Carriers and Their Role in Plant Agrosystem

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Abstract

The population explodes and the concerns of biomagnifications by the use of synthetic pest control methods are two major problems that have created the major food crop crises in the world. To eradicate the problem, various green practices like bioformulations, mixed cropping, etc. have been designed and implicated, but almost all of them had delivery constraints, and to minimize this, effective delivery model was needed. The researchers in the quest designed a model that was harmless, stable, and inert and that did not interfere with biocontrol activity against pest which can be used at time of harvesting and postharvesting as well as to increase the shelf life; such models were called as carriers. Various types of carriers have been studied and applied, but the rate of biocontrol is still yet to reach the optimum. So it becomes necessary to gain an insight into the constraints in effective biocontrol and retrospect the best practices to minimize the constraints.

This chapter throws light on carriers, their types, their formation and inoculation, and finally their role in plant agrosystem which will further help the researchers in designing the cost-effective and efficient carrier with minimum delivery constraints and eliciting maximum biocontrol to finally eradicate the use of synthetic pest control practices from the system.

12.1 Introduction

The plant diseases affecting the cultivation and production of crops are serious concerns in agriculture as they largely affect the quality and quantity of the crops. The human population in the world has now passed 7 billion, and it is

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V. Kumar et al. (eds.), *Probiotics and Plant Health*,
DOI 10.1007/978-981-10-3473-2_12

expected to reach up to 11 billion by 2100 with a prediction of a 70% chance of a continuous increase in population (EEA 2015). Such population increases lead to a greater demand for food. Since food is the basic necessity for life, the human population cannot compromise on food security, not even at the cost of earth's sustainability. It has been estimated in 2015 that the current global population is two to three times higher than can be sustained by current food production levels and is already utilizing 50% more resources than the earth is producing (http://www.worldpopulationbalance.org).

Moreover, the high pest population in developing countries is a complex problem with rapid increase of 1.2% annually in the human population adding to the ecological burden (Reece et al. 2011).

Consequently, our overburdened resources are declining very rapidly. There are 50,000 species of bacterial and fungal phytopathogens and 8000 species of weeds which largely reduce crop yield and quality (Ortiz-Hernandez et al. 2013). According to several studies, it has been suggested that specific crop losses due to pests may vary between 10% and 90% (Youdeowei 1989). In India, Singh and Shekhawat (1999) stated that crop losses due to pests may be as high as 80% if the crop is not well protected.

12.1.1 Major Outbreaks of the World

The major devastating effect on crops by pests worldwide is still the basis for the development of effective pest control policies, and so it should always be referred to study the nature of the outbreak (Table 12.1).

Table 12.1 Major Outbreaks of the world

Wheat and barley head scab	One of the most devastating plant diseases in the world and is ranked by the United States Department of Agriculture (USDA) as the worst plant disease to hit the United States after rust epidemics in the 1950s (Schmale and Bergstrom 2003). During the twentieth century, wheat and barley crops in the United States were largely attacked by a fungus *Fusarium graminearum* which led to serious loss of 60–70% in most susceptible cultivars (Zhang and Ye 1993). Since 1990, wheat and barley farmers in the United States have lost over $3 billion due to Fusarium head blight epidemics (Schmale and Bergstrom 2003)
Southern corn leaf blight epidemic (1970)	In 1970, a newly emerged race *Cochliobolus heterostrophus* (race T) attacked the hybrid corn plants with T cytoplasm which constituted 80% of the corn grown in the United States at that time (Hooker 1972)
The Great Bengal famine (1943)	One of the most tragic famines due to "brown spot disease" of rice caused by *Helminthosporium oryzae* which resulted in the loss of three million lives due to starvation and malnutrition (Sen 1981)
Irish potato famine (1845–1850)	One of the most devastating epidemics from Ireland resulted in a massive crop failure due to "potato late blight" caused by the fungus *Phytophthora infestans*. At present, late blight of potato accounts for the loss of US$3.75 billion annually in developing countries (Singh and Singh 2005)

12.2 Disease Management Practices and Failures

In India, disease management practices (Lamichhane et al. 2015) including the heavy use of synthetic pesticides to prevent the crop loss of 30–40% due to insect, pests, weeds, and diseases were estimated to be approximately US$2 billion in 1995 (Gautam and Mishra 1995), and worldwide crop loss due to pests in 1996 was estimated to be approximately $500 billion per year even after the annual application of 2.5 million metric tons of pesticides and synthetic chemicals which approximately were valued at $31.25 billion (Pimentel 1997).

At present worldwide, various synthetic pesticides worth 5.6 billion pounds are used in the agriculture sector (Grube et al. 2011), but in the history of agriculture, the use of pesticides has exerted a selection pressure on pests and pathogens which forced them to adapt according to their chemically modified habitats, and a consequence has been the evolution of "pesticide-resistant" varieties (Gould 1991) that was first documented by Melander in 1914. At least 447 pesticide-resistant arthropod species have been reported in the world (Callaghan et al. 1998). For example, resistance in the Colorado potato beetle (*Leptinotarsa decemlineata*) costs Michigan potato producers $16 million for crop losses in 1991 (Duchesne et al. 2001). In Brazil, the increased use of 234% in insecticides, 548% in fungicide, and 5414% in herbicides over a period of 15 years, from 1964 to 1979, resulted in an increase of only 16.8% in the production of 15 major crops (FAO 1986) that shows pest resistance against the disease management practices. Second failure of the practices was deposition of pesticide residue in food crops that eventually entered the food chain leading to biomagnifications of the pesticide. The total number of pesticide poisonings in the United States alone was 300,000 per year as estimated by EPA (1992). The studies on fruit samples of ber, grapes, and guava detected DDT, endosulfan, and HCH pesticides in almost all the samples as reported by Kumari et al. (2006). Chen et al. (2011) evaluated the residues of organophosphates and pyrethroids in fruits and vegetables collected from Xiamen, China, and found that out of 1135 samples, 37.7% contained pesticide residues. Dureja et al. (2015) stated that even the Crop Care Federation of India (CCFI) in organic farms uses chemical pesticides to protect their crops.

Thus, the current agricultural practices are not only contributing toward ecological degradation, but as the issue of food security is of prime importance, researchers are concerned to find better and safe alternatives to synthetic agrochemicals as food crops are highly susceptible to be attacked by many pathogens not only at all stages of their growth but also during postharvest storage which is largely controlled by pesticides (Gasic and Tanovic 2013).

The use of chemicals as pesticides is a common practice however with environmental concerns, and health safety biocontrol has been found to be the best practice in controlling the plant pathogens (Fig. 12.1). The **bacterial antagonism** is also an effective pest management practice (Chen et al. 2013). Plant symbionts or mutualists possess strong biocontrol potential as well as plant growth-enhancing capabilities (Fig. 12.2) (Tronsmo and Dennis 1977; Wilson and Pusey 1985; Cook 1990; Barkai-Golan 2001; Compant et al. 2005; Kavitha et al. 2003; Tewari and Arora 2014). In this context, bacterial populations in the soil which have the capability to

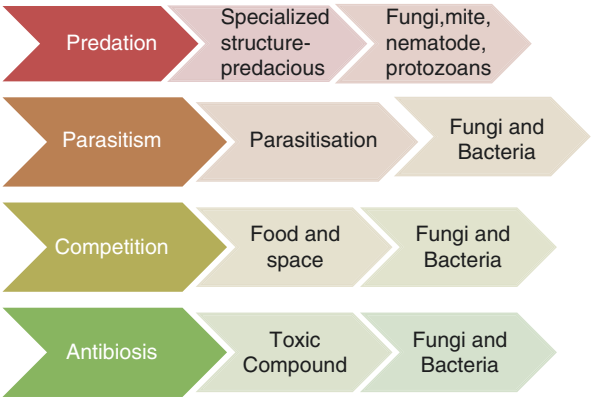

Fig. 12.1 Four mechanism of biocontrol

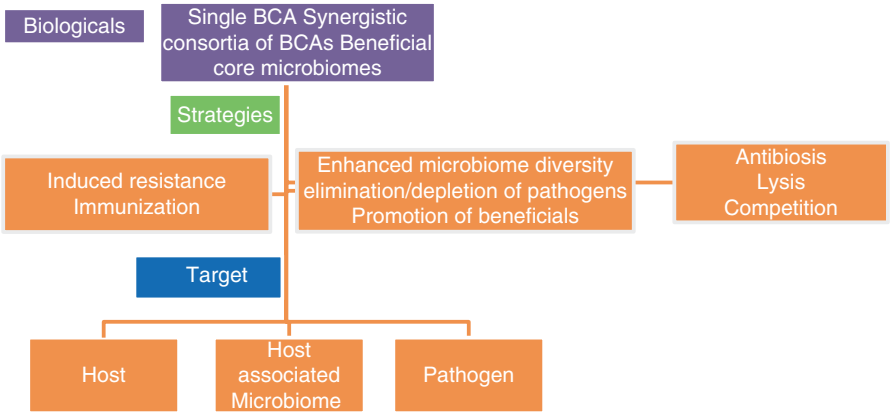

Fig. 12.2 Green revolution approach in agriculture

aggressively colonize the plant root system (i.e., rhizobacteria) and internal plant tissues (i.e., endophytic bacteria) are of considerable interest (Haas and Defago 2005; Backman and Sikora 2008; Lugtenberg and Kamilova 2009). Successful applications of antagonistic bacteria under field conditions have been evidenced from various case studies all over the world (Table 12.2). In Costa Rica, the use of dieldrin pesticide (over 12,000 ha) was stopped, and thereafter, the outbreak of six major pest infestations was suppressed by their natural enemies which started to colonize the area after cessation of pesticide use (Stephens 1984). Other examples illustrating the impact of natural enemies of plant pathogens are the use of *Bacillus thuringiensis* and the release of natural enemies like *Trichoderma* sp. on tomato crops in Colombia which over an area of 2000 ha have reduced the pesticide application from 20–30 times to 2–3, saving $650 per hectare (Belloti et al. 1990). In Sudan and Egypt, the total cost to protect the cotton crop from bollworm and white-fly reduced from 33.3% (in 1985–1986) to 19.3% (in 1988–1989) by using

Table 12.2 Some bacterial biocontrol agents against different pests of food crops

Bacteria	Target pest	Crop	References
Pseudomonas fluorescens	*Erwinia carotovora* *Gaeumannomyces graminis var. tritici* *Fusarium glycinia* *Sarocladium oryzae* *Puccinia ultimum*	Potato Wheat Wheat Soybean Sugar beet	Shaikh and Sayyed (2015), De Souza et al. (2003), and Shaikh and Sayyed (2015)
Pseudomonas putida	*Fusarium solani* *E. carotovora*	Beans Potato	Shaikh and Sayyed (2015)
Pseudomonas cepacia	*Fusarium oxysporum* *Bipolaris maydis*	Onion Maize	Shaikh and Sayyed (2015)
Azospirillum brasilense	*Pseudomonas syringae* *Fusarium* sp. *Rhizoctonia* sp. *Pythium* sp. *Sclerotinia* sp. *Pythium aphanidermatum* *Colletotrichum acutatum*	Tomato Cucumber	Bashan and Bashan (2002) and Hassouna et al. (1998)
Azospirillum lipoferum	*Heterodera avenae* (nematode)	Wheat	Bansal et al. (1999)
Azospirillum spp. *Bacillus pumilus* *Mesorhizobium loti*	*Striga hermonthica* (witchweed) *G. graminis* var. tritici *Sclerotinia sclerotiorum*	Wheat Mustard	Shaikh and Sayyed (2015) and Chandra et al. (2007)
Rhizobium leguminosarum bv. *phaseoli* strain RRE6	*Rhizoctonia solani*	Rice *Oryza sativa*	Mishra et al. (2006)
Rhizobium meliloti *Enterobacter* spp. *Streptomycetes*	*Macrophomina phaseolina* *R. solani* *F. solani* *Pythium* *Botrytis* *S. sclerotiorum*	Sunflower Okra Pea Apple Potato Tomato	Haque and Ghaffar (1993), Arora et al. (2001) and Shaikh and Sayyed (2015)

mechanical and biological control measures (Oudejans 1991). Sustainable agricultural practices including improved mechanical, cultural, and biological approaches could reduce pesticide application up to 50% saving $1 billion (Peschin 2002).

Among various groups of microbial biocontrol agents, bacteria are able to grow in wounds or damaged crop product but not on the undamaged surfaces of fruits, vegetables, etc. which make them suitable for their application not only in soil but also during storage or in the postharvest environment (Smilanick 1994; Bissonnette & Lalande 1988; Bouillant et al. 1997). Moreover, bacterial biopesticides are target-specific, rapidly multiplying, easy to handle, nontoxic, and economically suitable organisms with better survival and longevity (Usta 2013). Recent investigations in the search for more stable bacterial inoculants have drawn the attention of researchers toward endophytic bacteria. Endophytes remain well protected from fluctuating environmental conditions and biotic factors as they colonize the internal tissues of host plants and, therefore, have a competitive advantage over bacterial populations present in rhizosphere or

phyllosphere (Backman and Sikora 2008) and thus are promising biocontrol agents for the development of high-efficiency formulations. However, the bioformulations which exhibited potent biocontrol activity against their target pests in laboratories are not easy to use with equal efficiency under field conditions as undetermined factors in the environment as well as inter- and intraspecific competition with other organisms in their niche affect their growth, physiology, metabolism, and gene expression in several ways (Khare and Arora 2015), so well-formulated preparations of bacteria are done to increase the possibility of their optimum performance and commercial success in agro-food production (Bashan et al. 2014; Mari et al. 2003).

12.3 Commercial Bioformulation in the Market

As a part of green revolution and taking of a holistic approach, bioformulation can be defined as a ready-to-use formulation, containing living cells or their metabolites (of one or more strains), supported by nontoxic and inert compounds to maintain the viability and efficiency of cells or metabolites and to increase their shelf life.

Listed below are some of the important commercially available bioformulation (Table 12.3).

The percentage of application of biocontrol products still represents only 1% of the agricultural control measures to manage plant diseases, while chemical fungicide takes up the 15% stake in plant disease management.

The reason behind is the inefficacy in application of effective biocontrol. The various bioformulation types like **liquid formulation** (Singleton et al. 2002; Knowles 2005), **emulsions** (Brar et al. 2006; Gasic and Tanovic 2013), **dry formulations** (Gasic and Tanovic 2013; Brar et al. 2006; Knowles 2008), **dust formulations** (Knowles 2001), **powder seed treatment** (Woods 2003), **granules** (Tadros 2005; Knowles 2005; Lyn et al. 2010), **wettable powders** (Brar et al. 2006; Knowles 2005), and **water-dispersible granules** (Knowles 2008) also exhibited constraints in delivery, so as per Malusa et al. 2012, there are two widely applied methods which are **seed inoculation** and **soil inoculation**.

Seed coating methods have been relatively successful when applied to small volumes of soil under greenhouse conditions, but these are limited by failure of the biocontrol agents. In addition, antibiotic-producing biocontrol agents may have deleterious effects upon the seed if applied directly to the seed coat.

The field use of bioinoculation or bioformulation is largely hampered by the lack of suitable carrier. The scientists have been in process of finding effective carrier to introduce bioformulation in to the soil.

Table 12.3 Commercial bioformulation in the market

Bioinoculant used	Target pest	Food crop	References
Pseudomonas syringae	*Botrytis cinerea*, *Mucor piriformis*, *Geotrichum*, *Penicillium* sp.	Citrus and pome fruit	Shaikh and Sayyed (2015)
P. syringae ESC 11	*B. cinerea*, *Penicillium* spp., *M. piriformis*, *Geotrichum candidum*	Pome fruits and sweet potatoes	Mari et al. (2003)
Pseudomonas fluorescens	*Erwinia amylovora*	Almond, cherry, apple, potato, and tomato	Shaikh and Sayyed (2015)
Bacillus subtilis	Phytopathogenic fungi	Cotton and legumes	Shaikh and Sayyed (2015)
Streptomyces sp.	*Fusarium*, *Alternaria*, *Pythium*	Vegetable crops	Shaikh and Sayyed (2015)
Agrobacterium radiobacter	*Agrobacterium tumefaciens*	Fruit, nut, and ornamental	Shaikh and Sayyed (2015)
Streptomyces lydicus WYEC 108	*Pythium*, *Phytophthora*, *Fusarium*, *Rhizoctonia*	Food crops susceptible to root rot and damping-off fungi	Mishra et al. (2015)
Bacillus pumilus QST 2808	Powdery mildew, downy mildew, and rust fungi	Food crops susceptible to powdery mildew, downy mildew, and rust fungi	Mishra et al. (2015)
P. fluorescens A506	*E. amylovora*	Pome fruits	Stockwell and Stack (2007)
Streptomyces griseoviridis K61	*Fusarium*, *B. cinerea*, *Rhizoctonia*, *Phytophthora*	Vegetable crops	Mishra et al. (2015)

Biofertilizers prepared as carrier-based inoculants contain effective microorganisms which include rhizobia, nitrogen-fixing rhizobacteria, plant growth-promoting rhizobacteria, phosphate-solubilizing bacteria, and so on. Incorporation of microorganisms in carrier material enables easy handling, long-term storage, and high effectiveness of biofertilizers. Basically, the carrier-based inoculant of these bacteria can be prepared by a common procedure. In this chapter, type of carrier materials available for biofertilizers and preparation in general of carrier-based inoculants will be described. Various researchers as Arora et al. (2010) have defined bioformulations in diverse ways as biologically active products containing one or more beneficial microbial strains in easy-to-use and economical carrier materials.

12.4 Carriers in Modern Agricultural Practices

The vehicle that is used to deliver the live microorganism from in vitro conditions (laboratory) to in vivo conditions (Field) is known as **carrier.**

According to the *Handbook for Rhizobia* (Somasegaran and Hoben 1994), the properties of a good carrier material for seed inoculation are (1) nontoxic to inoculant bacterial strain, (2) good moisture absorption capacity, (3) easy to process and free of lump-forming materials, (4) easy to sterilize by autoclaving or gamma-irradiation, (5) available in adequate amounts, (6) inexpensive, (7) good adhesion to seeds, and (8) good pH buffering capacity. Needless to say, (9) nontoxic to plant is another important property (Fig. 12.3).

Properties of a Good Carrier

1. It should be stable.
2. It should be able to deliver.

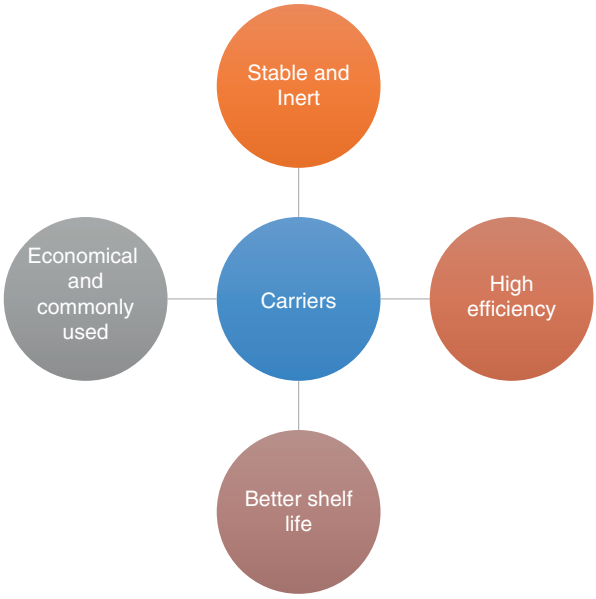

Fig. 12.3 Ray diagram to illustrate the properties of a carrier

3. It should be inert so that it does not interfere with microbial flora.
4. The bioformulation stabilized should be delivered with highest efficiency, that is, the carrier should be able to deliver the right number of viable cells under the right physiological condition at the right time (also defined as specific efficiency of the carrier).
5. It should provide better shelf life to the bioformulation.
6. It should be easily available and economical.

12.5 Types of Carriers

There are four types of carriers (Fig. 12.4):

1. Soils (peat, clay, silt, and inorganic soil) (Singh and Sharma 1973; Chao and Alexander 1984; Kotb and Angle 1986)
2. Plant waste material (mulch, sawdust, and compost), composts, farmyard manure, soybean and peanut oil (Kremer and Peterson 1982), wheat bran (Jackson et al. 1991), agricultural waste material (Sadasivam et al. 1986), sawdust (Arora et al. 2008), spent mushroom compost (Bahl and Jauhri 1986), and plant debris (Richter et al. 1989)

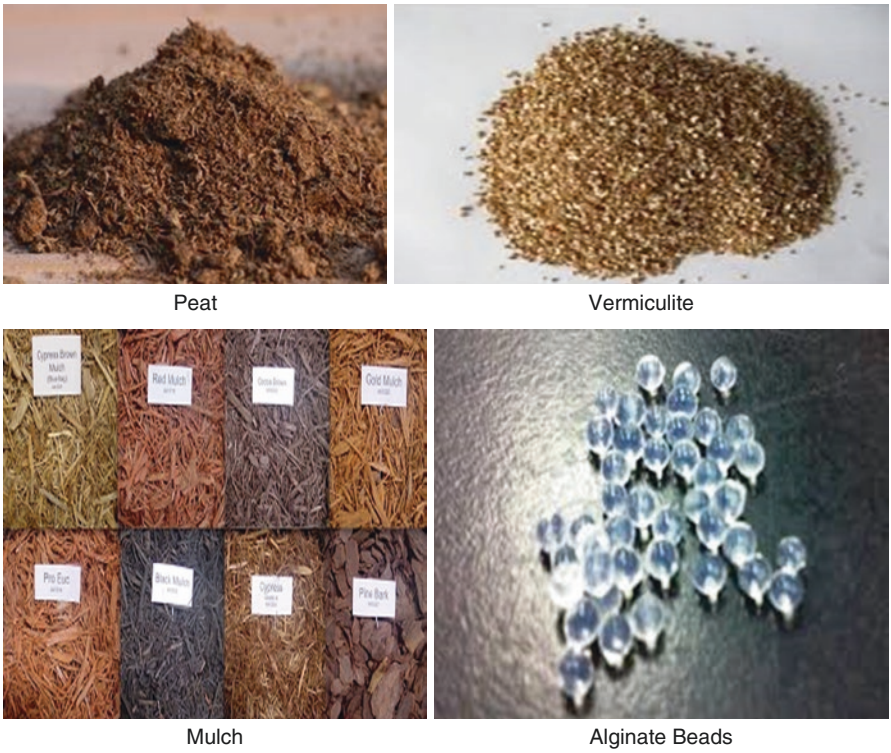

Fig. 12.4 Different types of Carriers in common use

3. Inert materials (polyacrylamide gels, alginate beads, talc)
   Vermiculite (Paau 1988; Sparrow and Ham 1983a, b), perlite (Daza et al. 2000), ground rock phosphate, calcium sulfate, polyacrylamide gels (Dommergues et al. 1979), and alginate beads (Aino et al. 1997; Sougoufara et al. 1989)
4. Plain Lyophilized Microbial Cultures

The carrier along with inoculants comes in four dispersal forms as in powders, slurries, liquids, and granules.

However in 1984, Taber et al. told about lignite-stillage carrier system for biocontrol of fungal pathogen. This carrier system was not only easy and economical but it acted as nutrient culture for biocontrol agent and was unique in the study as carrier and substrate system for impregnation of biocontrol agent to soil. After this study, many carrier-substrate systems were made for application of biocontrol agent.

Various types of material are used as carrier for seed or soil inoculation (Singh et al. 2014). For preparation of seed inoculant, the carrier material is milled to fine powder with particle size of 10–40 μm.

12.6 Sterilization of Carrier Material

Sterilization of carrier material is essential to keep high number of inoculant bacteria on carrier for long storage period.

Gamma-irradiation is the most suitable way of carrier sterilization, because the sterilization process makes almost no change in physical and chemical properties of the material. Briefly in the process of sterilization of carrier material, it is packed in thin-walled polyethylene bag and then gamma-irradiated at 50 kGy (5 Mrads).

12.6.1 The Necessity of Radiation Sterilization

The purpose of sterilization of carrier materials for biofertilizer can be for two reasons:

- To offer nutrient and place to the inoculant bacteria against the occupation by the contaminated and/or native bacteria so that the number of inoculant bacteria on carrier during the storage period before use can be kept.
- To prevent undesirable dispersion of pathogenic bacteria to agricultural field thus radiation sterilization is essential to reduce the risk of field contamination and infection.

Another way of carrier sterilization is autoclaving. Carrier material is packed in partially opened, thin-walled polypropylene bags and autoclaved for 60 min at 121 °C. It should be noted that during autoclaving, some materials change their properties and produce toxic substance to some bacterial strains. So before inoculation, the properties should be thoroughly screened.

12.7 Different Process of Formation of Carrier-Based Bioformulation

Most of the bacteria in biofertilizer have close relationship with plant roots. *Rhizobium* has symbiotic interaction with legume roots, and rhizobacteria inhabit on root surface or in soil rhizosphere. To achieve the successful inoculation of *Rhizobium* or rhizobacteria, large population of the bacterial strain must be placed close to the emerging root, so that the majority of nodules are formed by the inoculated rhizobial strain and that the inoculated rhizobacterial strain occupies the rhizosphere as a major member of rhizobacteria. If the population is not large enough, the native rhizobia/rhizobacteria will occupy most of the root nodules/rhizosphere, leading to unsatisfactory effect of inoculation. Therefore for effective inoculation, different techniques are employed with help of carriers.

12.7.1 Seed Inoculation

The most common way of inoculation is "seed inoculation" (Brockwell 1977; Bashan et al. 2014), in which the inoculant (bacteria-carrier mixture) is mixed with water to make a slurry form and then mixed with seeds. In this case, the carrier must be a form of fine powder. To achieve the tight coating of inoculant on seed surface, use of adhesive, such as gum, ethyl methyl cellulose, sucrose solutions, and vegetable oils, is recommended. Any locally available sticky material, which is nontoxic to bacteria and seeds, can be used as adhesive.

Peat is the most frequently used carrier material for seed inoculation (Bashan 1998). Peat-based rhizobial inoculant is already used in many countries, and a number of information are available on the properties and effect of the inoculants. However, seed inoculation may not always be successful, i.e., the inoculation resulted in low nodule occupancy of the inoculated rhizobial strain or low establishment of the inoculated rhizobacterial strain. This might be due to low population and/or low survival of the inoculated bacterial strain on the seed surface and in the soil.

12.7.2 Soil Inoculation

Seed inoculation may not always be successful, that is, inoculation resulted in low nodule occupancy of the inoculated rhizobial strain or low establishment of the inoculated rhizobacterial strain. This might be due to low population and/or low survival of the inoculated bacterial strain on the seed surface and in the soil. In such instance, "soil inoculation" will be adopted (Bashan et al. 2014), whereby a large population of a bacterial strain can be introduced into the soil. For soil inoculation in general, granular inoculant is placed into the furrow under or alongside the seed. This enhances the chance for the inoculated strain to be in contact with plant roots.

For soil inoculation, carrier material with granular form (0.5–1.5 mm) is generally used. Granular forms of peat, perlite, charcoal, or soil aggregates are suitable for soil inoculation.

12.8 Preparation of Carrier Material for Sterilization and Inoculation of Microorganism to Carrier

For this the following steps are followed:

- Prepare the appropriate amount of carrier material (10 kg is recommended).
- Divide into ten polyethylene packages (thickness, approx. 0.1 mm; size, approx. 20 cm × 30 cm with 1 kg carrier).
- Seal the packages using a heat sealer.
- If the carrier is a highly dry material, wet with an appropriate amount of water (to increase the indirect effect of radiation).
- If the presence of spore-forming bacteria is suspected in the carrier, add an appropriate amount of nutrient liquid medium (to promote the germination of spore).
  - Then irradiation is done by the following steps:
    Divide the carrier packages into two dose groups.
    Irradiate each group by 25 kGy or 50 kGy of γ-rays at room temperature in the atmosphere.
    In almost all cases, radiation sources are cobalt-60 or cesium-137.
    Irradiation dose can be controlled by changing the distance from the radiation source. The total irradiation time is dependent on the source activity. (option: instead of γ-rays, electron beams can be used for radiation sterilization).
    A margin of error of plus or minus 10% is allowed for irradiation dose. No limit for dose rate. A short interruption of irradiation during the total time for required dose can be allowed.
    After irradiation, preserve the irradiated packages at room temperature under the sealed condition until the inoculation of microorganisms.
  - Then confirmation of sterilization effect is done by the following methods:
    Prepare 1 g of carrier samples (nonirradiated, 25 kGy and 50 kGy irradiated samples).
    Mix with 9 ml of sterile water to make suspension.
    Dilute the suspension by serial tenfold dilutions using sterile water and spread on nutrient agar plates.
    Incubate (at 30 °C in general) and count bacterial colony number.
    Prepare 1 g of carrier samples (nonirradiated, 25 kGy and 50 kGy irradiated samples).
  - Finally inoculation of microorganisms to carrier is done by the following ways:
    Prepare starter culture for inoculation. Optionally, appropriately dilute with sterile water for moisture and cell number adjustment.

Inject the culture to the carrier package using a sterile disposable plastic syringe with a needle. Seal the needle hole with a waterproof tape.

– Keep the package at appropriate temperatures for maturation and storage as the temperatures suitable for maturation and storage are dependent on the inoculants microorganisms; however 30 °C for maturation and 20 °C–30 °C for storage will be suited for inoculants in most cases.

12.9 The Role of Carrier in Plant Disease Management

The essential criteria to be considered for carrier selection relating to survival of the inoculant bacteria are the following:

- Survival of the inoculants bacteria on seed. Seeds are not always sown immediately after seed coating with the inoculant bacteria. The bacteria have to survive on seed surface against drying condition until placed into soil.
- Survival of the inoculants bacteria during the storage period.
- Survival of the inoculant bacteria in soil. After being introduced into the soil, the inoculant bacteria have to compete with native soil microorganisms for the nutrient and habitable niche and have to survive against grazing protozoa. Such carrier materials that offer the available nutrient and/or habitable micropore to the inoculant bacteria will be desirable. In this sense, materials with microporous structure, such as soil aggregate and charcoal, will be good carrier for soil inoculants.

12.10 The Role of Carriers in Effective Delivery and Commercial Success of Bioformulation

In bioformulation preparation, carriers are the main ingredients that help to deliver bioinoculant to the field in good physiological condition and are crucial for the commercial success of bioformulations (Marjan et al. 2011). Since carrier materials play an important role in bioinoculant performance and survival in the field, they must be chosen carefully to assure easy field applicability at a minimum cost (Table 12.4). A carrier material must be easy to use, compatible with the seeding equipment at the time of seeding, stable under different field conditions and types of soil, able to help prolong the survival of the inoculated bacteria, have a long shelf life, and be harmless to nontarget organisms (Malusa et al. 2012; Bashan et al. 2014; Einarsson et al. 1993). Easy applicability of bioformulations is largely dependent on their physical form which is determined by the carrier material used in these preparations. Where various kinds of soil and organic materials like peat, clay, compost, agricultural waste, sawdust, wheat bran, etc. are used in solid formulations, liquid inoculants can be based on broth cultures, minerals or organic oils, or oil-in-water suspensions.

Table 12.4 Carriers materials used for biofertilizers

Carrier material	Inoculant bacterium	Characteristics
Sterilized oxalic acid industrial waste	*Rhizobium*	– Seed inoculation (Kaushal et al. 1996) – *Rhizobium* multiplication in carrier in ambient temperature up to 90 days – Carrier sterilization contributed significant increase in grain yield, nodule number, and nitrogen content
Alginate-perlite dry granule	*Rhizobium*	– Soil inoculation – *Rhizobium* strains survived in dry granules beyond 180 days – The inoculants can be stored in a dry state without losing much viability
Composted sawdust	*Bradyrhizobium*, *Rhizobium*, and *Azospirillum*	– Seed inoculation (Kostov & Lynch 1998) – Good growth and survival of the inoculant strains
Agriperlite, expanded clay, kaolin, Celite, diatom, porosil MP, Micro-cel, vermiculite	Agrobacterium radiobacter K84	– Crown gall control (Pesenti-Barili et al. 1991) – Screening was performed to find improved formulation of K84 cells – Effect of carrier storage temperature and carrier water content on survival of K84 was examined
Cheese whey grown cells in peat	*Rhizobium meliloti*	– Seed inoculation – Better survival at various temperatures during storage, even under desiccation
Mineral soils	*Rhizobium*	– Seed inoculants – *Rhizobium* survived better at 4 °C than at higher temperature
Coal	*Rhizobium*	– Seed inoculants (Paczkowski & Berryhill 1979) – Seven among eight tested coals supported the growth and survival of *R. phaseoli* strains. Most contained more than 107 rhizobia per g after 12 months
Granular inoculants amended with nutrients	*Bradyrhizobium japonicum*	– Soil inoculants (Fouilleux et al. 1996) – Bentonite granules, illite and smectite granules, or silica granules amended with glycerol and Na glutamate and inoculated with either peat or liquid *Bradyrhizobium japonicum* inoculants – Enhanced early nodulation of soybean and increased N content of grain
Soybean oil or peanut oil added with lyophilized cells	*Rhizobium*	– Seed inoculants – Provide more protection than peat-based inoculant when rhizobia are inoculated on seeds and exposed to condition of drought and high temperature
Perlite	*Rhizobium*, *Bradyrhizobium*, *Bacillus*	– Seed inoculants – Combination of a sucrose adhesive with the perlite carrier gave better survival of bacteria on seeds – Produced similar number of nodules, nodule dry weight, crop yield, and nitrogen content as peat-based inoculants

Table 12.4 (continued)

Carrier material	Inoculant bacterium	Characteristics
Wastewater sludge	*Sinorhizobium meliloti*	– Seed inoculants – Result showed the suitability of using sludge as a carrier because it had the same or a higher potential than peat to support survival of *S. meliloti*
Wheat bran, sugarcane bagasse	*Rhizobium*, *Bradyrhizobium*, and rock-phosphate-solubilizing fungus *Aspergillus niger*	– Soil inoculants (Hedge & Brahmaprakash 1992) – The number of cultured microorganisms was the highest with peat, followed by bran and sugarcane bagasse
Nutrient-supplemented pumice	*Rhizobium*	– Seed inoculants – Good storage and handling properties and could be mixed directly with the seeds during the sowing process

12.11 The Role of Carrier in Plant Agrosystem

12.11.1 As an Important Component of Bioformulation

There have been many articles stating the use of carriers and its roles in modern practices of plant disease management. The harmful effect of chemical pesticides is evident, and from the last two decades, efforts are being made to replace them with biopesticides, and for this, the isolates of plant growth-promoting bacteria with fungicidal property have to be successfully delivered to the soil, expressing maximum activity. To achieve this, isolates of biocontrol agents are formulated by using different organic and inorganic carriers by process of solid or liquid fermentation. The isolates are then applied as seed treatment, matrix priming, foliar spray, sucker treatment, soil treatment, seedling dip, and fruit spray (Bhattacharjee and Dey 2013).

12.11.2 To Increase the Shelf Life of Biocontrol Agent

One of the tables mentioned in *African Journal of Microbiology Research*, 2013 by R. Bhattacharjee and Utpal Dey shows the shelf life of different biocontrol agents in presence of different carriers (Table 12.5).

This chart clearly states how two bacterial strains formulated in different carriers have shown different shelf lives like *B. subtilis* formulated in talc had shelf life of only 45 days whereas when formulated in peat supplemented with chitin had shelf life of 6 months.

Even fly ash was found to be good carriers for biofertilizer strains, and it is comparatively cheaper than other carriers available in the market as stated by Kumar (2014), in his paper on fly ash as carrier to study the biocontrol, characterization, and shelf life of a locally isolated biofertilizer strains.

Table 12.5 The shelf life of different biocontrol agents in presence of different carriers

Formulation	Shelf life	Bacteria	Reference
Talc	12 months	*P. fluorescens* (p7nf tl3)	Ceaser and Burr (1991)
Talc	8 months	*P. fluorescens* (pf1)	Vidhyasekaran et al. (1997)
Talc	45 days	*B. subtilis*	Amer and Utkhede (2000)
Talc	6 months	*P. putida*	Bora et al. (2004)
Lignite	4 months	*P. fluorescens* (pf1)	Vidhyasekaran et al. (1997)
Peat with chitin	6 months	*B. subtilis*	Manjula and Podile (2001)

12.11.3 As a Facilitator in Microbial Activity

Arjomandzadegan et al. (2013), in their paper "Evaluation of Appropriate Carriers for Bio-control Agents of Apple Fire Blight," have mentioned about the carrier as an important role in biocontrol for survival of microorganisms. The aim of this study was to evaluate different compounds as carriers for *Pseudomonas fluorescens* and *Erwinia herbicola* that are used as biocontrol agents in Iran. Different compositions were prepared as carriers including peat, bagasse, bagasse-perlite, and bagasse-charcoal. The carrier was found to be of a good composition that could significantly retain bacteria viable for 6 months, and according to these criteria, all the formulae were suitable as carriers at 4 °C; however, bagasse was the best carrier at room temperature, because the numbers of bacteria were changed from 8.7×10^7 CFU/g after inoculation to 1.5×10^9 CFU/g after 6 months for *P. fluorescens* and from 2.53×10^8 CFU/g after inoculation to 1.13×10^8 CFU/g after 6 months for *E. herbicola*, and even the pH variation was not sensible in bagasse. These findings were suggestive for application of bagasse as a suitable carrier as it is nature friendly, cheap, and easily available in Iran.

12.11.4 As a Sole Source of Carbon and Energy

Vanvuurde et al. (2010) in their paper used processed manure as carrier to introduce *Trichoderma harzianum* to study population dynamics and biocontrol effect on *Rhizoctonia solani*. The antagonistic fungi could grow and sporulate on the processed manure that acted as the sole source of carbon and nutrients; thus, the incorporation of conidia in pellets of the processed manure was shown to be feasible on a laboratory scale that led to the survival of the fungus in the pellets during storage. At times the best carrier after evaluation from the rest is enriched to provide the maximum field efficiency of bioformulation. Such study was done by Naveen Arora et al. (2014) where they enriched the best carrier sawdust with molasses from the rest of the six carriers including talc, fuller's earth, rice husk, sugarcane bagasse, charcoal, and wheat bran that were also evaluated for the production of bioformulation. Molasses-enriched sawdust-based formulation showed 48.43%, 52.02%, and 57.41% enhancement in dry weight with *Rhizobium* sp., *Pseudomonas* sp., and their co-inoculant, respectively, after 60 days of sowing. Results showed that enrichment

of carrier is expected to permit the retention of cell viability thus increasing the effectiveness of the active material. In 2011, the similar growth studies were done on sugar beet by development of bioformulation of *Pseudomonas fluorescens* and *Bacillus coagulans* using organic and inorganic carriers by Jorjani et al.

12.11.5 As Single Carrier for Multiple Bioinoculants

The researchers have been in continuous process of identifying the best carrier with high efficiency and also identifying a single delivery base for multiple bio-inoculants. Naveen Arora et al. (2008) suggested sawdust as the most powerful carrier to deliver single as well as in combination bio-inoculant. The study was done on five carriers including alginate beads, charcoal, sand, sawdust, and sugarcane bagasse that were evaluated for the production of bio-inoculants. Sawdust proved to be the best carrier in maintaining the bacterial population for both individual and co-inoculation. The co-inoculants containing both rhizobial and pseudomonad population proved much better in enhancing the seedling biomass and the nodule number. The sawdust-based co-inoculant and mono-inoculant were much better than any other carrier-based inoculants taken in the study.

Similar study was done by Arora et al. (2014) by co-inoculation of PGPR (*Rhizobium* and *Pseudomonas*). The aim of this study was to determine potential five different carrier materials for survival of PGPR (*Rhizobium* and *Pseudomonas* strain) isolated from *Trigonella foenum-graecum* at room temperature for 8 weeks. Samples from the carrier materials (sterilized and non-sterilized) were taken every week and tested for the survivability and sustainability of the two different PGPR in it by determining viable cell count (CFUg-1). The result showed that after 8 weeks of storage treatment of carrier coriander husk, sawdust, and bagasse stored at room temperature (25–28 °C) was able to sustain the highest viable cell number of co-inoculation of *Rhizobium* and *Pseudomonas* followed by their individual inoculation in the carrier and determination of individual CFUg-1. These two carriers also had acceptable changes in pH value and moisture content followed by wood ashes and sand.

12.11.6 For Treatment of Seed and Enrichment of Seedling

The carriers have also helped in treatment and enrichment of seedling. The study done on the enrichment of cotton seedling and its damping off by the development of new bioformulations by Ardakani et al. (2010b) stated that formulations included a talc-based powder and bentonite-based powder as mineral carriers and peat and rice bran as organic carriers for increasing stability in interaction between PGPR and cotton plants. The results of a greenhouse experiment, where these products were applied to cotton seeds, showed that all treatments except TAL-B2 were effective (up to 62.5% control) as compared to untreated seeds. The efficacy of mineral carriers and organic carriers' treatments was much higher than that of the standard carboxin-thiram fungicide treatment at all stages.

12.11.7 Carriers as Nanoparticles and Use of Nanotechnology

In case of living microbial cells or biopesticides, nanotechnology is a newly emerging field with potent agricultural implication that includes nanocides which are encapsulated pesticide/biopesticide nanoparticles (Ghormade et al. 2011) or nanomaterial-immobilized microbial enzymes/metabolites (Kim et al. 2006). Nanoparticles of microbial metabolites or whole cell formulations induce systemic activity due to smaller particle size, higher mobility, and lower toxicity in comparison to conventionally used pesticides (Sasson et al. 2007). Integration of biomolecules (e.g., enzymes, bioactive compounds, secondary metabolites, etc.) or whole microbial cells with nanostructures leads to hybrid systems that have numerous applications in agriculture (Bailey et al. 2010).

12.12 Conclusion and Future Prospects of Existing Green Practices

The above findings clearly state that formulations containing live bacterial cells need utmost care during production, packaging, storage, and until the end use which adds extra cost to the product (Arora et al. 2010); therefore for cost-effective green revolution, there is an important role of carriers in plant agrosystem. Secondly careful selection of a biocontrol agent prior to the development of a commercial product is necessary to avoid any possible threat so that public acceptance, adoption, and registration of bacterial formulations would become easier (Handelsman 2002).

Tewari and Arora (2014) studied bio-preparations containing exopolysaccharides (EPS) derived from fluorescent pseudomonads against *Macrophomina phaseolina*, causing charcoal rot in sunflower. They found that EPS-based formulation not only effectively controlled charcoal rot but also enhanced crop yield under saline conditions. Fluorescent pseudomonads are also known to produce bioactive secondary metabolites such as antibiotics and biosurfactants that are inhibitory to phytopathogens.

The use of **biosurfactants** is also gaining importance in green practices due to their effective biocontrol potential and nontoxic nature. Raaijmakers et al. (2006) studied *Pseudomonas putida* 267 which provides excellent biocontrol activity against Phytophthora damping-off of cucumber by producing putisolvin-like cyclic lipopeptides (CLPs), biosurfactants similar to the efficacy of biosurfactants produced by *Pseudomonas koreensis*, as a crude extract was investigated successfully against *Pythium ultimum* in hydroponic tomato cultivation by Hultberg et al. (2009).

The use of nanofactories is an emerging technique in bioformulation development in which engineered bioinoculants are used to enhance communication with plants through quorum sensing that leads to **biofilm formation**. Biofilm formation not only maintains sufficient bacterial population in soil but also protects the bioinoculant from fluctuating environmental conditions and provides them a competitive advantage. N-Acyl-L-homoserine lactones, quinolone produced by genus *Pseudomonas*, and autoinducer-2 produced by *Bacillus* are examples of signaling

molecules which not only trigger biofilm formation but also enhance antibiotic production and biocontrol activity of bacterial inoculants in soil (Tewari and Arora 2013; Ryan and Dow 2008; McNab et al. 2003).

Similarly the application of **selected carrier materials** for the bacterial inoculants proves to be beneficial to protect the bacteria and have long been practiced (Ardakani et al. 2010a).

In view of safe agricultural practices and high yield, incorporation of carrier system to bioformulation is very necessary (Abd-Alla MH and Omar SA 2001). Most of the bacteria included in biofertilizer have close relationship with plant roots. *Rhizobium* has symbiotic interaction with legume roots, and rhizobacteria inhabit on root surface or in rhizosphere soil. To achieve the successful inoculation of *Rhizobium* or rhizobacteria, large population of the bacterial strain must be placed close to the emerging root, so that the majority of nodules are formed by the inoculated rhizobial strain and that the inoculated rhizobacterial strain occupies the rhizosphere as major member of rhizobacteria. If the population is not large enough, the native rhizobia/rhizobacteria will occupy most of the root nodules/rhizosphere, leading to unsatisfactory effect of inoculation, and so the carrier-based inoculation becomes a good alternative. The success of microbial inoculation to promote growth of plant is vastly influenced by the number of introduced bacteria into the soil (Catroux et al. 1999).

Therefore it is important to find out the duration of the bacterial survivability in the respective carrier materials to ensure the desired level of bacterial population remains viable for the inoculants to sustain efficient. Simultaneously the selected carrier materials must also have the properties such as cost-effectiveness, dissolve well in water so that bacteria can be released, and able to tolerate harsh environmental conditions (FAO 1998).

The studies done on carrier system and in process will one day lead to development of advanced agricultural practices of biocontrol that will completely eradicate the use of chemical pesticides and fertilizers (Reban 2002). Use of certain waste and industrial by-products as carrier materials in bacterial formulations has been studied for their significant role in bacterial formulations, and they were found quite promising (Bashan et al. 2014).

The preparation of biofertilizers is usually carrier-based containing effective microorganism. This enables easy handling, long-term storage, and high effectiveness of biofertilizer. These biofertilizers consist of majorly rhizobia, nitrogen-fixing rhizobacteria, plant growth-promoting bacteria, phosphate-solubilizing bacteria, and so on, and their carrier-based inoculants are prepared by very simple procedures. According to the results of previous studies (Shah-Smith and Burns 1997), when PGPR are formulated using inorganic or organic carriers, their stability and durability are increased. In addition, their application particularly as seed treatment becomes easier and more practical.

However it is yet to be stated that from the existing green practices which one is the best. It is the emerging agricultural need that decides the green practice that has to be implemented. Thus if every time even one of the green practices is used for pest management then it will completely replace synthetic pest control practices one day leading ecological stability.

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