constituents and useful microbes) to the soil is a scientific approach towards sustainable farming. It could be used to improve soil quality, promote plant growth and protect the crops from pathogenies [2]. Although an integral component of crop ecosystem, the active soil microbes are little recognised in agricultural management strategies, and their role needs to be augmented further [3].

Continuous and indiscriminate use of chemicals poses a negative impact on soil, environment, and ultimately

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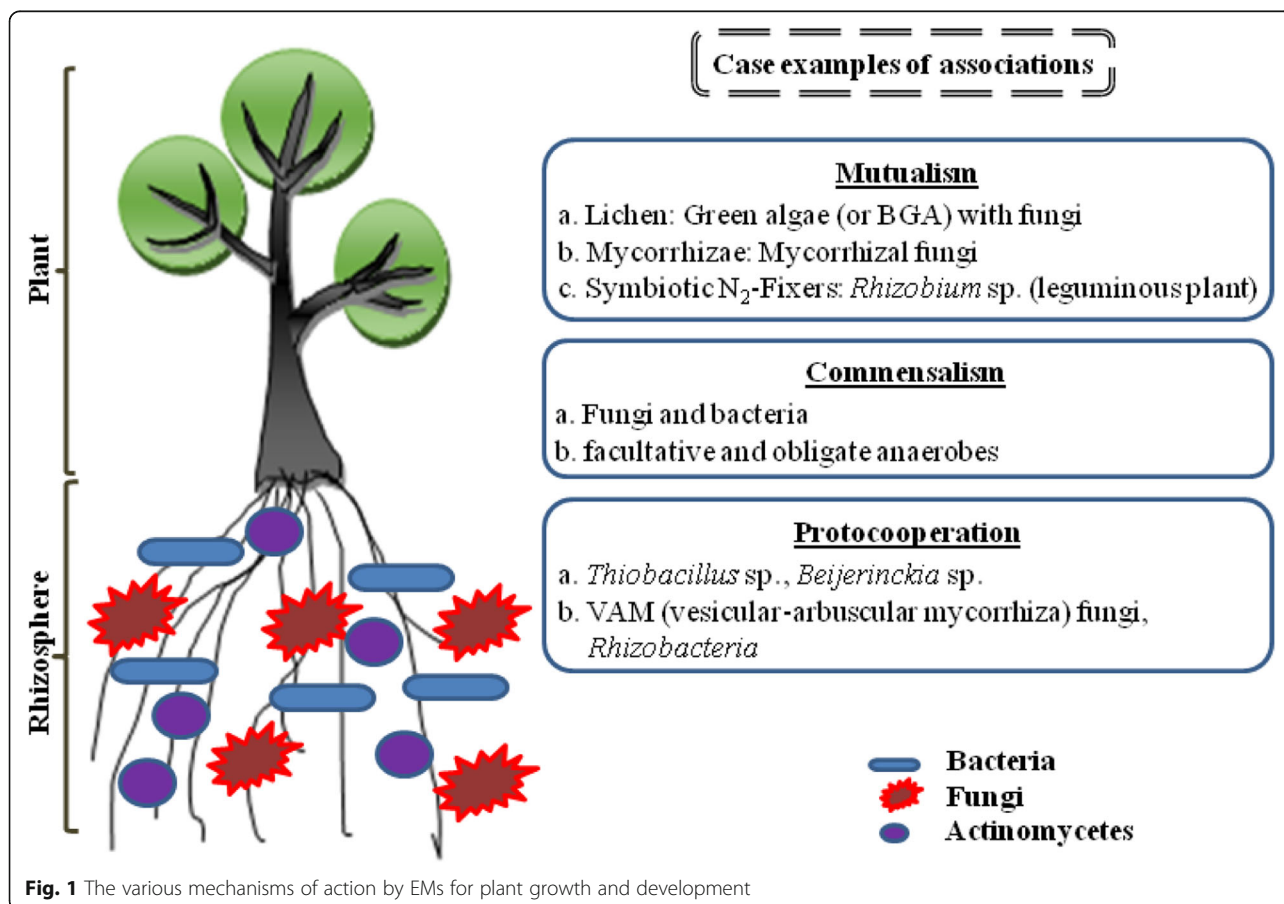
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human health, in the same sequence. Use of chemical fertilisers indiscriminately leads to soil pollution gradually deteriorating the soil fertility. Although productivity increased appreciably to feed the starving population with the onset of green revolution (in early 70s), the use of chemical fertilisers and pesticides glorified then had an obvious long-term adverse effect [4]. Since then, the synthetic chemicals have taken the front seat in current global agriculture. Excess accumulation of such chemicals in crops bioaccumulates and biomagnifies along the food chain thereby adversely affecting human and animal health. Chemical run-offs from agri-systems when flooded further add to the awe. So, concerted scientific investigations to utilize agricultural resources efficiently and enhance productivity through biological means instead of chemicals are underway. Applying organic practices along with effective microbes (EMs) for yield enhancement on a sustainable basis is a promising approach. Nutrients recycling becomes efficient through this by saprobes, and the urgency for chemical fertiliser dwindles [4–6].

Using microbes solely or in a consortium could enhance the productivity of most farming systems significantly as the microbes and plants have been evolutionarily interacting in nature [7, 8]. Among various microbial communities

active in agricultural farming systems, fungi, bacteria, actinomycetes and yeasts have been recommended as potential EMs [1]. Applying composts and animal manure in an agricultural system along with EMs (as EM formulations) to the soil environment promotes plant growth. As the EMs persist in the soil environment for a long time, their beneficial effects in the growth and development of the crops is manifested better [9, 10].

The sludge and organic wastes treated by the EMs could be applied as biofertilizer, wherein the EM participants as well as the essential nutrients could be healthy inputs for crop growth. The beneficial bacteria and fungi present in the biofertilizers help improve chemical and biological characteristics of the soil thereby ensuring agricultural productivity [11, 12]. In biofertilizer, various microbial communities, viz., fungi, bacteria, actinomycetes and yeasts, are used as inoculant and they majorly promote plant growth through activities like fixing  $N_2$ , phosphate and potassium solubilisation, exopolysaccharides secretion, biocontrol agent, organic matter decomposition, and siderophores production [3, 13]. The various mechanisms of action performed by the EMs promoting plant growth and development are graphically presented in Fig. 1. Diazotrophs like *Rhizobium* sp.



and *Bradyrhizobium* sp. are the major  $N_2$ -fixers [14]. *Azospirillum*, a free-living  $N_2$ -fixer reportedly enhances the growth in non-leguminous crops [15]. *Pseudomonas putida* and *Pseudomonas fluorescens* are not only good biocontrol agents but also stimulate crop growth through biological  $N_2$ -fixation and by enticing hormonal secretion for plant growth [16]. *Azotobacter* and *Azospirillum* application as EMs reportedly enhance strawberry production [17, 18]. Phosphorus, which is the key nutrient in soil and is present in complex unavailable forms, is made available for the plant through the activity of phosphate solubilising microbes. Han and Lee [19] reported the benefits of phosphate solubilising bacteria *Bacillus megaterium* and potassium solubilising bacteria *Bacillus mucilaginosus* in enhanced nutrient uptake by eggplant in nutrient-limited soil. Coinoculating two or more microbes might improve the yield and growth as compared to a single one due to the added benefits of their concerted efforts [19].

Photosynthetic and lactic acid bacteria, fermentative fungi, actinomycetes and yeasts or their combinations are a part of a formulated EM [20–22]. EMs as environmental probiotics are added to the prebiotic that result in formulated EM. In a formulated EM, the biotic (as environmental probiotic) and abiotic (as the prebiotic) are two important ingredients, wherein the microbes act as a probiotic and the carbon and other nutrient sources act as prebiotics. Kato et al. [20] and Raja and Bharani [22] collected beneficial microbes from nature and formulated the EM using lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus lactis*), photosynthetic bacteria (*Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*), yeasts (*Saccharomyces cerevisiae*, *Candida utilis*), actinomycetes (*Streptomyces albus*, *S. griseus*), and fungi (*Aspergillus oryzae*, *Mucor hiemalis*) [20, 22]. Formulated EM application along with organic fertiliser enhances the growth, nutrient uptake and grain yield of sweet corn as compared to chemical fertilisers [20]. As wood waste residue provides a suitable environment for the EM to thrive, and a high quality compost could be produced, EM formulation could be used in industrial wood waste management [21].

### Impact of EMs on various crops

EM formulations are available commercially as well as prepared by researchers themselves for pilot- and field-scale studies. The detail information of some commercially available as well as self-made EM formulations, along with the participating microbes on plant growth promotion is furnished in Table 1.

Commercial liquid formulation EM-1 containing lactic acid bacteria (*Lactobacillus plantarum*), yeast (*Candida utilis*), and actinomycetes (*Streptomyces albus*) decomposes fruits and vegetables refuse and the resultant

compost performs better in terms of increased leaf surface area, total leaves, total chlorophyll content, shoot length, plant height, branches and foliage count [22]. Another EM formulation composed of *Bacillus* sp. *Pseudomonas aeruginosa*, *Streptomyces* sp. was used in seed treatment of sunflower for improving crop performance, that also help in preventing sunflower from necrosis disease [31]. EM culture consisting of photosynthetic bacteria (*Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*), lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei* and *Streptococcus lactis*), yeasts (*Saccharomyces* sp.), and actinomycetes (*Streptomyces* sp.) reportedly produces bioactive substances including enzymes, controls soil-borne diseases and accelerates lignin decomposition in the soil [32]. EMs are mutually compatible, and live for an extended period [33]. EMs could suppress the growth and activity of the indigenous putrefactive microbes that add to malodours in plants [34]. Problems in handling of organic urban waste like bad odour, fly population control and pathogenic microbes' devaluation in piling of waste could be prevented by applying formulated EMs [34].

EM Application has been successfully tried on vegetable crops in New Zealand and Sri Lanka, herbage grasses in Holland and Austria, and apples in Japan. Bokashi (nutrients and EM-enriched compost) was applied in these studies, that increased the yield over a period of time [35]. As the first solid form of EM formulation for agricultural applications, Bokashi compost was prepared using organic refuse like saw-dust mixed with nitrogen-rich materials like rice husk, corn bran, wheat bran, fish meal and oil cake [36]. Bokashi base material is normally prepared by mixing molasses with water, followed by the addition of EM consortia. The resultant mixture is further mixed with dry ingredients (mixture of rice bran, oil cake, fish meal, etc.). The final mixture is allowed to ferment in airtight container, for 4–5 (in summer) to 7–8 (in winter) days under tropical conditions. After fermentation, a sweet and fermented odour suggests that Bokashi is ready for application. Bokashi reportedly facilitates nutrient release from soil, improves soil carbon mineralisation, and enhances the soil properties. It also increases the photosynthesis and protein activity in crops, increases crop resistance to water stress, facilitates spreading of the roots, and suppresses the pests and plant diseases in agricultural practices [35–37].

Liquid EM formulations have been used in agricultural practice extensively. Foliar application of EM formulation was compared with chemical fertilisers on onion, watermelon, garlic and tomato, and the yields were higher in EM application. Foliar application of EMs evades various biotic and abiotic factors and other limitations in soil microenvironment, thereby increasing the yield and quality of crops, fruits and vegetables [24].

**Table 1** A compilation of various EM formulations and their crop wellbeing benefits

Sl No.	EM formulation	Candidate organism(s)	Dosage and application pattern	Trial location	Prevailing edaphic and climatic variables	Target crop(s)	Plot size	Reported crop benefits	Ref.
1.	EM-1, (commercial, Environ Biotech, Mumbai, Maharashtra)	lactic acid bacteria ( <i>Lactobacillus plantarum</i> ), photosynthetic bacteria, yeast, fermenting fungi ( <i>Candida utilis</i> ) and actinomycete ( <i>Streptomyces albus</i> )	1 L EM-1 mixed with 20 L water and 2 kg Jaggery (pure cane sugar)	Sathyabama University, Chennai, Tamil Nadu, India (12°52'23" N, 80°13'21" E)	Total nitrogen 980 mg kg <sup>-1</sup> , P 901 mg kg <sup>-1</sup> , K 3014 mg kg <sup>-1</sup> , Organic carbon 36.5%, Humic acid 21 mg kg <sup>-1</sup>	Black gram ( <i>Vigna mungo</i> )	Study conducted in 5 x 3 m plot; sowing at 30x 10 cm spacing	Shoot length, total chlorophyll, leaves and foliage, plant height, leaf surface area and branches of crop were increased	[22]
2.	EM Bioaab (commercial, NFRD Foundation, Faisalabad, Pakistan)	Photosynthetic bacteria, lactic acid bacteria, yeast and actinomycetes	1 L diluted Bioabb (0.2% v/v) applied to soil mixed with <i>Parthenium</i> green manure; fortnight foliar spray till end	University of Punjab, Lahore, Pakistan (31°47'90" N, 74°26'62" E)	pH 7.5, Organic matter 0.87%, Total N <sub>2</sub> 0.045%, Available P 6.50 mg kg <sup>-1</sup> , Exchangeable K 100 mg kg <sup>-1</sup> , Mn B 108 mg kg <sup>-1</sup> , Fe 23.80 mg kg <sup>-1</sup> , Cu 10.80 mg kg <sup>-1</sup> , 1.80 mg kg <sup>-1</sup>	Wheat ( <i>Triticum aestivum</i> L.)	–	Shoot length, dry biomass, spike length, number of grains per spike, grain yield of crop increased	[23]
3.	EM Bioaab (commercial, NFRD Foundation, Faisalabad, Pakistan)	Photosynthetic bacteria, lactic acid bacteria, yeast and actinomycetes	1 L diluted Bioabb (0.2% v/v) applied to soil mixed with <i>Parthenium</i> green manure; fortnight foliar spray till end	University of Punjab, Lahore, Pakistan (31°47'90" N, 74°26'62" E)	pH 8.2, Organic matter 0.90%, Total nitrogen 0.05%, Available P 14 mg kg <sup>-1</sup> , Available K 210 mg kg <sup>-1</sup>	Pea ( <i>Pisum sativum</i> L.)	Study conducted in pots of 20 cm diameter filled with 2.5 kg soil (30 cm depth)	Nodule number, nodule biomass and grain yield of crop increased	[24]
4.	EM Bioaab (commercial, NFRD Foundation, Faisalabad, Pakistan)	Photosynthetic and lactic acid bacteria, yeast and actinomycetes	EM stock diluted at 1:1000 (EM:trap water ratio); 300 mL diluted EM applied to soil 7 days before sowing	University of Punjab, Lahore, Pakistan (31°47'90" N, 74°26'62" E)	pH 8.1, Organic matter 0.9%, EC 4.8 mS cm <sup>-1</sup> , Total nitrogen 0.05%, Available P 14 mg kg <sup>-1</sup> , Available K 210 mg kg <sup>-1</sup> , Fe 9.53 mg kg <sup>-1</sup> , Cu 1.71 mg kg <sup>-1</sup> , Zn 4.42 mg kg <sup>-1</sup>	Wheat ( <i>Triticum aestivum</i> L.)	Study conducted in pots of 20 cm diameter filled with 2 kg soil (30 cm depth)	Root and shoot dry biomass of crop increased	[25]
5.	EM – 1* (commercial, Punto EM, Roccastrone, Sanremo, Italy, <a href="http://www.italiaem.it/Home/em.html">http://www.italiaem.it/Home/em.html</a> )	–	EM-1* stock applied to soil every 7 days	Milan State University, Milan, Italy (45°46'01" N, 9°19'46" E)	Temperature 24 °C, pH 6.0–7.5, Relative Humidity 60%, Total nitrogen ≤1.4%, EC 0.25 dS m <sup>-1</sup> , Apparent density 120 kg m <sup>-3</sup> , Total Porosity 90% (v/v)	Bean ( <i>Phaseolus vulgaris</i> L.)	Study conducted by sowing one seed in each 22 cm size pot	Photosynthesis and crop yield increased	[26]
6.	Self-formulated	<i>Candida tropicalis</i> , <i>Phanerochaete</i>	EM stock mixed with poultry dropping	Indian Council of Agricultural Research,	pH 8.26–9.17, Available N 220–	Marigold ( <i>Tagetes</i> L.),	Study conducted in 2 x 2 m plot size	Carotenoid pigment content increased;	[27]

**Table 1** A compilation of various EM formulations and their crop wellbeing benefits (Continued)

Sl No.	EM formulation	Candidate organism(s)	Dosage and application pattern	Trial location	Prevailing edaphic and climatic variables	Target crop(s)	Plot size	Reported crop benefits	Ref.
		<i>Chrysosporium</i> , <i>Streptomyces globisporus</i> , <i>Lactobacillus</i> sp., photosynthetic bacteria	fertilized paddy straw	New Delhi, India (28°40' N, 77°12' E)	226 kg hm <sup>-2</sup> , Available P 27– 38 kg hm <sup>-2</sup> , Available K 585– 601 mg kg <sup>-1</sup> , Available P 0.31%, Organic Carbon 0.35–0.36%	Calendula ( <i>Calendula</i> L)		increased acid phosphatase, dehydrogenase and $\beta$ -glucosidase activities at flowering	
7.	Self-formulated	<i>Lactobacillus plantarum</i> , <i>L. casei</i> , <i>Streptococcus lactis</i> , <i>Rhodospseudomonas palustris</i> , <i>Rhodobacter sphaeroides</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida utilis</i> , <i>Streptomyces albus</i> , <i>Streptomyces griseus</i> , <i>Aspergillus oryzae</i> and <i>Mucor hiemalis</i>	80 mL EM applied to soil before sowing; with chemical fertilisers	–	Total soil N 3.4 g kg <sup>-1</sup> , Available P 0.025 g kg <sup>-1</sup> , K 0.44 g kg <sup>-1</sup> , C/N 13	Sweet corn ( <i>Zea mays</i> L. cv. Honey-Bantam)	Study conducted in pots of 0.02 m surface area filled with 3 kg fresh soil	Increased biomass and grain yields	[20]
8.	Oki Bac 174 (commercial, Oikos Chile Ltda, Lampa, Chile)	<i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i> , <i>Bacillus megaterium</i> , <i>Bacillus polymyxa</i> , <i>Bacillus macerans</i> , <i>Pseudomonas fluorescens</i> , <i>Pseudomonas putida</i> , <i>Nocardia corallina</i> , <i>Saccharomyces cerevisiae</i> and <i>Trichoderma viride</i>	50 g consortium and 100 g saccharose added to 10 L tap water: resultant solution applied at planting, and every two-month interval	El Nogal Experimental Station, University of Concepcion, Chile (36°35' N 43.2° S, 72° 04'39" W)	Temperature 13 °C, pH 6.74, Organic matter 5.77%, NO <sub>3</sub> <sup>-</sup> 11.6 mg kg <sup>-1</sup> , K 406.3 mg kg <sup>-1</sup> , P 77.3 mg kg <sup>-1</sup>	Blueberry ( <i>Vaccinium corymbosum</i> L)	Study performed in 30 x 30 cm pots and 30 cm deep per seedling; seedling done 1 x 3 m apart	Increased plant biomass	[28]
9.	Self-formulated	<i>Bacillus</i> sp., <i>Streptomyces</i> sp., <i>Azotobacter</i> sp., <i>Frutieria</i> sp.	Seeds immersed in 48 h old EM for 5 min and transferred to water agar (nine seeds per plate); incubated at 30 °C for 4 d	Balaji Nagar, Andhra Pradesh, India (14.4492° N, 79.9993° E)	–	Black gram ( <i>Vigna mungo</i> L)	–	Enhanced seed germination, root volume, shoot dry mass, total dry mass of crop	[29]
10.	Self-formulated (EM compost)	<i>Streptomyces albus</i> , <i>Propionibacterium freudenreichii</i> , <i>Streptococcus lactis</i> , <i>Aspergillus oryzae</i> , <i>Mucor hiemalis</i> , <i>Saccharomyces</i>	50 kg EM compost consisting of 50 kg traditional compost fortified with 200 mL concentrated EM; applied twice a year before planting of	Qu-Zhou station, North China Plain, China Agricultural University (115°01' E, 36°52' N)	Temperature 13.2 °C, pH 8.77, Rainfall 542.7 mm, Bulk density 2.65–2.58%, Organic carbon 5.82 g kg <sup>-1</sup> , Total N 0.59 g kg <sup>-1</sup>	Maize ( <i>Zea mays</i> L), Wheat ( <i>Triticum aestivum</i> L)	Study performed in 4 x 8 m plots	Increased soil C, N and nutrients content; increased crop yield	[30]

**Table 1** A compilation of various EM formulations and their crop wellbeing benefits (Continued)

Sl No.	EM formulation	Candidate organism(s)	Dosage and application pattern	Trial location	Prevailing edaphic and climatic variables	Target crop(s)	Plot size	Reported crop benefits	Ref.
		<i>Cerevisiae</i> , <i>Candida utilis</i> , <i>Lactobacillus</i> sp., <i>Rhodopseudomonas</i> sp., and <i>Streptomyces griseus</i>	crops (maize, wheat)		C/N 9.86, Available N 64.6 mg kg <sup>-1</sup> , Available P 26.1 mg kg <sup>-1</sup> , Available K 65.4 mg kg <sup>-1</sup>				



Foliar application alongside NPK amendment of soil resulted in 120% grain yield, 217% in nodular number and 167% in nodular biomass, while an additional green manure amendment increased the grain yield by 145% [25]. Compared to the control, foliar spray of EM formulation at 15-d interval for three times gave higher pod yields in okra [38].

### Role of EMs in agro-ecosystem

Plant growth by EMs may proceed either through direct or indirect action [39]. Direct action refers to soil amelioration, production of plant growth substances, soil fertility improvement by mobilising soil mineral components, N<sub>2</sub>-fixation, phosphate and minerals solubilization, phytohormones production, and deaminase activity. The indirect action, on the other hand, refers to the biocontrol activities that inactivate or kill plant pathogens thereby providing a healthy cropping environment.

Plant growth-promoting rhizobacteria (PGPR), plant growth-promoting bacteria (PGPB) and vesicular-arbuscular mycorrhizae (AMF) fungi are various growth promoting microbes (PGPM). PGPR provides a favorable environment for plant-microbe interaction. N<sub>2</sub>-fixers like *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Mesorhizobium*, *Allorhizobium* are potential mutually-benefitting plant growth promoting endosymbionts [39]. Genera like *Azospirillum*, *Enterobacter*, *Klebsiella* and *Pseudomonas* proficiently colonize root surfaces and fix nitrogen [40]. PGPB like *Pseudomonas fluorescens* and *Bacillus subtilis* induce PGPRs to produce plant growth-promoting substances [41]. As a classic case of symbiosis, the amino acids, carbohydrates, active enzymes and organic acids secreted by the plant roots are used by the EMs, and the EMs secrete amino acids, various vitamins, nucleic acids and hormones for the plant in return.

ACC (1-aminocyclopropane-1-carboxylic acid) deaminase producing microbes like actinomycetes would take up and metabolise ACC to  $\alpha$ -ketoglutarate and NH<sub>3</sub>, thereby decreasing ethylene concentration (excess concentration adversely affects growth) in plant. These actinomycetes individually or as coinoculants reportedly fix N<sub>2</sub>, solubilize phosphate, and produce siderophore in sugarcane [42]. Soil actinomycetes could produce numerous antibiotics and extracellular enzymes that inhibit plant pathogens. Numerous actinomycetes protect plants against diseases [43]. Soil salinity particularly in coastal belts is a challenge for crop growth and wellbeing [44], and *Enterobacter* sp. UPMR18 reportedly enables okra plant to withstand salt stress [45]. Okra plant has better germination percentage and higher leaf chlorophyll contents by rhizospheric EM symbionts [42].

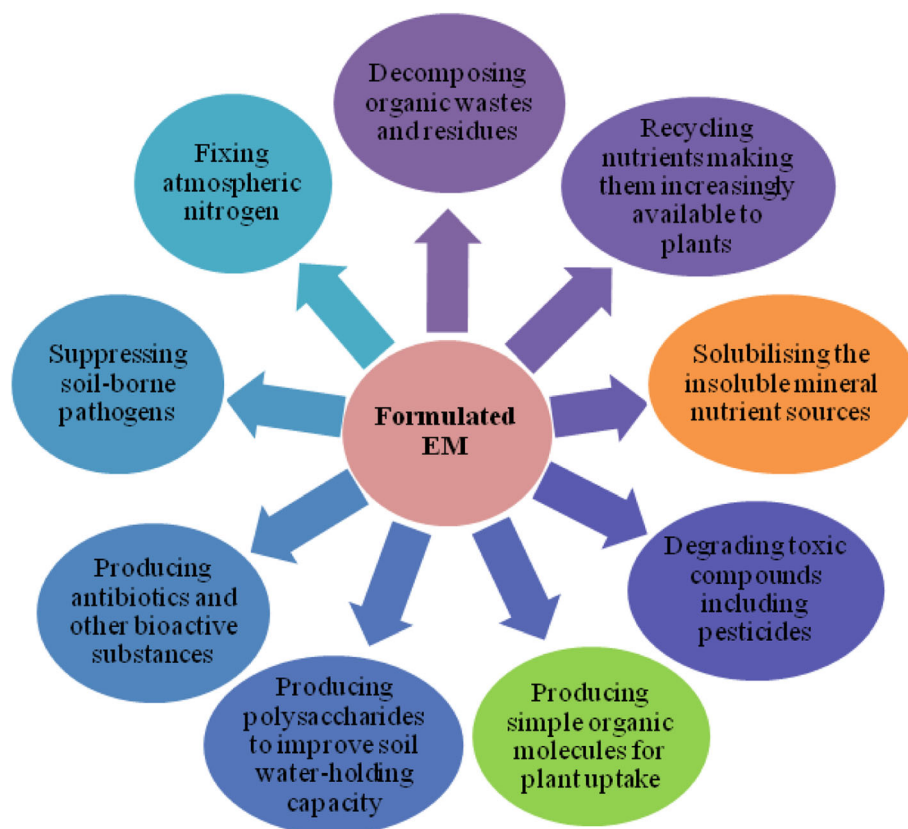
### Interactive role of participating microbes in a formulated EM

Coexistence of various microbial species is a prerequisite in a formulated EM. Microbial interactions occur through secondary metabolites, siderophores, quorum sensing system, biofilm formation, and cellular transduction signalling [46]. The ultimate interaction unit is the gene expressed in each organism in response to an environmental (biotic or abiotic) stimulus that is responsible for the production of molecules for microbial interactions [46]. The participating microbes in an EM formulation may interact with each other through mutualism, commensalism, and protocoeperation. A case example of mutualism is the blue green algae and fungus where they exchange nutrition among one another [46]. The algae get protection from environmental stress by the surrounded fungal hyphae which in turn gets carbon that is fixed by algae (algal photosynthesis). Similarly, a commensalism association is seen in cellulose and lignin degrading fungi to glucose and organic acids that are utilised by bacteria further [47].

Protocoeperation is a mutualism in which both the microbial partners benefit from each other without depending on each other for survival [48]. Here, favor is extended by one organism to its associate by providing carbonaceous products. Nutritional association for several vitamins, amino acids and purines is observed between bacteria and fungi in terrestrial ecosystems. Nutritional protocoeperation may be formed between various bacteria and fungi in which various vitamins, amino and purine are produced by certain microbes that could be utilised by the partner microbes. *Proteous vulgaris* and *Bacillus polymyxa* may form nutritional protocoeperation for nicotinic acid and biotin, respectively [49]. While formulating an EM, other various interactions of little relevance are antagonism, competition, parasitism, and predation. The survival of one microbe may be at stake (due to the inhibitory or lytic effect of the other partner) when these microbial associations are negative.

### Plant-microbe interaction

Plants constantly interact with an enormous soil microflora (Fig. 2). Ecological interactions like mutualism, commensalism, amensalism, protocoeperation and antagonism might contribute to the overall soil health and plant wellbeing [13, 46, 50]. Lichen is an association of green or cyanobacterial algae with fungus (ascomycetes). The alga is saved from environmental stresses by the fungal hyphae, while the fungus obtains nutrients and oxygen from the photosynthetic algae. Similarly, the leguminous plant acquires readily available fixed nitrogen source from *Rhizobium*, and the *Rhizobium* is protected by the leguminous plant from environmental stress in



**Fig. 2** Microbe-microbe and plant-microbe interactions for sustainable agriculture

return. *Frankia* (an actinomycete) forms a symbiotic association with *Alnus* and *Casuarina* (non-legume plants) supplementing them with the fixed nitrogen and obtains organic nutrients in return. Mycorrhizal fungi associate with the plant roots and obtain carbohydrates, while it increases the surface area for water, N, P and inorganic nutrients to be absorbed by plants in return. Endomycorrhizal symbioses help plant withstand environmental stress and enhance the soil structure by forming hydro-stable aggregates [51, 52].

Amensalistic association suppresses the growth of one partner by the other through toxins (like antibiotics) production. Here, a soil pathogenic microbe is inhibited by amensal partner where the later remains unaffected thereby benefitting crop growth. Some amensals also release harmful gases like hydrogen cyanide (HCN), ethylene, methane, nitrite, sulphides and other volatile compounds of sulphur [52]. In agriculture, synergism is seen between VAM fungus-legume plants and *Rhizobium*. In this association, nitrogen is fixed by *Rhizobium* for the plants to uptake the fixed nitrogen. Phosphorus uptake by plant is also elevated which results in increased crop yields and improved soil fertility.

Antagonism association is the most common in nature which is governed essentially by antibiotic production.

Here, an organism directly or indirectly inhibits the activities of the other, e.g., the soil *Bacillus* sp., *Pseudomonas fluorescens* and *Streptomyces* sp. produce antibacterial and antifungal antibiotics that help suppress various plant pathogens. *Thiobacillus* sp. reduces the soil pH up to 2.0 thereby restricting the growth of pH-sensitive microbial species. In lichen, the  $O_2$  produced by algae prevents anaerobic microbes from colonisation, while the cyanide produced by fungi is toxic to numerous other microbes [13, 46, 50, 52].

### Microbial (EM) formulation

#### Probiotics (EMs)

As mentioned earlier, the EMs predominantly consist of physiologically compatible lactic acid and photosynthetic bacteria, yeasts, fermenting fungi and actinomycetes [10, 53, 54]. Adding photosynthetic bacteria to the soil provides a healthy environment for growth of other EMs. VAM fungi increases the soil phosphate solubility and coexist with the  $N_2$ -fixing *Azotobacter* and *Rhizobium*. Lactic acid bacteria secrete lactic acid that sterilizes the soil, and suppresses the thriving harmful microbes (like *Fusarium*) and nematodes, and stimulates the decomposition of lignocellulosic organic materials in soil [55]. Bioactive substances like phytohormones and enzymes



produced by fungi help promote active cell/root division, while providing useful substrates for EMs, viz., lactic acid bacteria and actinomycetes [56]. Fermenting fungi help decompose organic matters and rapidly producing alcohol, esters and antimicrobial substances that help suppress harmful insects and maggots [57]. Actinomycetes are other critical antimicrobial producers from amino acids secreted by photosynthates that would suppress the harmful soil microbes. Thus, various EM species complement each other and form mutually-beneficial relationships in the soil [57]. EMs enhance the quality of soil profile and thereby facilitate crop growth and development [58].

#### Prebiotic/carriers for microbial inoculants

Microbial formulation is a carrier-based preparation to provide microbes with better survival for longer duration. Prebiotic carriers provide the desired nutrients to augment the EMs. EMs are formulated with the prebiotic to facilitate storage, commercialisation and easy field application. The affordability and availability are the two significant factors while selecting a carrier.

Few of the desirable characteristics of a quality carrier are lump-free material that is easy to process, moisture absorption capacity, ease of sterilization, cost efficient, plentifully available and a good inherent pH buffering capacity. While dry formulations are produced using solid carriers like soil (peat, coal, clay, and inorganics), organic (composts, soybean meal, wheat bran, and sawdust), or inert (vermiculite, perlite, kaolin, bentonite, and silicates) materials, liquid formulations can be prepared using mineral oil, organic oils, oil-in-water suspensions, molasses, humic acid, and landfill leachates [59, 60]. These have been detailed in Table 2.

#### Solid carrier base

A formulation can be prepared by mixing compatible beneficial microbes with the prebiotic. Microbial viability and shelf-life in formulation are important in formulation. Majority of formulations use charcoal, talc or other inert carrier material. *Pseudomonas fluorescens* formulation was mixed with talc and 1% carboxymethyl cellulose and used against leaf disease. Alginate-base formulation of *Bacillus subtilis* and *Pseudomonas corrugata* was easy to prepare and dry, and could be stored up to 3 years [68].

Compost is a good nutrient natural carrier for the EMs. It is biodegradable and non-polluting, usually processed from abundant natural waste materials. While supporting soil microbes to survive, it also facilitates plant growth. Composting has been established as one of the low-cost alternatives to minimize the volume of solid waste disposed of to the environment [59, 69]. This form of transformation of various organic wastes into

compost is safe and economical [70, 71]. By converting the biowastes into composts, the nutrients in the waste can be utilized better creating a zero-waste system [71]. The converted compost would contain a substantial amount of EMs that are helpful for plant growth and yield.

Talc and charcoal based formulations of *Bacillus* sp. increases the growth of mung bean and rice [72]. *Bacillus* sp. shows antagonistic effects against various phytopathogens, including *Rhizoctonia solani* (ITCC-186) and *Fusarium oxysporum* (ITCC-578). Likewise, alginate-base formulation of *Bacillus subtilis* and *Pseudomonas corrugata* (PGPRs) reportedly benefit the crops [68]. A solid base *Piriformospora indica* (root endophyte) formulation as bioinoculant enhances the growth of *Phaseolus vulgaris* L. [73]. This solid base formulation also increases the adaptability of *Phaseolus vulgaris* L. to greenhouse conditions.

#### Liquid carrier base

The EM could be formulated using aqueous, oil or polymer liquid base. The liquid base contains nutrients, cellular protection and additives to promote survival after seed or soil applications [74]. Such prebiotics in EM formulation are glycerol, vermicompost wash, indole acetic acid, and malic acid. Such a PGPM formulation of *Bacillus licheniformis*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Streptomyces fradiae* shows good microbial survival even after 120-d storage period [74, 75]. The seed germination and plant height increase by using liquid formulation treatment in sunflower [74, 75].

#### Means to apply EM formulations

The various ways to apply agricultural EM formulations include applying it directly into the soil (soil application), spraying it on leaves (foliar application), and soaking the seeds in it prior to sowing [58]. Seeds are soaked in 0.1% EM suspension for half an hour (for smaller seeds) or up to 4–6 h (for larger seeds). The seeds are carefully semi-dried before sowing ensuring that they do not clump.

In foliar application, the crops benefit from the EM through the foliage. Foliar spray is effective when applied in the evening or early morning. A dilution of 1:1000 with water is often recommended [58]. Foliar application of EM with soil application of fermented plant extract enhances the yields of cucumber and reduces the instance of pickle worm infection [56]. Foliar application of 0.1% EM improves the quality and enhances the yield of tea (by 25%), cabbage (by 14%), and sugar corn (by 12.5%) [32]. The impact of foliar-applied EM and seed treatment on groundnut, along with 0.1, 0.5 and 1.0% (v/v) EM concentrations foliar application on garlic, onion, tomato and watermelon at one- and two-week intervals are effective [32].

**Table 2** A compilation of possible prebiotics for EM formulation

Sl No.	Name of EM	Prebiotic/carrier/base	Carrier compositions	Compositional importance for microbes	Effect on EM shelf-life	Remarks	Ref.
1.	Self-formulated EM Bokashi (solid EM formulation but prepared using liquid carrier)	Molasses	Carbohydrates (major constituent), vitamins, minerals, water and fat (trace quantity)	Microbes used carbohydrates as energy and carbon sources, minerals as micronutrients and vitamins as enzyme cofactors	–	Beneficial effects of the fermented organic fertilizer based on molasses could contribute to crop growth will depend upon the organic and viable microbial content of EM Bokashi	[1]
2.	Self-formulated EM	Molasses (1–10% dilution of original molasses)	Carbohydrates (major constituent), vitamins, minerals, water and fat (trace quantity)	Microbes used carbohydrates as energy and carbon sources, minerals as micronutrients and vitamins as enzyme cofactors	–	EM used to treat sewage that reduces malodour, turbidity, TDS, TSS, BOD and COD contents significantly	[61]
3.	–	Landfill leachate	Organic carbon, Ca, Mg, Na, K, Fe, $\text{Cl}^-$ , $\text{SO}_4^{2-}$ , $\text{PO}_4^{3-}$	Microbes used organic carbon as energy and carbon source, $\text{SO}_4^{2-}$ and $\text{PO}_4^{3-}$ as micronutrients, and metal cations as micronutrients	–	–	[62]
4.	–	Landfill leachate	Organic carbon, fatty acids, $\text{NH}_4^+$ , $\text{PO}_4^{3-}$ , $\text{Cl}^-$ , Na, Mg, K, Ca, Mn, Fe, Ni, Cu, Zn, Pb.	Microbes used organic carbon as energy and carbon source, $\text{NH}_4^+$ and $\text{PO}_4^{3-}$ as micronutrients, and metal cations as micronutrients	–	–	[63]
5.	Self-formulated EM	Humic acid	Carboxyl and phenolic groups may contain cations ( $\text{Mg}^{2+}$ , $\text{Ca}^{2+}$ , $\text{Fe}^{2+}$ , $\text{Fe}^{3+}$ ).	Microbes used it as food (energy and carbon) source and used constituent cations as micronutrient or cofactors	–	The cell viability in EM formulation was increased and the corresponding EM application inhibited the wilt tomato plant	[64]
6.	Self-formulated EM	Coconut water and polyvinyl pyrrolidone or glycerol	Sugar, K, Na, Vitamins, polyvinyl pyrrolidone or glycerol	Microbes used sugar and glycerol as energy and carbon source and vitamins as cofactor	–	Carrier could contribute to maintain microbes and resultant formulation brings positive impact on chili and tomato seedlings	[65]
7.	Self-formulated EM	Glycerol (2% v/v)	Glycerol	Microbes used glycerol for growth and metabolism	–	EM formulation reduces the wilt infection and increased banana yield	[66]
8.	Self-formulated EM	Polyvinyl alcohol, xanthan gum or gelatin	Polyvinyl alcohol, xanthan gum or gelatin	Microbes supposed to use it as nutrient source	–	EM formulation enhances wheat yield. Additionally, increased microbial colonisation of root	[67]

Several soil applications of EM formulations for enhanced growth have been reported. Soil application of EM maintains the photosynthetic efficiency of bean plant 2 weeks longer [26]. Application of compost-based effective microbes (*Candida tropicalis*, *Phanerochaete chrysosporium*, *Streptomyces globisporous*, *Lactobacillus* sp.) along with chemical fertilizer dosage enhances the carotenoid pigment of calendula and marigold by 46 and 12%, respectively [27]. The specific microbes and their modes of action in plant growth are provided in Table 3 with the impact of rhizosphere-associated EM on plant growth in Table 4. The potential microbial candidates for EM formulation are compiled in Table 5.

### Climatic (abiotic) factors and the efficacy of EM formulation

Although EMs are meant to particularly promote plant growth in harsh (drought, salinity, CO<sub>2</sub>, high/low temperatures) climatic conditions [38], climatic factors could also affect the growth and survival of EMs thereby affecting plant growth and productivity [81].

There was an improved interaction between legume and rhizobia at elevated ambient CO<sub>2</sub> concentration [82]. Atmospheric CO<sub>2</sub> fortification facilitates the activity of *Rhizobium leguminosarum* over other strains [83]. The nitrogen content of plant tissues in common bean decreases at elevated atmospheric CO<sub>2</sub> condition [84]. Elevated CO<sub>2</sub> stimulates microbial growth in rhizosphere wherein the plant and rhizobia compete for nitrogen which leads to a low N-nutritional status in plant. The population of HCN-producing *Pseudomonas* sp. (inhibiting root parasitic fungi) is reduced at an elevated CO<sub>2</sub>

conditions, and the fractions of siderophore-producing and nitrate-dissimilating strains decrease. A study confirmed the dominance of *Pseudomonas* sp. in rye, and *Rhizobium* sp. in white clover at elevated CO<sub>2</sub> concentration [85]. *Pseudomonas mendocina* enhances lettuce growth at elevated CO<sub>2</sub> condition [86]. At elevated CO<sub>2</sub> condition, the plant biomass, foliar K concentration and water content increase.

Temperature variation could affect microbial activity for plant growth. Decreased temperature may enhance the activity of certain PGPM, while it may be the reverse in some other cases. The root and shoot significantly increase with the activity of *Mycobacterium* sp., *Pseudomonas fluorescens* and *Pantoea agglomerans* at 16 °C in winter wheat crop in loamy-sandy soil as compared to at 26 °C [87]. The rhizobia isolated from nodules of woody legume *Prosopis glandulosa* shows improved growth at 36 °C than 26 °C [88]. Bacteria colonising at various sites may respond differently to varying temperature, e.g., an endophyte *Burkholderia phytofirmans* reduces colonisation in tomato rhizosphere when the temperature increases from 10 to 30 °C while the endophytic colonisation remains unaffected [89].

Various reports on the effect of drought on the efficacy of effective microbes are available. *Azospirillum* strains improve the plant-water interaction. *Azospirillum* application increases wheat, maize and sorghum yields in water-limiting conditions. *Pseudomonas putida* or *Bacillus megaterium* and AM fungi (*Glomus coronatum*, *Glomus constrictum* and *Glomus claroideum*) association induce development and drought forbearance in plants [90].

**Table 3** EMs and their specific roles in plant growth promotion

Microbial category	Isolates	Association	Plant growth promotion	Ref.
Bacteria	<i>Rhizobium</i> sp., <i>Bradyrhizobium</i> sp.	Symbiosis (legume-rhizobium)	1. N <sub>2</sub> -fixation	[11, 13, 15, 76]
Bacteria	<i>Azospirillum</i> sp., <i>Azotobacter</i> sp.	Asymbiotic (non-legume)	1. N <sub>2</sub> -fixation	[14, 16, 17, 76]
Bacteria	<i>Pseudomonas fluorescens</i> , <i>Pseudomonas putida</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus</i> sp. ( <i>Bacillus subtilis</i> )	Rhizospheric soil	1. Biocontrol agent	[15, 77]
Fungi	<i>Trichoderma viride</i>			
Bacteria	<i>Bacillus</i> sp. ( <i>B. subtilis</i> , <i>B. megaterium</i> , <i>B. mucilaginosus</i> ) <i>Pseudomonas</i> sp., <i>P. fluorescens</i>	Rhizospheric soil	1. Phosphate solubilisation 2. N <sub>2</sub> -fixation	[15, 18, 70, 77]
Fungi	<i>Aspergillus</i> sp., <i>Penicillium</i> sp.			
Yeast	<i>Candida tropicalis</i>		1. Phosphate solubilisation	[77]
Fungi	Arbuscular mycorrhizal fungi (AMF)	Symbiotic	1. Provided tolerance to host plants against various stressful situations (heat, salinity, drought, metals and extreme temperatures)	[78]
Actinomycetes	<i>Streptomyces</i> sp.	Rhizospheric soil	1. Production of antibiotics, siderophores, antimicrobial enzymes, plant growth promoting substances 2. Phosphate solubilisation	[79, 80]

**Table 4** Rhizosphere associated EMs and their impacts on plant growth

Microbial category	Microbial consortium	Association	Plant growth promotion	Ref.
Bacteria	<i>Rhizobium</i> sp., <i>Bradyrhizobium</i> sp.	Symbiotic (legume-rhizobium)	1. N <sub>2</sub> -fixation	[14]
Bacteria	<i>Azotobacter</i> sp., <i>Azospirillum</i> sp.	Asymbiotic (non-legume)	1. N <sub>2</sub> -fixation	[15, 17, 18]
Bacteria	<i>Pseudomonas putida</i> , <i>P. fluorescens</i>	Rhizospheric soil	1. Biocontrol agents, 2. Biological N <sub>2</sub> -fixation 3. Nutrients solubilisation	[16]
Bacteria	<i>Bacillus megaterium</i> , <i>B. mucilaginosus</i>	Rhizospheric soil	1. Phosphate solubilisation 2. Potassium solubilisation	[19]
Bacteria and actinomycetes	<i>Bacillus</i> sp., <i>Pseudomonas aeruginosa</i> , <i>Streptomyces</i> sp.	Rhizospheric soil	1. Prevention of sunflower necrosis disease	[31]
Lactic acid bacteria, yeast and actinomycetes	<i>Lactobacillus plantarum</i> , <i>Candida utilis</i> , <i>Streptomyces albus</i>	Rhizospheric soil	1. Decomposition of organic matter	[22]
Lactic acid bacteria, photosynthetic bacteria, yeast, actinomycetes and fermentative fungi	<i>Lactobacillus plantarum</i> , <i>L. casei</i> , <i>Streptococcus lactis</i> , <i>Rhodopseudomonas palustris</i> , <i>Rhodobacter sphaeroides</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida utilis</i> , <i>Streptomyces albus</i> , <i>S. griseus</i> , <i>Aspergillus oryzae</i> , <i>Mucor hiemalis</i>	Rhizospheric soil	1. Enhancement of the growth pattern and nutrient uptake	[20]

Recombinant DNA technology application has been a successful approach to improve microbial property which in turn stimulates the plant to withstand drought [91]. Trehalose-producing microbes pose the ability to support and promote plant growth under drought stress. There are numerous microbial types that stimulate and promote plant growth under drought stress and these include *Burkholderia phytofirmans*, *Paenibacillus polymyxa*, and *Actinobacteria* [81]. PGPB may stimulate cell division of root and root hairs that eventually help plant to take-up water from deeper soil layer [92]. PGPB may support plant growth under drought situation by regulating abscisic acid and ethylene production [93].

### Effect of EMs on crops

There are several reports of beneficial effects of EMs on crops. This review discusses the effect of EM formulations particularly on cereals, pulses, oilseeds, and vegetable crops. The rationale behind these selected groups of crops is based on the economic value and their

popularity among the Indian farming community. EM formulations and their effect on crops are shown in Table 6.

### Cereals

Rice (*Oryza sativa*) is the staple cereal crop in India, and is a principal food for the half of the world's population. Approximately 480 MMT milled rice are produced annually to feed the increasing global population. Flooding is the conventional approach for rice cropping which means that there is a need for a huge quantity of water. Yet, around 50% of the rice cultivated area worldwide suffers from drought. Water deficit impacts the crops negatively and might result in a significant yield reduction, especially during critical stages of crop growth [104, 105].

Drought stress affects the crop yield significantly as the nutrient uptake by plants decreases. Thus, attempts to engineer draught-tolerant crops requiring less water while maintaining or enhancing the production are

**Table 5** Various EMs for formulation

Microorganisms	Species	Function	Ref.
Photosynthetic bacteria	<i>Rhodospseudomonas palustris</i> <i>Rhodobacter sphaeroides</i>	1. Produces amino acids, nucleic acids, bioactive substances and sugars 2. Conducts photosynthesis	[20, 24, 25, 30, 32]
Lactic acid bacteria	<i>Lactobacillus plantarum</i> <i>Lactobacillus casei</i> <i>Streptococcus lactis</i>	1. Suppresses and reduce pathogenic microbes 2. Expedites decomposition of organic matters	[20, 22, 24, 25, 30, 32]
Actinomycetes	<i>Streptomyces albus</i> <i>Streptomyces griseus</i>	1. Produces antimicrobial substances to inhibit pathogens 2. Enhances the decomposition of phospholipid compounds	[11, 22, 24, 25, 30, 31]
Fermentative fungi	<i>Aspergillus oryzae</i> <i>Mucor hiemalis</i>	1. Decomposes organic matter 2. Synthesises amino acids and glucose from carbohydrates 3. Control odours	[20, 29]
Yeast	<i>Saccharomyces cerevisiae</i> <i>Candida utilis</i>	1. Degrades dead plant tissue and stimulate root growth	[20, 22, 24, 25, 30, 32]

**Table 6** EM formulations and their effect on crops

Crop type	Crop	Microbes in EM formulations	Effect on crops	Ref.
Cereal	Rice	Arbuscular mycorrhiza fungi, <i>Glomus intraradices</i>	1. Improved growth of rice crops under drought pressure	[94]
		<i>Bacillus subtilis</i> , <i>Pseudomonas fluorescens</i>	1. Biocontrol agents with antagonistic nature	[41]
		<i>Azospirillum brasilense</i> , <i>Phyllobacterium brassicacearum</i>	1. Growth of plants under drought situation and nutrient limitation	[95]
		<i>Pseudomonas putida</i> (or <i>Bacillus megaterium</i> ) and AM fungi ( <i>Glomus coronatum</i> , <i>Glomus constrictum</i> , <i>Glomus claroideum</i> )	1. Plant development and drought tolerance	[90]
Pulses	Rajmah	<i>Pseudomonas lurida</i> -NPRp15, <i>P. putida</i> -PGRs4, <i>Rhizobium leguminosarum</i> -FB1	1. Effective for plant growth through N <sub>2</sub> -fixation	[96]
	Bean	<i>Rhizobium</i> sp., <i>Bacillus megaterium</i> (M-3), <i>Bacillus subtilis</i> (OSU-142)	1. N <sub>2</sub> -fixation 2. Phosphate solubilisation	[97]
	Soybean	<i>Bradyrhizobium japonicum</i>	1. Symbiotic N <sub>2</sub> -fixation	[98]
Oilseed	Sunflower	<i>Rhizobium</i> sp., <i>Trichoderma hamatum</i>	1. Increase in chlorophyll, root and shoot length, mineral (N and P), total carbohydrate and protein contents of crop	[98]
		<i>Pseudomonas putida</i>	1. Drought tolerance	[31]
		<i>Trichoderma harzianum</i>	1. <i>Plasmopora halstedii</i> targeted	
		<i>Bacillus</i> sp., <i>Pseudomonas aeruginosa</i> , <i>Streptomyces</i> sp.	1. Inhibited sunflower necrosis disease	[99]
	Groundnut	<i>Azospirillum brasilense</i>	1. Enhanced tap root growth	[100]
		<i>Pseudomonas fluorescens</i>	1. Stimulated lateral root growth	
		Actinomycetes	1. Hydrogen cyanide, lipase, siderophores, and indole acetic acid production 2. Inhibited soil born pathogens such as <i>Sclerotium rolfsii</i>	[45]
Vegetables	Okra	<i>Enterobacter</i> sp. UPMR18	1. Helped in salt tolerance under salinity stress	[101]
		<i>Trichoderma harzianum</i>	1. Suppressed fungal infections	[102]
		<i>Pseudomonas aeruginosa</i> , <i>Trichoderma viride</i>	1. Biocontrol against <i>Fusarium oxysporum</i> , <i>F. solani</i> , <i>Macrophomina phaseolina</i> , <i>Rhizoctonia solani</i> and <i>Meloidogyne javanica</i>	[103]

being made. Genetically variant drought tolerant variety could have enhanced proline and abscisic acid production, stabilized superoxide dismutase activity for photosynthesis and improved root system [106]. Using a consortium of advantageous microbes is a prospect towards enhancing drought-resistant plant. Soil microbes like AMF attached to plant roots interact with specific microbial communities to develop an array of activities to enhance crop growth and yield under drought stress conditions [107].

Rice readily forms mycorrhizal associations in upland conditions. However, this is uncommon in flooded conditions as the anoxic condition develops at plant-soil interface. To encourage arbuscular mycorrhiza (AM) symbiosis, aerobic non-flooded farming conditions to boost establishment of AM fungi in the rice roots may be resorted [108]. AMF stimulate the metabolic response in plant under drought stress [109]. AMF *Glomus intraradices* enhances rice growth under drought condition where the shoot weight increases by 50% with AM symbiosis compared to the non-AM plants [94]. The photosynthesis increases by 40% along with the accumulation

of antioxidant molecule glutathione. AM symbiosis reduces hydrogen peroxide and decreases oxidative damage to the lipids [94, 110]. Positive association in *Pseudomonas putida* or *Bacillus megaterium* and AM fungi (*Glomus coronatum*, *Glomus constrictum* or *Glomus claroideum*) in drought situations positively affect crop development and drought forbearance [90]. PGPR like *Azospirillum brasilense*, *Phyllobacterium brassicacearum* and AM fungi increases crop survival in drought and nutrient limitation like situations. Biomass growth and grain yield increase in rice by applying these microbial formulations [95, 111].

### Pulses

Majority of the global population depend on pulses for the major amount of their protein requirements. Pulses primarily include chickpea, rajmah, black gram, green gram, beans and lentil. Of these, black gram is a major food crop in India [41]. Green gram (*Vigna radiata*) and black gram (*Vigna mungo*) grown under tropical and subtropical conditions are important food legume as protein source. As these are almost free from gassiness



causing factors, green gram and black gram seeds are preferred to feed babies.

India is on the way to develop alternative agricultural practices to obtain higher pulses yield to fulfil the need of its larger population. EM formulation enhances crop growth and yield in both leguminous and non-leguminous pulses. *Pseudomonas lurida*-NPRp15 and *Pseudomonas putida*-PGRs4 either individually or in combination with *Rhizobium leguminosarum*-FB1 are effective in growing rajmah [96]. While individual inoculant increases plant dry biomass, nitrogen, phosphorus, potassium, zinc and iron contents, *Pseudomonas lurida* NPRp15 and *Rhizobium leguminosarum* FB1 (or *Pseudomonas putida* PGRs4, *Pseudomonas lurida* NPRp15 and *Rhizobium leguminosarum* FB1) combination enhance the root and shoot dry weight, nutrient uptake, nutrient content, nodulation and pod yield in rajmah [96]. The EM of *Rhizobium*, phosphate-solubilising *Bacillus megaterium* (M-3) and N<sub>2</sub>-fixing *Bacillus subtilis* (OSU-142) has also been encouraging on bean plant. These are effective in nutrient uptake, nodulation, shoot and root dry weight, seed yield and plant growth. These are equally more effective as compared to chemical fertiliser as well [97].

### Oilseeds

Sunflower (*Helianthus annuus*) is an important oilseed crop and is the second most important source of global vegetable oil. India ranks 4th by area and 8th in production of sunflower. Karnataka, Andhra Pradesh and Maharashtra are the major sunflower growers contributing about 91% of the total sunflower cultivation area and 82% of total sunflower production [112]. Wheat bran compost containing *Rhizobium* sp. and *Trichoderma hamatum*, either individually or in combination, shows an increase in total chlorophyll, root and shoot lengths, minerals (nitrogen and phosphorus), carbohydrate and protein contents in sunflower [98]. Applying symbiotic nitrogen-fixer *Bradyrhizobium japonicum* (strain TAL-102) EM as biofertilizer or in combination with farm (or green) manure in soybean has positive benefit. Coinoculating *Bradyrhizobium japonicum* and biofertilizer with farmyard manure exhibits the highest biomass of the shoot, number and biomass of pods compared to other treatments [113]. EM formulation of *Azospirillum brasilense* and *Pseudomonas fluorescens* individually or mixed has been applied on groundnut plant through seed treatment, soil application, seedling root tip and foliar spray [99]. *Azospirillum brasilense* enhances tap root growth, whereas *Pseudomonas fluorescens* is effective in lateral root growth. A consortium mix enhances leaf numbers and shoot growth. Out of all treatments, soil application is the most effective [99].

Drought tolerance of oilseed plant could increase by applying suitable microbial formulation. Sunflower seeds treated with *Pseudomonas putida* could withstand drought [31]. Besides drought tolerance, EM is effective against plant pathogens in sunflower. Applying *Trichoderma hazianum* suspension on sunflower crop prevents it from *Plasmopora halstedii* (downy mildew). Seed treatment of sunflower with mixed consortium of *Bacillus* sp., *Pseudomonas aeruginosa* and *Streptomyces* sp. is fruitful against sunflower necrosis viral disease [31]. EM formulation increases disease resistance in groundnut; actinomycetes could prevent stem rot disease (by *Sclerotium rolfsii*) [100]. Actinomycetes inhibit *Sclerotium rolfsii* by producing various antibiotics/chemical agents, viz., hydrogen cyanide, lipase, siderophores and indole acetic acid [100].

### Vegetable plant

Okra (*Abelmoschus esculentus*) is grown throughout the tropical and warm temperate regions for its fibrous pods eaten as a vegetable. It is attacked by numerous insect pests. Various insect pest's infestation that decrease the pod yield in okra include fruit borers, shoot borers, leaf hoppers, sucking insects, chewing insects, aphids, root feeding insects, and mites. They suck the cell sap of the plant thereby destroying the plant vigor. The crop is tolerant to the most of the insect pests in wet season, while leaf hoppers and aphids may cause damage during dry season [114]. Although chemical control of the pests is generally practiced for higher yield, use of chemicals alone is not advisable due to shorter interval in the periodical harvest. Thus, it becomes relevant to look for effective and eco-friendly alternatives.

Wokozim, kissan supreme tonic (KST) and EM formulation (foliar) applications in okra for pest control show that KST application is the most effective against sucking pest complex and pod borers resulting in the increase in pod yield. It has been shown that although EM application results in low pod yield as compared to KST and Wokozim but it results in higher final yield (8431 kg ha<sup>-1</sup>) as compared to 8012 kg ha<sup>-1</sup> in control [38]. Siddiqui et al. [101] suggested that *Trichoderma*-enriched compost was more eco-friendly as against inorganic fertilisers, and enhanced crop yield by benefitting okra cultivation. EM formulation of *Enterobacter* sp. UPMR18 enhances the salt tolerance property of okra; while enhancing salt tolerance it also enhances its germination percentage as well as chlorophyll content [45].

Strains of *Pseudomonas aeruginosa* alone or with *Trichoderma viride* (entophytic bacteria) exhibit substantially enhanced disease resistance in okra against *Fusarium oxysporum*, *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Meloidogyne javanica* (the root knot nematode). It brings positive impact on plant growth by improving plant height, fresh shoot

weight and root length [102]. *Brucella* K12 strain, a Cr(VI) reducing bacterium, reportedly enhances the growth/yield of okra in Cr-contaminated soils [103].

## Conclusions

EMs enhance plant growth and productivity by fixing atmospheric  $N_2$  and supplementing the plants with the fixed nitrogen as ammonia. Additionally, the release of trace elements, secreted antioxidant, exopolysaccharides, bioactive compounds (vitamins, hormones and enzymes) by the EMs stimulate plant growth and productivity. Biocontrol agents secreted by the EMs protect the plants from harmful microbes as also from environmental stress. EMs contain primarily the photosynthetic and lactic acid bacteria, fermentative fungi, yeasts, actinomycetes, among others and could be formulated by adding a solid or a liquid carrier to it. The resulting EM formulation could be applied to the soil by spraying on leaves (foliar application), soaking seeds in it (seed treatment) and through irrigation (fertigation/soil application). The review discusses the impact of various EM formulations on cereals, pulses, oilseed and vegetable plants. Application of EM formulation improves grain productivity, biomass accumulation, photosynthesis efficiency and drought tolerance in cereals. It increases the trace elements, biomass, shoot weight, root weight, modulation, pod production in rajmah, and nodulation, root-shoot weight and seed yield in bean. It increases shoot weight, pod number and biomass in soybean. EM formulation positively affects root-shoot growth, chlorophyll, nitrogen, phosphorus, carbohydrate and protein content, drought tolerance, virus resistance, leaf number and fungal disease resistance in sunflower and groundnut. In vegetable plants like okra, EM formulation improves the shoot-root growth, plant height, chlorophyll content, pod yield, fungal disease resistance, Cr-resistance and insect pest resistance. Thus, for sustainable and more promising green agriculture, microbial formulations have an important and indispensable role to play in modern agriculture for cropping of cereals, pulses, oilseeds and vegetables.

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## Authors' contributions

KN and HS prepared the primary manuscript. PKS and AC helped in providing results, information and preparation of tables and figures. SM conceptualised, revised, corrected and edited the manuscript. All the authors have read and approve the final manuscript. KN and HS contributed equally as first authors.

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## Availability of data and materials

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## Competing interests

The authors declare no competing and/or conflict of interests.

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