Plant Archives Vol. 25, Supplement 1, 2025 pp.1529-1538 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.211

USE OF BIOFORMULATIONS AND BIO-CONTROL AGENTS IN AGRICULTURE: A REVIEW

Nitish Ahlawat and Kaamini Kumari*
Department of Soil Sciences and Agricultural Chemistry, Lovely Professional University, Punjab, India
*Corresponding author E-mail: drkaaminik@gmail.com

(Date of Receiving : 24-08-2024; Date of Acceptance : 07-11-2024)

ABSTRACT

Addressing the pressing issues of increased food demand, declining crop productivity under varying agroclimatic conditions, and the deteriorating soil health resulting from the overuse of agricultural chemicals, requires innovative and effective strategies for the present era. Microbial bioformulation technology is a revolutionary, and eco-friendly alternative to agrochemicals that paves the way for sustainable agriculture. This technology harnesses the power of potential microbial strains and their cell-free filtrate possessing specific properties, such as phosphorus, potassium, and zinc solubilization, nitrogen fixation, siderophore production, and pathogen protection. The application of microbial bioformulations offers several remarkable advantages, including its sustainable nature, plant probiotic properties, and long-term viability, positioning it as a promising technology for the future of agriculture. To maintain the survival and viability of microbial strains, diverse carrier materials are employed to provide essential nourishment and support. Various carrier materials with their unique pros and cons are available, and choosing the most appropriate one is a key consideration, as it substantially extends the shelf life of microbial cells and maintains the overall quality of the bioinoculants. An exemplary modern bioformulation technology involves immobilizing microbial cells and utilizing cell-free filters to preserve the efficacy of bioinoculants, showcasing cutting edge progress in this field. Moreover, the effective delivery of bioformulations in agricultural fields is another critical aspect to improve their overall efficiency. Proper and suitable application of microbial formulations is essential to boost soil fertility, preserve the soil's microbial ecology, enhance soil nutrition, and support crop physiological and biochemical processes, leading to increased yields in a sustainable manner while reducing reliance on expensive and toxic agrochemicals. This manuscript centers on exploring microbial bioformulations and their carrier materials, providing insights into the selection criteria, the development process of bioformulations, precautions, and best practices for various agricultural lands. The potential of bioformulations in promoting plant growth and defense against pathogens and diseases, while addressing biosafety concerns, is also a focal point of this study.

***Keywords* :** Biofertilizer, Bio-control agent, Sustainable, Agriculture, Microbes.

Introduction

In the last few decades, rampant chemical fertilization and biomagnification of hazardous chemicals in the food chain has posed a threat to human health and destroyed the health of the soil. The deterioration of soil fertility and decline in the indigenous beneficial soil microbial population led to decreased crop production. Hence, an alternative and green approach is needed to maintain agricultural productivity without reliance on chemical fertilization. The use of microbial bio-formulations offers an alternative approach for utilizing beneficial plant microorganisms to achieve good plant growth and productivity. The use of bio-formulated products, especially biofertilizers, has been widely popularized as an alternative to the agrochemicals (Khan *et al.*, 2020a; Pathak *et al.*, 2022; Ayilara *et al.*, 2023). Therefore, the term bio-formulation can be represented as the 'development of material containing living but valuable microbial strains, using suitable carrier materials for their productive use in agriculture, industry, bioremediation, etc (Balla *et al.*, 2022). The key ingredients of a bio-formulated product/ bioformulation are potential microbes, possessing plant growth promoting properties including nutrient solubilizers, nitrogen fixers, biocontrol agents, and bioremediation (Pirttila *et al.*, 2021). The major goals of microbial formulations preparation are: (i) to create an appropriate environment for the bioinoculants functioning, ii) to provide physical and chemical protection for an extended period of time to circumvent a rapid reduction in cell viability during storage, (ii) to support the competition of inoculants with the indigenous soil microbiota, and (iii) to reduce losses engendered from depredation by the local micro-fauna. Another goal, however, is to provide a sufficient source of live bioinoculant cells that are accessible for interaction with plants and the soil microbiome (Vassilev *et al.*, 2020). It has been observed that direct use of plant beneficial microorganisms in the green house or small scale is fine but on field or large scale, viability issue of the microorganisms gets enhanced. Indeed, it is necessary to obtain a significant number of microbial cells (at least 106-107) in order to obtain a positive response of the formulated product (Bashan *et al.*, 2014; Vassilev *et al.*, 2020). The abiotic substrates, which have the ability to provide a safer environment for microbial cells and can accommodate viable and physiologically active cells, are called as carrier substances. Solid or liquid materials are used as ‘carriers’ for the development of various microbial formulations, depending on the product type (Naik *et al.*, 2020). The solid formulations are produced in solid, powdery, or granular form and are based on either inorganic or organic carriers. Various carrier materials such as peat, vermiculite, coal, compost, perlite, agro-industrial waste, polysaccharides, etc. are used to produce the most important solid formulations. In contrast, liquid based formulations also contain microbial cultures with desirable properties, modified with additives that improve the viscosity, constancy, and dispersibility of the cell suspension (Mishra and Arora, 2016). In recent years, formulation technologies have paid more attention to the immobilization of cells, since the tactic of gel cell immobilization is the technological solution that can better ensure the quality and standardization of the formulated product. In addition, particular attention has recently been paid to cell-free formulations (Tewari *et al.*, 2020). These formulations resemble fermentation broth and encompass various metabolic products, including metal chelators (siderophores), antibiotics, enzymes, notably those with lytic capabilities, toxins, and soluble phosphate. Collectively, these components have the potential to exert a beneficial influence on plant growth. Delivery of bioformulations is a mandatory step, done either by inoculating the soil directly or by treating plants/seeds (Rocha *et al.*, 2019a). The escalating concern over the inadequate uptake of chemical fertilizers by plants and their detrimental impact on ecosystems, alongside a global rise in apprehension regarding pollution, greenhouse gas accumulation, and an increased emphasis on plant-based food production, has led to a surging demand for biofertilizer agents. Farmers are increasingly embracing biofertilizers to sustainably and organically cultivate their crops. To date, numerous biofertilizers have been successfully commercialized for various environmental conditions and crops. However, a significant obstacle to the widespread success of biofertilizers in agroecosystems is the lack of knowledge in selecting and correctly applying them. This knowledge gap erodes the confidence of farmers in biofertilizers. Hence, there is a critical need to disseminate knowledge within farming communities about the scientifically sound methods of selecting and applying correct microbial bioformulations according to their native environment and crops.

Typically, a formulation is a mixture of an active ingredient in a formulated product with inert (inactive) substances (http://npic.orst.edu/ factsheets/ formulations. html). However, regarding bioformulation we see that there is no uniform definition available and various authors define it in their own way. Burges and Jones (1998) stated bioformulation comprises aids to preserve organisms, to deliver them to their targets, and once there to improve their activities, whereas Arora *et al.* (2010) define the term bioformulation to preparations of microorganism(s) that may be partial or complete substitute for chemical fertilization/pesticides. But any operative definition must include an active ingredient, a carrier material, and an additive. The active ingredient is mostly a viable organism; it may be live microbe or spore and its survival during storage is very essential for successful formulation development (Auld *et al.* 2003; Hynes and Boyetchko 2006). Suitable carrier material is inert that supports active ingredient (cells) and assures that the cells are easily established in or around the plant and provide better chances of enhancing plant growth or killing target pest. Carrier materials also increase the shelf life of the product (Burges and Jones, 1998). Some inert carrier materials are fine clay, peat, vermiculite, alginate, and polyacrylamide beads, diatomaceous earth, talc, vermiculite, cellulose (carboxymethyl cellulose), and polymers specially xanthan gum (Digat, 1989).

Additives such as gums, silica gel, methyl cellulose, and starch protect from harsh environment conditions and improve physical, chemical, and nutritional properties of formulations (Schisler *et al.*, 2004; Hynes and Boyetchko, 2006).

Types of formulation available

Broadly two types of bioformulations are available, liquids and solids (Burges and Jones, 1998), although in these days there are so many other types of bioformulation available and being used all over the world.

Solid Formulations

Solid formulations include granules (GR), microgranules (MG), wettable powders (WP), wettable/water-dispersible granules (WG, WDG), and dusts (Larena *et al.*, 2003; Abadias *et al.*, 2005; Guijarro *et al.*, 2007a). They are produced by adding binder, dispersant, wetting agents, etc. (Tadros, 2005; Brar *et al.*, 2006; Knowles 2008).

Granules (GR)

Granules are dry particles and contain active ingredient, binder, and carrier. Concentration of active ingredients in granules is 5–20 % (Brar *et al.*, 2006). On the basis of particle size, they are classified as coarse particles (size range 100–1000 μm) and microgranules (size range 100–600 μm). The granules should be noncaking, non-dusty, and free flowing and should disintegrate in the soil to release the active ingredient. They are usually safer having no risk of inhalation and mostly used in soil treatment. Granular formulations are more concerned with storage and increased shelf life (Callaghan and Gerard 2005). Most commonly used granules are wheat meal granules (Navon, 2000), corn meal baits, granules formed with gelatinized cornstarch or flour (Tamez *et al.*, 1996), gluten (Behle *et al.*, 1997), cottonseed flour and sugars (Ridgway *et al.*, 1996), gelatin or acacia gum (Maldonado *et al.*, 2002), sodium alginate (Guijarro *et al.*, 2007b), diatomaceous earth (Batta 2008) and semolina (durum) wheat flour (Andersch *et al.* 1998). MET52 ®, a granular bioformulation of *M. anisopliae* var. anisopliae strain F52, is widely used in biocontrol of black vine weevil (*Otiorhynchus* spp.) larvae in soft fruit and ornamental crops (Ansari and Butt, 2012). Sterile rice is used as organic carrier, whereas alginate prill is being utilized in "SoilGard" preparation. This granular formulation contains *Trichoderma virens* as active ingredients and marketed by Certis LLC for eradication of soil borne diseases caused by *Pythium, Rhizoctonia*, and *Fusarium.* Selection of different carriers may affect activity of active ingredients in field conditions. In a study Mejri *et al.* (2013) measured bioherbicidal activity of deleterious rhizobacterium *Pseudomonas trivialis* X33d by taking two granular formulations and found that semolina–kaolin (pesta) showed higher brome suppression activity in wheat field in comparison to kaolin–talc-based granular formulation, whereas BioShield™, formulated as a granule containing Serratia entomophila, is sold in New Zealand for control of grass grub larvae in established pasture (Young *et al.*, 2010). Although granular formulations are very effective, their application is also limited due to inactivation of active ingredient in ultraviolet (UV) light. In a study by Bailey *et al.* (1996), Bt product used to control apple moth caused by *Epiphyas postvittana* lost more than half of its activity within a day on exposure to sunlight, whereas BioShield, a *Serratia entomophila* containing granular formulation, is very sensitive to UV light and osmotic and desiccation stress and requires subsurface application (Johnson *et al.*, 2001). Some UV protectants such as Tinopal, Phorwite, Intrawhite, and Leucophor; uric, folic, 2-hydroxy-4-methoxy-benzophenone, p-aminobenzoic, 2-phenylbenzimidazole-5-sulfonic acids; and dyes such as Congo red, methyl blue, safranin, brilliant yellow, and buffalo black may overcome UV inactivation of organism when added in formulation medium or coated on formulation product (Warrior *et al.* 2002; Cohen and Joseph 2009). Stilbene-derived optical brighteners are also more effective in baculoviruses containing formulation as these absorb UV radiation and emit visible blue wavelengths and enhance the infectivity (Goulson *et al.* 2003). Recently Fernandes *et al.* (2015) reviewed tolerance of selected entomopathogenic fungal strains to UV radiation.

Wettable Powders (WPs)

Wettable powders (WPs) are one of the oldest types of formulations. They consist of 50–80 % technical powder, 15–45 % filler, 1–10 % dispersant, and 3–5 % surfactant by weight to 8 J. Mishra and N.K. Arora achieve a desired potency formulation (measured in international units) (Brar *et al.*, 2006). These dry formulations are of much interest as they are readily miscible with water and can be easily added to a liquid carrier, normally water, just before its application. WPs have a longer shelf life and by controlling moisture content, their shelf life may exceed 18 months. Longer shelf life is also related to their firm marketplace. Agricultural materials and industrial waste by-products such as wheat bran–sand mixture, sawdust–sand–molasses mixture, corn cob–sand–molasses mixture, bagasse–sand–- molasses mixture, organic cakes, cow dung–sand mixture, compost/farm manure, inert charcoal, diatomaceous earth, and fly ash (Table 1.1) can also be used to prepare powder formulations (Khan *et al.* 2007). Recently Cheng *et al.* (2015) prepared a WP containing 60 % B. cereus freeze dried powder, 28.9 % diatomite as carrier, 4 % sodium lignin sulfonate as disperser, 6 % alkyl naphthalene sulfonate as wetting agent, 1 % K2HPO4 as stabilizer, and 0.1 % β-cyclodextrin as ultraviolet protectant, and in his preliminary study, they found this formulation was effective in biocontrol of postharvest disease in comparison to chemical used. Woo *et al.* (2014) reviewed current application of Trichoderma-containing products in agriculture, and it was found that 55.3 % of Trichoderma formulations are commercialized as WPs.

Wettable/Water-Dispersible Granules (WG, WDG)

Wettable/water-dispersible granules (WG, WDG) are also known as dry flowable. They have been designed to make WPs more user and environment friendly, non-dusty, free-flowing granules quickly dissolving in water. They contain wetting agents and dispersing agents similar to those used in WPs, but the dispersing agent is usually at a higher concentration. Like WPs, WDG also show excellent shelf life. WDG formulations have wider role in nematode control and capture 90 % of the total market available for nematode-based products. Antagonistic fungus, *Ampelomyces quisqualis*, is used to control powdery mildew caused by several pathogenic species in grapes, tomato, apples, strawberries, and cucurbits, formulated as WDG (Falk *et al.*, 1995). Chumthong *et al.* (2008) produced water-soluble granules containing Bacillus megaterium for biological control of rice sheath blight and showed that these granule formulations exhibited good physical characteristics, such as high-water solubility and optimal viscosity, suitable for spray application.

Dusts

Dusts are also one of the oldest formulation types and contain very finely ground mixture of the active ingredient (usually 10 %) with particle size ranging from 50 to 100 µm. Although they have been used since a long time and in some instances more effective in killing (Ifoulis and Savopoulou-Soultani 2004), there have always been handling and application problems associated with dusts (Harris and Dent, 2000). Dust containing beauverial protein extract (weighing about 5 kDa) is also being used in biocontrol. Biofox C has been formulated as dust containing nonpathogenic *F. oxysporum* and used in basil, cyclamen, tomato and carnation (Kaur *et al.*, 2010)

Liquid Formulations

Liquid formulations are also known as flowable or aqueous suspensions and consist of biomass suspensions in water, oils, or combinations of both (emulsions) (Schisler *et al.*, 2004). A typical liquid formulation contains 10–40 % microorganisms, 1–3 % suspender ingredient, 1–5 % dispersant, 3–8 % surfactant, and 35–65 % carrier liquid (oil or water) (Brar *et al.*, 2006). Liquid formulation may be of the following types.

Suspension Concentrates (SCs)

SCs are produced by adding solid active ingredient(s) with poor solubility in water and satisfactory stability to hydrolysis (Tadros, 2013). SCs are diluted in water before use. Their storage and solubility can be improved by addition of surfactants and various additives. Farmers generally prefer suspension concentrates to wettable powders because they are non-dusty and easy to measure and pour into the spray tank.

Oil-Miscible Flowable Concentrate (OF)

OF is stable suspension of active ingredient(s) in a fluid intended for dilution in an organic liquid before use (Singh and Merchant, 2012).

Ultralow Volume (ULV) Suspension (SU)

They are suspension ready for use through ULV equipment. ULV are aerial or ground spray equipment and generate extremely fine spray (Singh and Merchant, 2012).

Oil Dispersion (OD)

OD is a stable suspension of active ingredient (s) in water-immiscible solvent or oil (Michereff *et al.*, 2009). ODs have validated a growing importance over the past decade. Recently Mbarga *et al.* (2014) developed a soybean oil-based formulation and found that *Trichoderma asperellum* containing OD had great potential for the control of cacao black pod disease with increased half-life of the conidia in comparison to aqueous suspension. Some protective measures are required with regard to handling fungi containing OD formulations. As in prolonged storage, active ingredient (conidia) may be settled out of suspension or densely compacted in the bottom of the container (Butt *et al.* 2001). Some of the Trichoderma containing liquid formulations used in biocontrol are Trichojet, Enpro-Derma, and Trichorich-L (Woo *et al.*, 2014). Oil-based formulations have been proven better in foliar spray and considered effective in enhancing the activity of entomopathogens (Feng *et al.*, 2004). Oil evaporates much less, so it remains in contact for greater time and can be applied as an emulsion (oil in water) (Luz and Batagin, 2005) or in some cases as an invert emulsion (water in oil) (Batta, 2007).

Encapsulation

Encapsulation involves coating or entrapping microbial cells within a polymeric material to produce beads which are permeable to nutrients, gases, and metabolites for maintaining cell viability within the beads (John *et al.*, 2011). Based on the size of the polymeric bead produced, two types of techniques, i.e., macro -encapsulation (size ranging from few millimeters to centimeters) and microencapsulation (size ranging from 1 to 1000 µm, generally less than 200 µm), are used (Nordstierna *et al.*, 2010). Macro-encapsulation techniques are advantageous than micro-encapsulation (for further details on microencapsulation review by Rathore *et al.*, 2013 can be seen). Encapsulation provides good protection to active ingredient from harsh environmental factors. Currently, gelatin, starch, cellulose, and several other polymers are used for encapsulation of active ingredients (Amiet Charpentier *et al.*, 1998; Park and Chang 2000; Cheze-Lange *et al.*, 2002). Protection may enhance to some extent by coating capsule with dyes (Cohen *et al.*, 1990). For further detail on encapsulation, chapter by Schoebitz *et al.* can be seen from this very book. Although both liquid and solid formulations have been extensively used in Aero systems, dry formulations are generally preferred over wet formulations because they provide extended shelf life and are easier to store and transport (Burges and Jones, 1998). The development of a bioformulation is proving a hectic job and earlier work done in this field is not sufficient. The increasing demand for developing new formulations to replace chemical pesticides and fertilizers has created interest amongst entrepreneurs in this field, and they are funding various projects for the development of cheaper and effective technology. Some technological advances in development of Bt-based products have provided substantial aid in its commercial production. For example, Micellar-enhanced ultrafiltration (MEUF) is a technique being used to separate dissolved organic compounds like thuringiensin from aqueous streams (Tzeng 12 J. Mishra and N.K. Arora *et al.*, 1999). Similarly in situ product removal (ISPR) involves biochemical product removal during fermentation process and successfully applied in removal of Bt toxin proteins (Agrawal and Burns 1996), whereas cross-flow microfiltration (CFM) has been utilized for extraction of all kinds of proteins and harvest of recombinant yeasts (Persson *et al.*, 2004)

Factors affecting the efficacy of microbial bioformulation

The efficiency of microbial formulations can be altered by various biotic and abiotic factors. These factors affect the acclimatization, viability, activities, and overall performance of microbial formulation. Some key factors that can impact microbial formulation efficiency are listed below (Mawar *et al.*, 2021; Rojas-Sánchez *et al.*, 2022):

Strain Selection: The selection of appropriate microbial strains is vital, as different strains have varying abilities to thrive in different environmental conditions and they only perform desired functions at their best in their loving environment conditions.

Carrier: The choice of carrier materials or additives in the formulation directly influences the protection, delivery, and release of the microbes. These materials should be selected to enhance microbial survival and activity.

Storage Conditions: Proper storage conditions, including temperature, humidity, and packaging, are critical to maintaining the viability of the microbes in the formulation.

Shelf Life: The shelf life of the formulation can significantly impact its efficiency. Microbial formulation having shorter shelf lives may require more frequent application, while longer shelf lives can reduce the need for frequent reapplication.

Environmental competition: The ability of microbes to adhere to surfaces and colonize their intended habitat is crucial because microbes in formulations may face stressors such as UV radiation, chemical exposure, and competition with native microorganisms. Interactions with native microorganisms or other introduced strains can affect the performance of the formulated microbes.

Application Method: The method of application, whether through spraying, irrigation, injection, or other means, can impact the distribution and effectiveness of the formulation in the target area.

Environmental Conditions: External environmental conditions, such as seasonal variations and climate changes which determine the biotic and abiotic factors (pH, Temperature, salinity, soil type, microbiota, etc.) of such regions can affect the efficiency of microbial formulations.

Quality Control: Rigorous quality control measures during the manufacturing process are critical to ensure consistency and reliability in microbial formulations because contamination of any foreign microorganisms greatly affects bioformulation efficiency.

Genetic Stability: In some cases, the genetic stability of the microbial strains in the formulation should be considered to ensure that they maintain their desired traits over time.

Apart from above mentioned factors, numerous other factors are also responsible for influencing the efficiency of microbial formulations. Optimizing these factors based on the specific application and environmental conditions is essential for maximizing the working efficiency of microbial formulations.

Role of Bioformulation

Plant growth promoting microorganisms (PGPM) are those beneficial microbes that help in plant's growth and development through protection from biotic and abiotic stresses and by maintaining nutrient availability (Upadhayay *et al.*, 2022a; Upadhayay *et al.*, 2022b; Khan *et al.*, 2020b; Khan *et al.*, 2022). Therefore, the implementation of PGPM as a microbial-based formulation is the current time to ensure high crop productivity with better nutritional values of plants and maintain the high nutritional status of soil (Geetha and Balamurugan, 2011; Accinelli *et al.*, 2018).

Enhancer of crop yield and nutritional quality

The main application of biofertilizers in agriculture is to ensure food security and the nutritive value of plants for the good health of consumers like humans. After the green revolution, the continuous use of chemical fertilizers was able to fulfill food quality, but it is diminishing the nutritional value of plants and soil. Nitrogen (N), phosphorus (P) and Potassium (K) are essential macronutrients for proper plant growth and act as major limiting factors in terms of crop production as these elements play a vital role in plant metabolism, growth, and development. N, P, and K are present in different forms in soil, but the plants do not take the majority forms (Khan *et al.*, 2019). Hence, most of the soil land in the entire world lacks plant-available nutrients (Karamesouti and Gasparatos, 2017). Therefore, in agriculture practice, the use of chemical fertilizers to increase the NPK content in soil increased, resulting in the leaching of excessive minerals into the soil environment. Plants uptakes nitrogen, phosphorus, and potassium through their roots from the soil, so the application of N-fixation bacteria, phosphate solubilizing bacteria (PSB), and potassium solubilizing bacteria as biofertilizers will increase the available NPK in soil and influence the plant nutritional status along with yield (Figure 2). "BioGro" inoculant is a mixture of microbial strains isolated from rice crop soils. The application of this inoculant increases the grain yield and nutrients like N and P content in rice (Nguyen *et al.*, 2017). Colla *et al.* (2015) reported a significant increment in the growth of shoot, root biomass, and leaves number by 23%, 64%, and 29%, respectively, and an increase in yield (8.3% to 32.1%), depending on the growing season and high nutritional grain quality along with enhancement in protein, K, P, Fe, and Zn concentrations after direct treatment with consortium of arbuscular mycorrhizal (AM) fungi (*R. intraradices* and *F. mosseae*) and *T. atroviride* as compared with untreated. The seed inoculation with the liquid formulation of *Pseudomonas flouorescens* increased the plant growth, biomass, and grain yield, and reduced the recommended dose of N fertilizer in maize (Sandini *et al.*, 2019). A study to identify the best combination of bioformulation and chemical fertilizers for maximum chickpea production in hilly areas found that bioinoculants (N-fixers and PSB) with 20 Kg N/ha urea concentration resulted in high crop yield in chickpea and enhanced the rhizosphere and soil nutrition in comparison to alone biofertilizer, chemical fertilizer, and untreated control, as bioformulation increased the survivability of microbes (Joshi *et al.*, 2019). This combinational approach for applying bio and chemical fertilizer to improve production with economic efficiency was also found applicable in sugarcane (Pereira *et al.*, 2018). These studies showed that the correct combination of appropriate doses of chemical and biofertilizers could boost plant growth, which will help reduce the amount of chemical fertilizers.

Role as biocontrol agents

Bio-control agents (BCA) and inducers of induced systemic resistance (ISR) have been widely studied to reduce the use of chemical fungicides in agriculture crops. In most cases, BCA can control plant pathogens directly or indirectly by developing a nonphysical relationship with host-pathogen (Figure 2). Another way to prevent the plant from biotic stresses is the competition for micronutrients and space to colonize and survive in the rhizosphere (Upadhayay *et al.*, 2021). BCA colonization at pre-empty infection sites allows them to consume available plant resources and leaves the pathogen for nutrient and space scarcity. In a study of Lindow (1987), plant foliar colonization of *Pseudomonas syringae* strain on pear plants resulted in less infection caused by *Erwinia amylovora* than untreated plants. Another way to control plant infection against pathogenic microorganisms and insects is to induce an Induced systemic response (ISR) defense system in plants (Pieterse *et al.*, 2014). *Bacillus* spp are reported to produce cyclic lipopeptide compounds that result in plant ISR mechanism elevation through jasmonic acid (JA)/ethylene and salicylic acid (SA) pathways against phytopathogens. Chitin amended talc-based bioformulation of Pseudomonas fluorescens Pf1 reduced the disease effect of *Macrophomina* root rot in Moong bean by inducing the expression of the defense-related proteins and phytochemicals accumulation at the site of infection, which decreased the colonization of pathogens in the root (Saravanakumar *et al.*, 2007). In this study, chitin amendment increased the growth and survival of chitinolytic microbes through acct as a carbon source in bioformulation (Bell *et al.*, 1998). Singh *et al.* (2014) found that seed coating of chickpea with a bioformulation using gum arabic as an adjuvant led to higher plant growth and an elevated amount of phenolic compounds in fungal pathogen *Sclerotium rolfsii* infected chickpea, in comparison to untreated control and single inoculations. Similarly, Saravanakumar *et al.* (2007) studied a mixture of three Pseudomonas fluorescens Pf1, TDK1, and PY15 strains to reduce the rot disease in rice with an increase in grain yield (Saravanakumar *et al.*, 2007). In both studies, these consortia led to the activation of the plant host defense mechanism by elevating the level of defense-related enzymes, proteins, and phenolic content in the plant, which causes the ISR mechanism activation in the host to deal with biotic stresses. While in another application of Trichoderma strains with two synthetic fungicide agents (acibenzolar-S-methyl and thiamethoxam) decreased disease indices of phytopathogen *Pyrenophora tritici-repentis* in wheat by inducing plant defense system and activating pathogenesis-related enzymes which directed for ethylene signaling (Perelló and Bello, 2011). The combination of microbial-based bioformulation with chemical compounds has resulted in more growth and caused less disease occurrence, so the use of the biological and chemical combinatorial approach for healthy plant and crop production will reduce the fungicide application. There is a robust future for new development and research in applying multi-strain carrier based bioformulation in agriculture to manage biotic stresses.

Controlling abiotic stress

The use of microbial bioformulations is often seen as a viable alternative to improve the crop yield under different abiotic pressures (Singh *et al.*, 2021). Abiotic stress like drought, waterlogging, low or high temperature, salinity stress, and deficient or excessive mineral content negatively influence plant growth, yield, and nutritional quality of seeds. Recently, a research study documented improved cowpea's biomass and crop yield under water-deficient conditions following treatment with silicon dioxide and starch-based- P. putida bioformulation (Rocha *et al.*, 2019b). The study of Sohaib *et al.* (2020) reported that a bacterial consortium promotes high nitrogen and phosphorus content in straw and grains with better wheat plant growth and crop productivity by mitigating the salt stress and reducing ethylene production in organic compost biogas slurry-based carrier bioformulation. Accelerated ethylene production is known to occur in stress conditions and induce senescence by degrading chlorophyll pigments, mineral misbalancing, and inhibiting protein synthesis under salinity stress. This result was also supported by previous research that highlighted the application of ACC deaminase containing bio-inoculants prevented ethylene's output, which protects the plant from senescence (Zahir *et al.*, 2011). The above-mentioned carrier-based bioformulation surges the survival of the above bacterial consortia until three months, which is best to protect the wheat plant. The same kind of effect was also reported by using PGPB like Pseudomonas fluorescens YsS6, *Pseudomonas migulae* 8R6 in peat-based bioformulation in tomato plants (Ali *et al.*, 2014), and application of liquid-based alone or combination of different ACC deaminase producing microbes UW3 (Pseudomonas sp.) and UW4 (P. sp.) rhizobacterial isolates CMH3 (*P. corrugata*) in both barley and oats under high salt stress (Chang *et al.*, 2014). Under abiotic stress, plant's survival mechanisms induce through complex signaling pathways, which remarkably enhance by PGPR through the array of mechanisms (Wang *et al.*, 2019). Under stress, plant activates signaling pathways with sensors, receptors, and ion channels. Specific protein kinases, like AtHKT1 in Arabidopsis thaliana, detect signals, triggering downstream gene activation via secondary messengers like reactive oxygen species and inositol (Gupta *et al.*, 2022). These messengers induce calcium oscillations, driving stress-responsive protein formation (Ali *et al.*, 2017). In a study, Bacillus subtilis priming was reported to modulate the HKT/K+ transporter gene (HKT), improving the K^+/Na^+ ratio by reducing Na+ uptake (Zhang *et al.*, 2008). In another study, Pseudomonas fluorescence and P. putida regulate the At3g57530 gene, impacting calcium and calcium-dependent protein kinases (CDPKs). Rhizobacteria offer drought resilience through RIDER (Rhizobacterial-Induced Drought Endurance and Resilience). RIDER involves PGPR-induced changes like producing phytohormones, exopolysaccharides, cyclic metabolic pathways, and reinforcing antioxidant defenses with compounds like amino acids, polyamines, sugars, and heat shock proteins (Saharan *et al.*, 2022). Additionally, the *Piriformospora indica* fungal endophyte was also found to enhance drought resistance by upregulating antioxidant enzymes, drought-related genes, and CAS mRNA levels in stressed leaves (Sun *et al.*, 2010). In a research endeavor, chickpea seeds were subjected to an experimental treatment involving the use of sodium alginate and $CaCl_2$ as carriers for *Paenibacillus lentimorbus* B-30488. This treatment led to a notable proliferation of beneficial bacteria in the soil and the formation of biofilms. Subsequently, this enhanced bacterial activity played a pivotal role in improving the chickpea plants' resilience to drought stress by positively modulating their physiological responses to dehydration (Khan *et al.*, 2011). Use of sodium alginate and calcium chloride increases the biofilm production and better seed attachment in this bioformulation and leads to overcoming the drought effect in plants. So further, these bioformulations may also be used in the phytoremediation of marine soils.

Challenges and limitations in utilizing microbial formulation

In recent years, there has been a growing interest in harnessing the power of beneficial soil microorganisms for the production of biofertilizers, aimed at boosting plant productivity. This approach has witnessed significant successes, yet it is not without its set of challenges and constraints. The complexities of replicating their positive effects on plants under ever-changing environmental conditions at field conditions pose a primary hurdle. Furthermore, there is a need to raise awareness within farming communities about the scientific methods of applying microbial bioformulations in the field and the ecological importance of these microbial formulations. Education and outreach efforts are crucial to foster their adoption and successful application. Ethical concerns may also arise, particularly when considering the use of genetically modified microorganisms or non-native species in these formulations. The acceptance of such practices within society can play a pivotal role in their adoption. Additionally, the existing native soil microorganism populations can present significant barriers to the successful implementation of these inoculants. The consistency of microbial biofertilizers across diverse environmental conditions and crop types is not guaranteed. Selecting the right microbial strains for specific agricultural contexts can be a challenging task. Moreover, the efficacy of these strains can vary based on factors like soil type, temperature, pH, and moisture levels. Another limitation is the limited shelf life of microbial formulations. Over time, the viability of microorganisms in these formulations can diminish, reducing their effectiveness in the field. To maintain the consistency and effectiveness of these products, rigorous quality control during production is essential. Studies have revealed issues of contamination and the presence of unintended bacterial strains in commercial biofertilizers such as Herrmann and Lesueur (2013) performed the analysis on 65 commercial biofertilizers, and revealed that merely 37% of these products met the criteria for being labeled as "pure." In contrast, a significant 63% of the tested biofertilizers exhibited contamination by one or more bacterial strains. Furthermore, in 40% of the cases, the tested products lacked the specified strains entirely and were instead found to contain contaminants. A shortage of suitable carriers for these formulations, inadequate storage facilities to prevent contamination and the unpredictability of their effectiveness due to extreme weather conditions add to the list of constraints. Additionally, the credibility of biofertilizer application can be undermined by the absence of crucial labeling information, such as expiration dates and the identification of microorganisms used in production. Most biofertilizers also exhibit selectivity in their actions, limiting their compatibility with certain chemical pesticides or fertilizers, which can affect integrated pest management or nutrient management programs. To overcome these challenges and limitations, continuous research, development, and collaboration among scientists, agricultural practitioners, and policymakers are imperative. It is crucial to explore and leverage the potential benefits of microbial formulations while actively addressing their drawbacks to advance sustainable agricultural practices.

Conclusion and Future prospects

The primary focus in advancing agricultural productivity to meet the needs of our growing global population lies in investing in the development of microbial formulations. This greener approach supports plant growth and environmental sustainability. While bacterial strains often perform well in laboratory settings, their efficacy in field conditions is hindered by factors such as poor survivability, inappropriate carrier selection, or ineffective delivery methods. To ensure the success of bioformulations, the process begins with the critical task of selecting microbial strains carefully. These chosen strains must possess a competitive edge against native microflora while demonstrating beneficial functions even under stressful conditions, all the while maintaining their bio-efficacy once released. Creating an effective bioformulation demands several essential steps, including proper isolation and characterization of the microbial strains for their plant growth-promoting traits. Additionally, rigorous testing for pathogenicity is necessary to ensure bio-safety. Moreover, the selection of an ideal carrier is crucial to enhance the shelf life of the bioformulation and preserve its efficacy. Field conditions play a vital role in determining the success of a bioformulation. Therefore, it is imperative to assess the survival of the formulated product in real-world agricultural settings. The overall cost of developing and implementing the formulated product should be considered to ensure its feasibility and practicality on a larger scale. Shifting the research focus towards the development of broad temperature and elevation ranged bioinoculants based bioformulation, harnessing their potential metabolites, holds the key to advancing sustainable and safe practices. Rather than solely concentrating on the isolation and characterization of new bacterial bioformulation, this approach offers several benefits by utilizing bioinoculants bioformulation that relies on potential metabolites, we can significantly enhance field efficacy while simultaneously addressing biosafety concerns. These bioformulations can be tailored to deliver targeted benefits, promoting plant growth, disease resistance, and nutrient uptake without the risk associated with introducing entirely new bacteria into the environment. Moreover, there is a pressing need to explore ways to stabilize these bioformulations and increase their shelf life. By doing so, we ensure their long-term viability and practicality for widespread agricultural adoption, promoting cost-effectiveness and convenience. To achieve this, research efforts should be directed toward identifying numerous inexpensive and non-toxic carrier materials. These materials can play a crucial role in preserving the bioformulations' effectiveness and longevity, allowing farmers easy access to sustainable solutions without imposing harmful consequences on the environment or human health. Lastly, to truly replace agricultural chemicals and make agriculture more sustainable and productive, it is essential to investigate effective delivery methods. Implementing innovative delivery techniques can ensure that bioinoculant bioformulation reaches their target areas efficiently, maximizing their beneficial impact on crops and reducing the need for conventional chemical interventions. By emphasizing these research areas developing specific bioinoculants bioformulation based on potential metabolites, stabilizing formulations, exploring eco-friendly carrier materials, and optimizing delivery methods-we pave the way for a more sustainable, productive, and environmentally friendly approach to agriculture.

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