

Chapter 10

Microbial Bioformulations: Present and Future Aspects



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Contents

10.1	Introduction.....	243
10.2	Current Situation of Bioformulations.....	245
10.3	Consortia- or Inoculant-Based Bioformulations.....	246
10.3.1	Inoculums Production Approaches.....	248
10.3.2	Outcome of Inoculants/Formulations.....	249
10.3.3	Effects of Co-inoculations vs Mono-inoculations.....	251
10.4	Problems, Challenges, and Approaches.....	252
10.5	Conclusions.....	252
	References.....	253

10.1 Introduction

One of the hardest tasks these days is to employ sustainable agricultural practices. Moreover, agricultural practices around the globe are not uniform, but usage of chemical pesticides and fertilizers is common. Around 890 man-made chemically prepared various chemicals are certified that can be used as pesticides and insecticides (Stenersen 2004). These chemicals play a crucial role in improving the crop yield and inhibiting diseases, but they are also leaving their harmful effect on environment (Fenske and Day 2005; Colt et al. 2007). The condition in developing countries is very bad; despite the fact that the usage of agrochemicals in these countries is only 20%, they encounter about 99% of mortality rate due to pesticide poisoning (Kesavachandran et al. 2009). Chief victims are farmers due to high exposure and lack of awareness. About 20,000 workers die because of exposure to pesticides every

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year (Pimentel et al. 1992). According to the WHO, majority of the reports show that pesticides used for agricultural practices have very malignant influence on human well-being; short-term effects include headaches, nausea and vomiting, rashes, respiratory failure, coma, shock, etc. (Moses et al. 1993), whereas long-term effects include reproductive problems, cancer, and neurological disorders, and in serious cases it can cause death (Sanborn et al. 2007). Further, wildlife, aquatic ecosystems, and environment also get disturbed (Berny 2007). Continuous usage of pesticides drastically influences the microbial systems present in soil, and it kills large diversity of microbial population which is beneficial for the agriculture or crop production (Dorigo et al. 2009). Different types of pesticides have different effect on microbial populations and greatly influence the microbial diversity (Johnsen et al. 2001; Spyrou et al. 2009). Chemically synthesized fertilizers are another important factor on which the majority of our agriculture depends. Different studies were conducted to determine the long-term effects of these fertilizers on fertility of soil, and reports show that regular usage can elevate the strength of total nitrogen, organic matter, and different nutrients in soil as compared to the primary values present at the start of experiment (Liang et al. 2013; Mitchell et al. 1991; Mandal et al. 2007). But quality and productivity of soil is gradually deteriorating due to excessive usage of chemical fertilizers. They also influence the microbial diversity and their functions (Nakhro and Dkhar 2010). Formulations based on microbes are known as “bioformulations.” In other words, formulations which constitute of several valuable strains of microbes, which are immobilized or trapped on an inert carrier material, that can be employed to enhance plant growth and inhibit plant pathogens and can increase fertility of soil are known as bioformulations (Mendes et al. 2011). Bioformulations are found to be more effective than synthetic chemicals because formulations synthesized by a single microbe can interact with plant pathogens and can have a role in plant growth promotion and disease inhibition (Arora et al. 2010). The abiotic substrates that have the ability to provide protective environment to cells and can deliver cells viably under proper physiological environment are used as carrier molecules. Various types of substrate can be employed as carriers such as inert substances (perlite, vermiculite, polymers), some liquids, and soils (clays, peat, coal) (Bashan et al. 2014). Majority of the studies executed emphasize on the (i) development of better carrier molecules; (ii) search for microbes which can enhance crop yield; and (iii) enhancement of metabolic state and potential of the cells, so that they can be used as intercellular storage devices and can survive within carrier molecules (Kadouri et al. 2005). Bioformulations have to go through various stressful conditions during storage and production, where microbes have to survive through different situations like desiccation, hot conditions, etc. Microbes should retain high survival rates and sustain their capabilities to enhance plant development for extended durations. Various strategies are used by microbes for their survival like formation and aggregation of polyhydroxyalkanoates (PHA) or osmolytes. Higher level of tolerance toward desiccation is shown by microbes which are osmoadapted and osmolytes such as glycine betaine or trehalose are aggregated by them, in comparison to the cells which are non-osmoadapted (Bonaterra et al. 2005). Those cells which have higher PHA levels have more survival potential than those cells which have lower PHA levels; this is due to the fact that PHA imparts caliber to the cells so that they can withstand against the

unfavorable physical and chemical strains (Morel et al. 2012). The most remarkable microbial-plant synergism is seen in the diazotrophic microbial relationship with plants. Diazotrophs may be symbiotic or free-living microbes which have potential to fix as well as reduce atmospheric nitrogen into ammonia; some examples of diazotrophs include rhizobia, *Azotobacter*, *Gluconacetobacter diazotrophicus*, *Azospirillum*, and *Azoarcus*. Certain phytohormones, ACC deaminase, phosphate-solubilizing molecules, iron-sequestering siderophores, and other molecules are produced by some plant growth-promoting bacteria (PGPB) and diazotrophs. *Bacillus* and *Pseudomonas* come under non-diazotrophic, plant growth-promoting bacteria (Morel and Castro-Sowinski 2013). The most commonly studied symbiotic relationship includes legume-rhizobia association for agricultural improvement and productivity enhancement. Such type of associations provides enough evidence that unification of different valuable microbes, exhibiting distinct routes of plant growth enhancement, has cumulative and synergic influence on plant development and crop yield (Morel et al. 2012). Several reports also suggest that combo of secondary metabolites produced by plants with bioformulations may amplify the agricultural productivity. However, nature-friendly bioformulations are not so popular in agro-market because of some limitations associated with them (Morel et al. 2015). The constraints include sustenance of microbial biota and vigor, unpredictable field performance, inconsistency in quality, and inadequate shelf life. There are some queries which are yet to be answered so as to gain trust of consumers and to make agricultural practices chemical free (Arora et al. 2010). To answer the questions, it is essential to ascertain the work done in the previous reports and also to know the idea in which mainstream research is going so as to determine the future aspects for the development of superior bioformulations. This chapter deals with the current and future aspects of bioformulations.

10.2 Current Situation of Bioformulations

For the elimination of plant pathogens, microbial-based formulations are being utilized all around the globe, but the supportive information about its usage all over the world is very limited (Leggett et al. 2011; Naderifar and Daneshian 2012). One of the major reasons is difference in terminology. Majority of the developing countries use the term “biofertilizers,” whereas in developed countries the term “bioinoculant” for crop yield and improvement is used, but in both of the cases either compounds are isolated from living organisms or whole organism is employed for the enhancement in nutrient uptake by plants so as to improve crop yield and soil quality (Vessey 2003; Chen et al. 2006; Prasad et al. 2018). Many producers (farmers) around the globe regularly employ biofertilizers and biopesticides into their different types of crops. The most advanced and prevalent market for formulations is European biofertilizer market as compared to all other domains, and growth from \$2566.4 million in 2012 to \$4582.2 million was observed in 2017, at an annual growth rate of 12.3% from 2012 to 2017 (PRWEB 2014). In 2012, biofertilizer market was highest in North America and is expected to develop at the rate of 14.4%

in the duration of 2013–2018 (Micro Market Monitor 2015). China is the chief grower of wheat and rice along with cabbage and onions as well as promotes the use of biofertilizers (Grand View Research 2015). In India, there are around 151 biofertilizer manufacturing units which are run by government and nongovernment agencies (Mahajan and Gupta 2009). Mainly nitrogen-fixing biofertilizers were used in maximum as compared to all other biofertilizers, and in 2012 their worldwide demand increased over 78% (Agro news 2014). In controlling plant diseases, *Bacillus thuringiensis* (BT)-based biopesticides are more popular and constitute for 95% of the overall microorganisms employed (Bravo et al. 2011). Around 322 BT products generate a revenue of \$210 million annually (CAB International Centre 2010). Usage of fungal and non-BT biopesticides is also growing. Various agencies have carried out market research survey, but outcomes of these kinds of surveys are not very reliable and are questionable. This is because criteria employed in market research survey may deviate since many firms and agro-based industries involve subcategories such as biochemicals, plant-induced protectants (PIPs), microbes, plant growth regulators, pheromones, insect growth regulators, and essential oil in the name “biopesticide,” while others use only the products which are microbial in origin (Gelernter 2007). Around \$672 million was biopesticide turnover in 2005 (description of category was not included) (Thakore 2006), and worldwide market of biopesticide was about \$280 million in 2007 (for true microbial agents) (Harwood et al. 2007). CPL (2006) and BCC (2010) are business consultancies which are vigorously conducting direct market survey and generating a reliable data, and their reports show that biopesticide market is enhancing at a rate of 10% every year, globally. Biopesticide market was expected to exceed by \$2.5 billion by 2015, via global industry analysis 2015. Other research surveys conducted by BCC on biopesticide suggested that total sale of biopesticide in 2008 was \$1.2 billion and in 2009 it was \$ 1.6 billion. And it was expected to increase in 2014 to around \$3.3 billion and in 2017 around \$10 billion (Marrone 2007). Region-wise research reports highlight that the United States is the largest region of biopesticide globally, while Europe is the fast-growing regional market for biopesticide and represents an average annual growth rate of 15.0%. Asia Pacific is also an emerging market for biopesticides, where sales were expected to be around \$362 million in 2012. Latin America has shown very little increase as compared to other regions. In 2005, market was about \$70 million, and in 2010 it reached only \$88 million, with an average annual growth rate of 5.0% (Industrial Equipment News 2011). Table 10.1 shows the types of microbes, main crops, and areas in world utilizing microbes as bioinoculants/bioformulations.

10.3 Consortia- or Inoculant-Based Bioformulations

Majority of the bioformulations available are mostly composed of single strain or mixed cultures or are in co-inoculations with other microbes. Use of such co-inoculations helps in overall development and plant growth promotion. When mycorrhiza co-inoculated with rhizobia, it displayed enhanced performance with legumes. This association helps to enhance the nutritional value of most nodulated plants and

Table 10.1 Types of crop, microbes, and agricultural market of bioinoculants

Market by type	Market by mode of application	Market by microbial type	Market by type of crop	Market by type of geography
Biocontrol agents	Seed inoculant	Bacteria: rhizobacteria N ₂ fixing P-solubilizing Cyanobacteria Others	Cereals and grains	North America: USA, Canada, Mexico, other North American countries
Plant resistance stimulants	Soil inoculant	Fungi: <i>Trichoderma</i> sp., AM fungi <i>Aspergillus</i>	Oil seed and pulses	Europe: Germany, France, Spain, Italy, Denmark, other European countries
Plant growth-promoting microbes	Foliar spray	Others: Azolla-Anabaena	Fruits and vegetables	Asia Pacific: Australia, China India, Japan, other countries
Plant stress manager	Other inoculants		Other crop types	South America: Brazil, Chile, Argentina. LAMEA countries (Latin America, Middle East, and African countries)

also raises the tolerance toward drought and other osmotic stress in pigeon pea (Bhattacharjee and Sharma 2012), lucerne (Ardakani et al. 2009), soybean (Gao et al. 2012), broad bean (Jia et al. 2004), and chickpea (Tavasolee et al. 2011). Several reports also suggest that plant growth is stimulated after the employment of nodule-forming bacteria with Phosphate solubilizing bacteria (PSB) in leguminous plants (Messele and Pant 2012). Currently, several studies have been conducted related to consortia formulation development by different researchers and patents have also been filed (Paikray and Malik 2010). Maiyappan et al. (2010) conducted a study in which a bioformulation consortium was prepared (as a wettable powder) which involved nine strains of the following genera—*Frauteria*, *Bacillus*, *Azotobacter*, and *Streptomyces*, and this formulation was found to be useful for black gram. Similarly, consortium bioformulation was prepared using *Burkholderia* species MSSP plus three other plant growth-promoting bacteria and was examined for the development of *Cajanus cajan*, by employing different carriers like sawdust, rock phosphate, bagasse, wheat bran, cocoa peat, charcoal, rice husk, and paneer whey, and this consortium was found to be effective in enhancement of pigeon plant growth, when used as a formulation (Pandey and Maheshwari 2007). Studies conducted by Tajini et al. (2012) demonstrated that in bean plants, when arbuscular mycorrhizal (AM) fungi and rhizobia are inoculated in combination, they help in enhancing uptake of nitrogen and potassium in plants as compared to single inoculation. Further, according to some researchers, the consortia can be prepared using *Azotobacter*, microalgae, and cyanobacteria, which can be employed as biofertilizer and bio-stimulator (Zayadan et al. 2014). Figure 10.1 shows the basic comparison of plant growth promoting rhizobacteria (PGPR) and bioformulations used in agroecosystem.

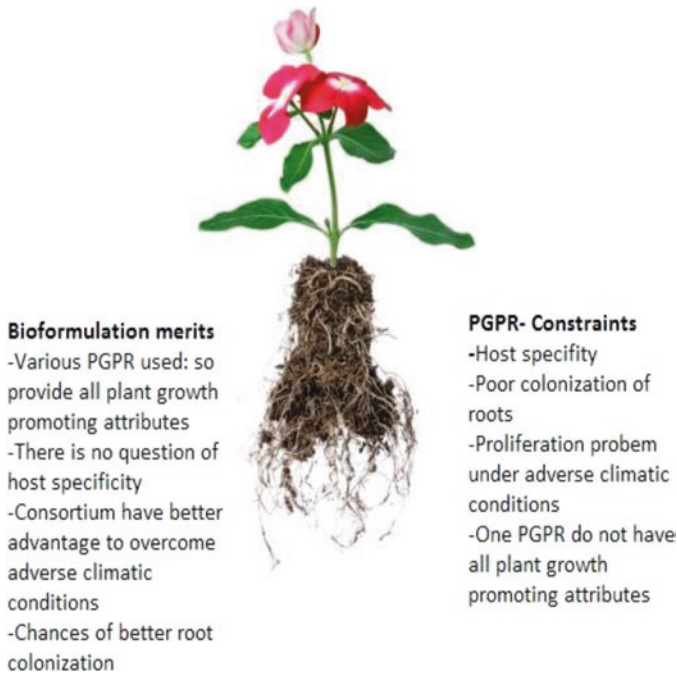


Fig. 10.1 Some comparisons of bioformulations with PGPR under agro-climatic conditions

10.3.1 Inoculums Production Approaches

10.3.1.1 Inoculants Based on Carriers

A carrier is usually locally available and reasonably affordable material that has efficient water-holding capacity and is competent enough to slowly liberate its viable cells into soil. Agro-industrial residues, peat, compost, vermiculture, charcoal mixed with soil, bentonite, and perlite are some of the commonly available and economically friendly organic materials that are utilized for making microbial inoculants. After a suitable carrier is selected, it is sterilized and amalgamated with definite microbes such as phosphate-solubilizing or nitrogen-fixing microbes under sterile environment. Such carrier-based bioformulations can be stored only for 3–4 months and have high cell density. When stored at room temperature, cell density decreases at a very high rate (Trivedi et al. 2005).

10.3.1.2 Inoculants Based on Clays

The most widely utilized formulation in agricultural domain is “clay.” It may be employed in a number of forms such as powder, suspension, or granules. Use of clays can increase the shelf life of microbial strains as they have large pore size and surface area. It offers suitable conditions for microbes to flourish (Smith 1992).

10.3.1.3 Liquid Formulations

Liquid bioformulations are made by using specific microbes which are prepared in a mineral-based medium, broth-based medium, or organic oil-based suspension (Schisler et al. 2004). When these liquid formulations are employed, the microbial inoculants come in direct contact with soil or seeds and perform their action; however, protective environment for microbial strains is absent. This kind of formulation is not suitable for long-term storage and is highly susceptible to contamination during storage. Suitable environmental conditions are required for maintenance so that microbial strains do not lose their viability and efficacy (Brar et al. 2006).

10.3.1.4 Encapsulation

In bio-encapsulation procedure, active strains are incorporated into solid or liquid matrix, where materials like polystyrene, alginate, agarose, polyacrylamides, agar-agar, polyurethane, carrageenan, synthetic polymers, etc. are utilized as matrix (John et al. 2011). The stabilization is done by chemical polymerization. It can be stored as long as 5 years.

10.3.1.5 Use of Biofilms

Biofilms are based on microbial cell aggregates. Biofilm involves four stages in its development which are (a) primary adherence, (b) irrevocable immobilization by generation of exo-polysaccharide, (c) prematuration stage, and (d) maturation stage. Biofilms prepared by employing fungal and bacterial strains have been found adequate (Seneviratne et al. 2008).

10.3.2 Outcome of Inoculants/Formulations

10.3.2.1 Mycorrhizal Fungi Inoculants

Arbuscular mycorrhizal fungi belonging to phylum *Glomeromycota* have potential to form symbiotic relationship with majority of the land plants (Schubler et al. 2001; Prasad et al. 2017a). They use their host as a carbon source and help the host plant by promoting its growth by providing nutrients, water, and minerals. AMF influence the soil microbes and form mycorrhizospheric zone in the soil (Linderman 1988). The AMF present in mycorrhizosphere may have positive (Albertsen et al. 2006) or negative (Cavagnaro et al. 2006) or no effects (Olsson et al. 1996) at all on the growth of microbes or microbial biomass. Several studies show that certain bacterial species behave differently in the vicinity of specific AMF; this suggests that bacteria and AMF might have high degree of specificity between them (Artursson et al. 2006). Therefore, AMF associated with specific bacteria may prove to be useful for plant

development by providing essential nutrients, enhancing branching of roots (Barea 1997). AMF also provide protective benefits by inhibiting the proliferating pathogens by forming the bacterial populations which limits the invasion of pathogens (St-Arnaud and Vujanovic 2007). *Glomus intraradices* shows beneficial effects on the development of bacterial fauna and saprotrophic fungal biomass (Albertsen et al. 2006).

10.3.2.2 *Azospirillum* Inoculants

Agricultural benefits of *Azospirillum* are well known (Okon and Labandera-Gonzalez 1994). *Azospirillum* inoculation highly influences the root exudation and development; the utilization of these phyto-stimulatory plant growth promoters can also affect the microbial fauna present in rhizospheric zone (Dobbelaere et al. 2001). In some studies, *Azospirillum brasilense* sp 245 was found to be useful in plant growth, as it helps in generation of auxins, gibberellins, and cytokinins (Steenhoudt and Vanderleyden 2000). Naiman's study suggested that when *Azospirillum* and *Pseudomonas* are co-inoculated on wheat fields, they displayed different effects on the bacterial communities flourishing on rhizospheric zone of the wheat. This inoculation altered the carbon source usage of soil microbial communities (Naiman et al. 2009). Carbon source utilization is directly related to the total microbial population capable to utilize every carbon source, and their growth reflects the functional capabilities of the microbial community. The two *A. brasilense* strains (42M and 40M) isolated from roots of maize when inoculated altered the physiological profiles of the microbial flora and fauna linked to rice (De Salamone et al. 2010).

10.3.2.3 *Rhizobia* Inoculants

As per several reports, rhizobia have enhanced effect on crop yield and plant development. They help in nitrogen fixation and uptake of nutrients like iron and phosphorus; stimulate plant hormones; promote the growth of favorable bacteria and fungi; and check fungal and bacterial diseases, insects, and pests. The entire rhizospheric biodiversity can be seen in the form of different functional groups which is mostly influenced by the alterations in the residual nitrogen rather than the effects of inoculation (Antoun and Prévost 2005). Further, the exact mechanisms which play key role in these changes are still not clearly known and need to be explored (Saharan and Nehra 2011).

10.3.2.4 Biocontrol Agents

Majority of rhizobacterial products have been employed as biological control for plant disease prevention instead of effecting plant nutrition uptake or dealing with abiotic stress factors (Berg 2009). Variety of microorganisms like *Bacillus subtilis*

(Dawar et al. 2010), *Trichoderma harzianum* (Mohiddin et al. 2010), and *Pseudomonas fluorescens* (Peighami-Ashnaei et al. 2009) have antagonistic effect against the diseases caused by *Sclerotium* species, *Pythium* species, *Rhizoctonia* species, and *Fusarium* species, which leads to high yield or plant growth promotion. *P. fluorescens* (Pal et al. 2000), *Pochonia chlamydosporia* (Kerry 2000), and *B. subtilis* (Khan et al. 2001) when employed are found to be effective against the diseases triggered by nematodes. Some *Pseudomonas* species are antagonist against the activity of tomato plant pathogen *Ralstonia solanacearum* (Kozdroj et al. 2004). Biocontrol agents like *Corynebacterium glutamicum* (Vahjen et al. 1995), *P. fluorescens* (Natsch et al. 1998), and *Streptomyces melanosporofaciens* (Prevost et al. 2006) when applied show transient effects on soil ecosystem and fungal flora and fauna, and it designates that the efficacy of the biocontrol agent may be for limited duration.

10.3.3 Effects of Co-inoculations vs Mono-inoculations

In most of the inoculations, usually single strain is applied which has displayed inconsistent results in the fields. This issue can be solved by employing different strains or different species of valuable microorganisms in the single formulation of microbes. By applying consortium of bioinoculates, there is no need of genetic engineering (Janisiewicz 1996) as different strains involve different working mechanisms to enhance the plant development, promise the efficiency and reliability that it will have positive effects on crops (Marimuthu et al. 2002). It was reported that when PGPR or AMF were inoculated, they induced slight modification on the bacterial community structure present in wheat rhizosphere (Roesti et al. 2006). The type of PGPR consortium utilized had additional effect on the bacterial community structure as compared to the AMF. Further PGPR strains employed produce an anti-biotic, i.e., 2-4-diacetylphloroglucinol, which has antifungal effect; however, it does not affect the growth of AMF, and associative or synergic outcome of PGPR and AMF co-inoculation was seen.

When *Pinus pinea* was inoculated with two different strains of *Bacillus*, i.e., *Bacillus pumilus* CECT105 and *Bacillus licheniformis* CECT5160, both of these *Bacillus* strains encourage the seedling development of *P. pinea* (Probanza et al. 2002). But this positive effect was not observed when both the *Bacillus* strains were co-inoculated, maybe as a result of competition effect (Probanza et al. 2002). Further, the combo of *A. brasilense* and *B. subtilis* did not display any synergic or associative effects on tomato plants in comparison to their single inoculations. Therefore, it may be concluded that when inoculants are employed in combination, they may not necessarily show synergic effects, instead they may display competitive effects, and as a result the growth and development could be minimum or gradually disappear. Similarly, the effects on microbial flora and fauna present in soil are also uncertain or unpredictable.

10.4 Problems, Challenges, and Approaches

The current researchers are becoming more aware and focusing their work to deal with growing problems like increasing urbanization and growing population. It is expected that by 2050, about 10 billion people would inhabit the planet. With increase in population, many issues will also arise, putting more pressure to produce food, fiber, and energy resources with simultaneous sustainable approach. The growing demands cannot be avoided; however, this will put immense risk to nonrenewable resources like fossil fuels, water, energy resources, agricultural soil, etc. Further, with more and more expanding industries, contamination is also at its highest peak (Browne et al. 2013). Emission of greenhouse gases is causing the rise in earth's temperature, disturbing the environmental stability, and giving rise to various stressful scenarios which affect both agriculture and natural systems (Duarte et al. 2006). Problems like salinity of soil, drought, nutrition deficiency, diseases, soil erosion, pests, crop destruction due to natural calamities, loss of biodiversity, deforestation, landscape fragmentation, use of chemicals, etc. are affecting humans either directly or indirectly (Vitousek et al. 1997).

In view of these issues, sustainable agricultural practices and various approaches are being implemented to meet the demands without hampering the natural ecosystems; this can be achieved only when there is balance between the three major interacting domains, i.e., environment, society, and economy. The balanced interaction cycle between these three domains can finally give the true meaning to the "sustainable development" approach (Altieri 2004). In the concept of sustainability, the vital issues are regarding depletion of nonrenewable resources, controlling pests and pathogens, suitable methods for recycling soil nutrients, dealing with abiotic stress, maintaining the vitality of soil microbes (which depends on soil microbes), etc. for the global ecosystems and human welfare (Zancarini et al. 2013; Prasad et al. 2014, 2017b). Majority of these issues can be resolved by using microbial services (Zolla et al. 2013). Microorganisms can be exploited after the identification of their beneficial functions/features in terms of both cost-effective and ecological sustainability.

10.5 Conclusions

The discussion in this chapter regarding development and uses of some novel bioformulations will definitely be useful in sustainable agroecosystem. The application of microbial consortium is an essential constituent of agroecological practice, which is a reliable technology whose time has come to sustain the soil and fulfill the requirement of food and feed. These microbes in the bioformulations have been successfully employed in several parts of the globe and with encouraging results, that with time this notion of utilizing microbial consortium as bioformulations will certainly grow. In the case of underdeveloped, developing, and developed world, where agricultural inputs are synthetic and quite expensive, the application of

bioformulations conquers an insignificant but developing role in organic agriculture development. Additionally, the microbial consortium in bioformulations also acts as biocontrol and stress manager and in many phytoremediation approaches. The cost-effectiveness and easy availability of bioformulations have made it a choice of farmers and scientists, since this is also a step toward organic agriculture. According to one survey carried in the USA, it was found that both organic and conventional growers are taking interest in growing and consuming organic products, since such products are not having side effects on health as caused by chemically growing agriproducts. Rzewnicki (2000) suggested that in the coming years the bioformulations demand will have huge market potential. Globally, the sale of organic products grew by 8% in the year 2010, which is expected to increase by 27% by the year 2020 (Komorowska 2014). In relation to organic production using organic manure and bioformulations, encouraging growth has been seen and still continued in the main European markets and in the case of the USA, and the viewpoint regarding bioformulations for coming years is very positive. Interestingly, China's organic market has grown four times in the last 5 years only. Moreover, in the case of Brazil, the organic produce has shown the growth rate of 40%, which is quite amazing. Regarding Asia, the market analysts forecast that organic sales using bioformulations is expected to grow by 20% a year over the next coming 3 years. All over the globe, 37 mh of land are now farmed organically, and most of the land also utilizes the microbes in one form or another. India has to work hard to achieve the good growth of organic farming using microbial bioformulations. The agricultural universities and institutions working on bioformulations development can work on these aspects using the help of state agricultural departments.

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