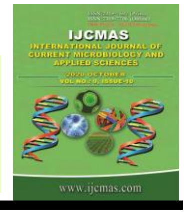
Int.J.Curr.Microbiol.App.Sci (2020) *9*(10): 3931-3946

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 10 (2020)**
Journal homepage: http://www.ijcmas.com



Review Article https://doi.org/10.20546/ijcmas.2020.910.453

Biopesticides: Production, Formulation and Application Systems

Varsha Bharti* and Shahida Ibrahim

Division of plant pathology, Sher-e- Kashmir University of Agricultural Science and Technology of Jammu Chatha, India

**Corresponding author*

Keywords

Biopesticides, Microbial component, Semiochemicals

Article Info

Accepted:
25 September 2020
Available Online:
10 October 2020

A B S T R A C T

Increasing public concern about the potential damage of chemical inputs in agricultural production systems has challenged industry to develop new and effective pest management and control strategies against insect pests, diseases and weeds. These new strategies must be less harmful to the environment than the current, chemical-based ones, and they must also safeguard the health of agricultural workers and consumers. Bio-pesticides are the formulated form of active ingredients based on microorganisms such as bacteria, viruses, fungi, nematodes or naturally occurring substances, including plant extracts and semiochemicals. Not all the natural products are biopesticides; some are chemical pesticides if they act on nervous system of the pest. The formulation process leads to a final product by mixing the microbal component with different carriers and adjuvants for better protection from environmental conditions, greater survival of the biological agents, as well as improved bioactivity and storage stability. Biopesticide formulation can be divided into liquid and dry formulation. Application of products must be easy, economical, effective, and timely to the appropriate site of action. The application of biopesticides fits the modern strategy of integrated pest management (IPM) which combines all suitable control techniques harmoniously with one another and integrates them with other crop production practices, to suppress pest populations below economic injury levels, while maintaining the integrity of the ecosystem.

Introduction

Agriculture has had to face the destructive activities of numerous pests like fungi, weeds and insects from time immemorial, leading to radical decrease in yields. With the advent of chemical pesticides, this crisis was resolved to a great extent. But the over dependence on chemical pesticides and eventual uninhibited use of them has necessitated for alternatives mainly for environmental concerns. Degraded soils and groundwater pollution has resulted in nutritionally imbalanced and unproductive lands. Violative pesticide residues also sometimes raise food safety concerns among domestic consumers and pose trade impediments for export crops. Therefore, an eco-friendly alternative is the need of the hour (Gupta and Dikshit, 2010).

The United States Environmental Protection Agency (EPA) defines biopesticides as, "certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals". They pose less threat to the environment and to human health. The most commonly used biopesticides are living organisms, which are pathogenic for the pest of interest. These include biofungicides (*Trichoderma),* bioherbicides (*Phytopthora*) and bioinsecticides (*Bacillus thuringiensis*). There are few plant products also which can now be used as a major biopesticide source. Plant-incorporated protectants include substances that are produced naturally on genetic modification of plants. Such examples are incorporation of Bt gene, protease inhibitor, lectines, chitinase etc into the plant genome so that the transgenic plant synthesizes its own substance that destroys the targeted pest. The potential benefits to agriculture and public health programmes through the use of biopesticides are considerable (Kandpal, 2014).The interest in biopesticides is based on the advantages associated with such products which includes Inherently less harmful and less environmental load. Designed to affect only one specific pest or, in some cases, a few target organisms. Often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems. When used as a component of Integrated Pest Management (IPM) programs, biopesticides can contribute greatly (kandpal, 2014).

Types of biopesticides

Biopesticides fall into three major categories:

Microbial pesticides

Microbial pesticides contain a microorganism (bacterium, fungus, virus, protozoan or alga) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest. For example, there are fungi that control certain weeds, and other fungi that kill specific insects. The most widely known microbial pesticides are varieties of the bacterium *Bacillus thuringiensis*, or Bt, which can control certain insects in cabbage, potato, and other crops. Bt produces a protein that is harmful to specific insect pest. Certain other microbial pesticides act by out-competing pest organisms. Microbial pesticides need to be continuously monitored to ensure that they do not become capable of harming non-target organisms, including humans (Mazid, 2011).

Plant- Incorporated-Protectants (PIPs)

PIPs are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Btpesticidal protein, and introduce the gene into the plants own genetic material. Then the plant, instead of the Bt bacterium manufactures the substance that destroys the pest. Both the protein and its genetic material are regulated by EPA; the plant itself is not regulated (Mazid, 2011).

Biochemical pesticides

These are naturally occurring substances such as plant extracts, fatty acids or pheromones that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are synthetic materials that usually kill or inactivate the pest. Biochemical pesticides include substances that interfere with growth or mating, such as plant growth regulators, or substances that repel or attract pests, such as pheromones. Because it is sometimes difficult to determine whether a natural pesticide controls the pest by a non-toxic mode of action, EPA has established a committee to determine whether a pesticide meets the criteria for a biochemical pesticide (Mazid, 2011).

Biopesticides in India

Biopesticides represent only 2. 89% (as on 2005) of the overall pesticide market in India and is expected to increase drastically in coming years. In India, so far only 12 types of biopesticides have been registered under the Insecticide Act, 1968 (Table 1). Neem based pesticides, *Bacillus thuringensis*, NPV and *Trichoderma* are the major biopesticides produced and used in India. Whereas more than 190 synthetics are registered for use as chemical pesticides. Most of the biopesticides find use in public health, except a few that are used in agriculture (Table 2). Besides, i) transgenic plants and ii) beneficial organisms called bio-agents: are used for pest management in India (Kandpal, 2014).

Production

Mass production technology

There are two major step for inoculum production

Solid state fermentation
Liquid state fermentation

Solid state fermentation: when organism grown on the surface of the medium**.** Example: nutrient agar, potato dextrose agar.

Liquid state fermentation: when organism grown the beneath the surface of the medium.

Example: Nutrient broth, potato dextrose broth (Askew and Laing, 1993).

Production of *Bacillus thuringiensis*(B.t) and *Bacillus sphaericus* (B.s)

Culture Maintenance and Preservation

There are over a thousand strains of *B. thuringiensis* active against agriculturally important insects. Similarly there are several hundred isolates of *B. sphaericus*, 25 percent of which are larvicidal to mosquito larvae. (Moazami, 2000). The most important single need for the production of microbial insecticides is a supply of reproducible, reliable, authentic cultures of the microorganism. The principle bacteria used in the control of insects (B.t. and B.s.) are relatively easy to maintain

Liquids

Coconut milk (waste product), crude sugar, e.g., jaggery, whey (waste product), molasses, corn steep liquid, inorganic nitrogen and (NH4)2SO4 (Moazami, 2000).

Material of plant origin

Legumes and other seeds, chick peas, peanuts, lima beans, cowpeas, soya beans, bambara beans, kidney beans, cotton seed meal, peanut cake, soy peptone, cotton seed, hydrolysate, horse beans, lentils; cereals, corn, guinea corn millets, wet mash from breweries, wheat flour, wheat bran carbohydrate, dextrin, maltose, sucrose, glucose Plant extracts, potato tubers, sweet potato roots, minced citrus peels, ground seed of dates, carrots; Tubers, cassava, yams, sweet potatoes; Yeast powder, fodder yeast. (Moazami, 2000)

Materials of animal (non mammalian) origin: Fishmeal

Materials of mammalian origin: Blood, chicken slaughterhouse residue

Minerals: Minerals are essential in the nutrition of organisms. Five metallic ions are considered to be particularly important in the growth and sporulation of bacilli: Mg++, Mn++, Fe++, Zn++, and Ca++. These are all normally present in the carbon and nitrogen sources used in fermentations and there may be no need to include these ions in the fermentation media (Moazami, 2000).

Fermentation

The fermentation of the different isolates of B.t., regardless of subspecies, have some general characteristics in common. They all use sugar (usually glucose, molasses, or starch), producing acid during the fermentation. In general, they have similar requirements for proteins or protein hydrolysates, can use NH4+ salts, and respond similarly to minerals. However, the individual isolates are unique entities, and a particular medium that may support good growth or toxin production by one isolate may be less satisfactory for another. Different isolates of B.t. may produce toxins with different spectra of activities.

After sterilization, the pH of the fermentor is pH 6.8-7.2. Immediately after inoculation, the pH falls steadily as the glucose is utilized, reaching a pH of about 5.8-6.0 after 10- 12 hours. At this point, the pH starts to rise at the same rate that it fell, reaching pH 7.5 after 25 hours.

The rise in pH slows gradually, reaching a pH of 8.0 after about 30 hours. The pH may continue to rise, reaching pH 8.8 after 50-60 hours. With some cultures, the initial drop in pH may only reach pH 6.4-6.6. In such fermentations, there may be little or no increase in pH as the fermentation continues, reaching a pH of 8.0 at about 30 hours. The pH may continue rising, reaching a pH of 8.8 after 50-60 hours.

The pH in B.s. fermentations in contrast to B.t. fermentations moves continuously upward throughout the growth and sporulation of the bacteria. Since the bacteria do not use sugars as a source of carbon, acids are not formed. Rather, ammonia accumulates in the broth, probably due to deamination of amino acids. The final pH may range from 8.0 to 9.0 depending upon the protein content of the medium. It is possible to control the pH by the addition of acid, and this may enhance toxin production by some strains but not by others.

The "log-phase" of any bacterial fermentation is that period during which the organism is vigorously growing and rapidly dividing. This first phase lasts 16-18 hours. Sporulation is complete 20-24 hours after inoculation, although the cells have not yet lysed. Lysis is complete by 35-40 hours (Moazami, 2000).

Use of new genetic-engineering technology

Biological control is the most important alternative to chemical pesticides in protecting crops from pests, pathogens, and weeds. Major breakthroughs in molecular biology and biotechnology since the early 1980s indicate that quick improvement in the competitive ability of biological control methods is possible and the biopesticide scan play a major role in crop protection in the future. It has become possible to improve some of the critical properties that earlier hampered the usefulness of many biocontrol agents. Valuable genes from completely unrelated organisms can now be utilized for biological control purposes.

Biological control using recombinant DNA (genetic engineering) technology can be achieved in several different ways: control agents may be improved; crop plants can be engineered to carry better resistance genes; or organisms associated with the plant may be modified to provide protection. All these approaches have successfully been used in several different ways experimentally.

Product development has been very active in the area of incorporating resistance genes mainly from Bt-directly into plants. Successes include potato, tomato, tobacco, and cotton. General root colorizing bacteria of plants have also been engineered to produce insecticidal toxins, which protect against pests such as the corn rootworm.

Another bacterium living in the vascular tissues of corn has also been modified to give protection against the corn borer. None of these modified plants or associated organisms is available commercially yet. Similar approaches are used for the biological control of plant pathogens and weeds, but research has been most active in the area of insect control (Moazami, 2000).

Engineering biological control agents

The genetic improvement of biological agents is a relatively new concept. For this, a great deal must be known about the biology, ecology, and behaviour of the organism. This is a very crucial step (Moazami, 2000)

Engineering crop plants

The first published reports of successful engineering of crop plants to produce insecticidal or antifeedant proteins appeared in 1987. The crop plants were tobacco and tomato, producing the delta endotoxin of *Bacillus thuriengensis*t o make them resistant against caterpillars. To date, transgenic crop plants have been produced of at least 27 different species, including potato, cabbage, sugarbeet, rice, soybeans, corn, rapeseed, sunflower, walnut and poplar (Moazami, 2000).

Instead of being inserted directly into the crop plant genome, the protective insecticidal genes can be engineered into associated organisms. Two bacteria have been successfully tested for this purpose. *Pseudomonas fluorescens*, which colonizes the root systems of crops, has been engineered to express *Bacillus thuringiensis* (Bt) toxins, and thus provide continuous protection against such pests as corn rootworm. The genes for all the major proteins that account for the insecticidal properties of Bt have been cloned and sequenced. Now we have nucleotide sequences for more than 20 Bt genes that encode proteins active against leopidopterans, eight genes encoding proteins active against dipterans, and two genes encoding proteins active against coleopterans (Moazami, 2000).

To increase the environmental stability and effectiveness of the various Bt toxins in the field, genes encoding proteins active against beetles and caterpillars have also been cloned into the rhizobacterium *Pseudomonas fluorescens*. After fermentation, the bacteria are killed and the cell walls hardened chemically. The endotoxins are thereby microencapsulated, resulting in insecticides with greatly enhanced residual activity. Large-scale field trials with this product have been performed, and the product obtained full registration in 1991 (Moazami, 2000).

Through genetic-engineering techniques, the *Autographa californica multinucleocapsid nucleopolyhedrosis virus* (AcMNPV) has been engineered to kill insects more quickly by expressing either enzymes or toxins soon after host invasion. Of particular interest is the possibility of making viruses produces insect neurohormones, which can cause rapid physiological disruptions in minutely defined target hosts. This strategy is in its early stages of development, but there is little doubt that within the very near future we will have viruses with extended or specifically designed host ranges, capable of killing insects within 24 to 48 hours. These genetically engineered viruses should have an advantage for use against hosts that are not easily controlled by Bt.(Moazami,2000).

Very little is known about the genetics of entomopathogenic fungi. The first transformation system for an entomopathogenic fungus was developed using *Metarhiziumanisopliae* protoplasts mixed with a fungicide-resistant plasmid. A benomyl-resistant strain of *M.anisoplieae*has thus been obtained. Fungal enzymes involved in the penetration of the insect cuticle have now been identified. Knowledge of these genes and gene products will eventually lead to the possibility of genetic alteration of fungal pathogens that possess those genes.Transformation systems for some fungi exist already and may soon be applied to the entomopathogenic species(Moazami,2000).

Formulation

Formulation refers to the preparation of a product from an active ingredient by the addition of certain active (functional) and non-active(inert) substances (Grewal, 2005).

Principles of formulation

Formulated organisms are suspended in a suitable carrier which is supplement by additives to maximize survival in store, optimize application to the target and protect the organisms after application. In contrast to chemical active ingredients, they are particulate and live or proteinous in nature, making them relatively sensitive to storage conditions and the environment (Bergis and jones,1998). There are a number of amendments used in preparation of formulation and some important ones are listed below (Table 3).

Four basic function of formulation

To stabilize the organism during distribution and storage.

To aid handling and application of the product so that it is easily delivered to the target in the most appropriate manner and form.

To protect the agent from harmful environmental factors at the target site, thereby increasing persistence.

To enhance activity of the organism at the target site by increasing its activity, reproduction, contact and interaction with the target pest or disease organism (Seaman, 1990; Mollet, 2001).

Regarding their physical state, biopesticide formulations can be divided two formulations.

Liquid formulations
Dry formulations.

Liquid formulations can be water-based, oil-based, polymer-based, or combinations. Water-based formulations (suspension concentrate, suspo-emulsions, capsule suspension, etc.) require adding of inert ingredients, such as stabilizers, stickers, surfactants, coloring agents, antifreeze compounds, and additional nutrients.

Dry formulations can be produced using different technologies, such as spray drying, freeze drying, or air drying either with or without the use of fluidized bed. They are produced by adding binder, dispersant, wetting agents, etc. (Tadros, 2005; Brar, 2006; Knowles, 2008).

Biopesticides are usually formulated as: dry formulations for direct application – dusts (DP), seed dressing formulations – powders for seed dressing (DS), granules (GR), micro granules (MG), dry formulations for dilution in water – water dispersible granules (WG), and wettable powders (WP); liquid formulations for dilution in water – emulsions, suspension concentrates (SC), oil dispersions (OD), suspo-emulsions (SE), capsule suspensions (CS); ultra low volume formulations (Knowles, 2005, 2006).

Dusts

Dusts (DP) are formulated by sorption of an active ingredient on finely ground, solid mineral powder (talc, clay, etc.) with particle size ranging from 50-100 µm. Dusts can be applied directly to the target, either mechanically or manually. Inert ingredients for this formulation are anticaking agents, ultra violet protectants and adhesive materials to enhance adsorption. Concentration of active ingredient (organism) in dust is usually 10%.

Although they have positive effects under certain circumstances, they also pose serious inhalation hazard for users. This is an old formulation type that had been used for many years before granules were developed and they became restricted on the account of their adverse health impact on users. Other dusts are manufactured very simply and they are still used today in many parts of the world (Knowles, 2001).

Powders for seed treatment (DS)

Powder for seed treatment are formulated by mixing an active ingredient, powder carrier and accompanying inert to facilitate product adherence to seed coats. This type of formulation is applied to seeds by tumbling seeds with the product designed to adhere to them. Powders for seed treatment are a very old type of formulation, a traditional product form for coating seeds, and they also contain a red pigment as a safety marker for dressed seed (Woods, 2003).

Granules (GR)

Granules (GR) are similar to dust formulations, except that granular particles are larger and heavier. Coarse particles (size range 100-1000 microns for granules and 100-600 microns for micro granules) are made from mineral materials (kaolin, attapulgite, silica, starch, polymers, dry fertilizers and ground plant residues) (Tadros, 2005). Concentration of active ingredient (organisms) in granules ranges from 5-20%. The active ingredient either coats the outside of the granules or is absorbed into them. Granule products are very simply manufactured, their active ingredient is processed by mixing a powder blend with a small amount of water to form a paste which is then extruded and dried if necessary. Another way of production is applying a liquid active ingredient to coarse absorptive material. After that granules can be coated with resins or polymers to control the rate of effectiveness of active ingredient after application. Granular biopesticides are mostly used to apply products to soil in order to control weeds, nematodes, and insects living in soil, or for plant uptake by root. Once applied, granules release their active ingredient slowly. Some granules require soil moisture to release their active ingredient (Knowles, 2005; Lyn, 2010).

Wettable powders (WP)

Wettable powders (WP) are dry, finely ground formulations to be applied after suspension in water. Wettable powders are produced by blending an active ingre- dient with surfactant, wetting and dispersing agents and inert fillers, followed by grinding to a required particle size (about 5 microns). These products can raise serious health and safety issues for manufacturers because of their dustiness, which can cause inhalation and skin and eye irritation problems if strict safety precautions are not taken. For these reasons and because of their dustiness during application, wettable powders are gradually suppressed by suspension concentrates or water dispersible granules, which have been the most widely used pesticide formulations (Knowles, 2005). Regarding solid biopesticide formulations, much attention has been focused on WPs because of their long storage stability, good miscibility with water and convenient application using conventional spraying equipment (Brar, 2006). Water dispersible granules (WG) have been developed to overcome problems of dustiness of powder formulations.

Water dispersible granules (WG)

Water dispersible granules are designed to be suspended in water, i.e. granules break up to form uniform suspension similar to that formed by a wettable powder. Compared to powder products these WGs are relatively dust-free, and with good storage stability. Water dispersible granules can be formulated using various processing techniques, such as extrusion granulation, fluid bed granulation, spray drying, etc.

The products contain wetting agent and dispersing agent similar to those used in wettable powders, but the dispersing agent is usually at higher concentration. Water dispersible granules are usually more expensive than older types of formulations (dusts, wettable powders) but their safety and greater convenience regarding application make them still desirable for many users (Knowles, 2008).

Emulsions

Emulsions consist of liquid droplets dispersed in another immiscible liquid (dispersed phase droplet size ranges from 0.1 to 10 µm). Emulsion can be oil in water (EW), which is a normal emulsion, or water in oil (EO), an invert emulsion. Both products are designed to be mixed with water before use. To avoid instability the proper choice of emulsifiers for stabilization is extremely important. In the case of invert emulsions, losses due to evaporation and spray drift are minimal because oil is the external phase of the formulation (Brar, 2006). However, lower shelf stability and occasional phyto- toxicity may affect the overall performance of emulsions. Studies are currently being conducted to screen a variety of oils and emulsifying agents in order to improve initial invert emulsion formulations for biopesticides (Verner, 2007).

Suspension concentrate (SC)

Suspension concentrate (SC) is a mixture of a finely ground, solid active ingredient dispersed in a liquid phase, usually water. The solid particles are not dis- solved in liquid phase, so that the mixture needs to be agitated before application to keep particles evenly distributed. The composition of suspension concentrate is complex and it contains wetting/dispersing agents, thickening agents, antifoaming agents, etc. to ensure a required stability. They are produced by a wet grinding process and have particle size distribution ranging from 1-10 µm. During the grinding process, inert ingredients adsorbed onto particle surfaces prevent re-aggregation of small particles.

These small particles often exhibit improved bioefficacy in use because greater particle surface area offers easier access of the active ingredient to plant tissues. Because they are water-based, they offer many advantages, such as of pouring and measuring, safety to the operator and the environment, and economy. Therefore they are becoming a very popular type of formulation (Woods, 2003; Knowles, 2005).

Table.1 Biocontrol agents

S. No.	Name of the Biopesticide
1.	***Bacillus thuringiensis var. israelensis***
2.	***Bacillus thuringiensis var. kurstaki***
3.	***Bacillus thuringiensis var. galleriae***
4.	***Bacillus sphaericus***
5.	***Trichoderma viride***
6.	***Trichoderma harzianum***
7.	***Pseudomonas fluoresens***
8.	***Beauveriabassiana***
9.	***NPV of Helicoverpa armigera***
10.	***NPV of Spodopteralitura***
11.	**Neem based pesticides**
12.	**Cymbopogan**

Table.2 Different bio pesticides used against various plant pathogens

Trade name	Biocontrol organism	Class	Target disease	References
Rhapsody/ Serenade	*Bacillus subtilis*	Fungicide , soil foliage	*Rhizoctonia, Fusarium, Alternaria, Aspergillus*	Marrone ,2002 Quarles, 2005
Blightban A506	*Pseudomonas fluorescens*	Fungicide Bactericide	Fire blight, frost damage, bunch top	Quarles,2011
Green light	Neem oil	Fungicide,	Powdery mildew, rust, anthracnose, leaf spot	Cao *et al*,2010
Madex HP	Codling moth granulosis Virus	Insecticide	Fruit moth	Arthurs *et al*, 2004.
Soil Gard	*Trichoderma virens*	Fungicide	*Pythium, Rhozoctonia* and root rot	Rose *et al*, 2004.

Table.3 Different type of amendments and example materials for formulation

Amendment type	Examples
Liquid carriers	Vegetable oil
Mineral carriers	Kaolinite clay, diatomaceous earth.
Organic carriers	Grain flours
Stabilizers	Lactose ,sodium benzoate
Nutrients	Molasses ,peptone
Binders	Gum Arabic, carboxymethy cellulose
Desiccants	Silica gel, anhydrous salts
Thickeners	Xanthan gum
Surfactants	Tween 80
Dispersants	Microcrystalline cellulose
UV protectants sunscreens	Oxybenzone
Light blockers	Lignin (PC 1307)
Stickers	Pregelatinized corn flour

Table.4 Different Sprayer technology used for application of control agents against pests

Sprayer / application technology	Control agent	Pest	References
Electrostatic knapsack mistblower; hydraulic.	Bt	Diamondback moth	Perez *et al.* (1995)
Spinning discs	Nematode	Diamondback moth	Mason *et al.* (1998, 1999)
Grooved and smooth spinning discs	Nematodewith polyacrylamide	Leaf miner	Piggott (2000), Piggott *et al.* (2000)
Refrigerated aerial l spraying	Predator pupae	*Trichogramma*	Bouse and Morrison (1985)
Dispersing pheromones in paraffin-wax capsules	Insectsex pheromones	Fruit moth	Atterholt et *al.* (1994)

Oil dispersions (OD)

Oil dispersions (OD) are dispersions of solid active ingredients in non-aqueous liquid intended for dilution before use. The non-aqueous liquid is most often an oil, the best choice is some kind of plant oil. In that way retention, spreading and penetration can be improved. Oil dispersion provides several important characteristics, such as an ability to deliver water sensitive active ingredients and an ability to use an adjuvant fluid in- stead of water which can increase and broaden pest control. This formulation is produced in the same way as suspension concentrate. Inert ingredients for this type of formulation should be carefully selected to prevent instability problems (Verner, 2007).

Suspo-emulsions (SE)

Suspo-emulsions (SE) can be considered as a mixture of suspension concentrate and emulsion. The product is very demanding to formulate because it is necessary to develop a homogenious emulsion component simultaneously with a particle suspension component which will remain stable in the final formulation of the product. Careful selection of appropriate dispersing and emulsifying agents is necessary to overcome the problem of heteroflocculation between solid particles and oil droplets. In addition, extensive storage stability testing of this formulation is necessary (Knowles, 2008). In spite of the complexity of this formulation, the use and importance of suspo-emulsions has been remarkable and will continue to increase.

Capsule suspension (CS)

Capsule suspension (CS) is a stable suspension of micro-encapsulated active ingredient in an aqueous continuous phase, intended for dilution with water before use. Bio-agent as its active ingredient is encapsulated in capsules (coating) made of gelatin, starch, cellulose and other polymers. In that way the bio-agent is protected from extreme environmental conditions (UV radiation, rain, temperature, etc.), and its residual stability is enhanced due to slow (controlled) release. The most frequently applied method of encapsulation uses the principle of interfacial polymerization. Encapsulation in microcapsules has been extensively used to give smaller size and high efficiency to fungal biopesticide formulations (Winder, 2005; Brar, 2006). Microcapsule suspensions need to be stabilized with surfactants and thickeners in the same way as sus- pension concentrate and similar additives are used. Despite clear benefits of this controlled release formulation, its commercial development is rather slow. The slow progress is partly due to the complexity of formulation and partly to its high production cost (El-Sayed, 2005; Chen, 2013).

Ultra low volume liquids (UL)

Ultra-low volume liquids (UL) are formulations with very high concentration of active ingredient which is extremely soluble in crop-compatible liquid (ultra-low volume liquid). UL products are not intended for dilution with water before use and often contain surface active agents and drift control additives. Ultra-low volume liquids are easy to transport and use. UL liquid biopesticides can be formulated in a similar way using a suspended biocontrol agent as an active ingredient (Woods, 2003).

Application system

Delivery of products must be easy, economical, effective, timely to the appropriate site of action, and compatible with current agronomic practices and equipment. Formulated microbes can be delivered to seed, seed pieces, tubers cuttings seedlings, transplants mature plants, or soil, these application methods are

Seed treatment

For optimal protection of germinating seeds and seedlings against disease, the biofungicides need to be delivered in a manner that allows the organism(s) to colonize the spermospere and the developing rhizosphere at a density that is high enough to suppress the pathogen (Cook and Baker, 1983). Biocontrol agents can be percoated or encapsulated onto the seed, mixed with the seed at the planting, applied in-furrow, or incorporated into the soil-mix or seed bed (Thomashow and Weller,1990).

Precoating of seed usually involves formulations of dry powders or oil-and polymer-based liquids with dormant microbes that are capable of surviving a period of desiccation (Pauu, 1988). Additives, such as xanthum gm and gum Arabic are sometimes used to increase adhesion of the microbial product to the seed. A specialized seed-coating process, termed seed encapsulation, involves enveloping the seed, microbe and possibly other components such as pesticides or micronutrients, in a gelatinous or polymer gel-matrix, thereby prolonging survival of microbial agents on seed. An example of a seed encapsulation product is GEL-COAT, which is an alginate hydrogel preparation patented as a delivery system for entomopathogenic nematodes. The seed encapsulation method of delivery has the distinct advantage of user safety and reduced environmental hazard, since the active ingredients are effectively sealed until they are released during seed germination. Factors to consider in selecting a formulation for coating seeds include inoculum density achievable on the seed, stability of the coating, both for microbe viability and coat integrity, and the feasibility and cost of production (McIntyre and Press, 1991). Formulations consisting of fine dusts or powders, wettable powders, or liquids can be applied to seed with or without sticker materials at the time of planting. Delivery at the time of planting usually ensures a high number of viable microbes and may allow growers to apply the product directly into the plant box. Drawbacks to this delivery method include possible variability in efficacy resulting from a reliance on the grower's ability to apply the seed treatment correctly and the extra task for growers (Boyetchko *et al*, 1996).

Soil treatment

If seed treatment is not a practical option, e.g., if direct inoculation onto seed is harmful to the microbe due to dessication, or presence of inhibiting compounds (Gindrat, 1979), biocontrol agents can be applied to soil. Soil treatment is most effective when the agents are applied as a post-fumigation treatment or at time of planting. In sterile soil or growth mixes, colonization by pathogens may be reduced by establishing a high population of the biocontrol agent. This creates a "suppressive soil," making subsequent colonization by other less beneficial organism's difficult (Lumsden *et al.*, 1995). Dust, powder and granular formulations can be broadcast and incorporated into soil,where as wettable powder, water-dispersible granular, and liquid formulations can be delivered in furrow (Lewis,1991). Soil application may also be a useful method for controlling overwintering pathogen propagules in soil. For example, the product CONTANS, a water dispersible granular formulation of the hyperparasite *Coniothyrium minitans* can be incorporated into soil to reduce the number of *Sclerotiniasclerotiorum* (Boyetchko *et al.*, 1996).

In greenhouse crops, a simple yet effective method of delivering biocontrol agents to soil or growth medium is by direct injection into an irrigation system, such as overhead boom or spaghetti systems. This type of delivery is advantageous in that it allows precise control of the concentration and total volume of microbial suspension being applied, and requires minimal labour to treat large numbers of plants. The one drawback to this type of delivery system is that it requires specialized injection equipment (Boyetchko *et al.*, 1996).

Treatment of plants

Biocontrol products can also be applied to plant roots, wounds and foliage by drenching, dipping or spraying. Formulated bacteria can be applied directly to roots as a dip or drench (Funk, 1997).Spores of the biofungicide *Phelbia gigantea* in an aqueous suspension can be brushed onto freshly cut stumps of pine to prevent entry of *Heterobasidion annosum* (Rishbeth, 1975), thereby protecting exposed wounds. Alternatively, spores can be incorporated into chain saw oil so that they are delivered at the same time the tree is harvested. Formulations of bacteria or fungi used as foliar sprays vary according to the crop to be treated, the pest to be controlled, and the anticipated delivery system. The two formulations most commonly used for foliar sprays are liquids and slurries, with the slurries usually reconstituted from either dry or moist carrier-based formulations. Emulsifiers, stickers, spreaders, and other adjuvants and additives aid in application, dispersal and adhesion of the microbes on plant surfaces, and protect the microbes from adverse environmental conditions, such as desiccation, unfavourable pH, and UV radiation (Harvey, 1991 and Shieh, 1995). A broad range of spray application equipment and techniques is available for applying chemical pesticides (Table 4) including high volume (1000 L/ ha), medium volume (350 L/ha), low to very low volume (3-150L/ha), and ultra low volumes (0.5-3.1 L/ha), controlled droplet application, and electrostatic spraying (Auld, 1992). If control agents are to be applied using the same techniques, formulations must have the necessary physical properties.

Steinke and Akesson found that surface tension and viscocity of the suspension to be sprayed are important factors in reducing droplet size and maintaining the necessary dispersion and control of droplets. Density of the suspension was not an important factor. Successful application of biocontrol agents using different spray techniques has been achieved. For example, Bt-based products have been applied to numerous crops using conventional ground or aerial spraying methods. Highly concentrated ultra-low volume liquid formulations of Bt-based products have also been used to control insect pests on such crops as cotton and banana (Sheih, 1995). And to control spruce bud worm over large areas of coniferous forests(Bryant,1994). A low –volume electrostatic rotary atomizer has been used to apply *Verticilliumlecanii,* an entomopathogenic fungus, to successfully control the aphid *Aphis gossypii*. In addition,ultralow-volume equipment, such as spinning disk sprayers, are now commonly used for application of baculoviruses in forests (Cory and Bishop,1995).

In conclusion

Organic food indicates huge scope for growth of Bio-pesticides sector in India. At the same time increasing population can be fed by organic farming dependence is a big question and unless organic farming yield can be brought equal to that of conventional farming involving the use of agrochemicals etc, the organic farming may not be feasible at the moment.

Rich traditional knowledge base available with the highly diverse indigenous communities in India may provide valuable clues for developing newer and effective bio pesticide.

A new more active strain of *B.t* was produced which has increased the performance and acceptance of commercial products and broadened its use against other insect pests.

Commercial biopesticides should be economical to produce, have persistent storage stability, be easy to handle, mix and apply, and provide effective control of target pests.

References

Arthurs, S.P. and Lacey, L.A. 2004. Field evaluation of commercial formulations of the codling moth granulosis virus: persistence of activity and success of seasonal applications against natural infestations of codling moth in Pacific Northwest apple orchards. *Biological*

*Control*31(3):388-397.

Askew, A. and Laing, D.1993. An adopted selective medium for the quantative isolation of *Trichoderma. British society for Plant pathology* 40(5):686-690.

Atterholt, C. A., Delwiche, M. J., Rice, R. E. andKrochta, J. M. 1994. Controlled release of insect sex pheromones from paraffin wax and emulsions. *Journal ofControlled Release* 57(3): 233–247.

Auld, B.A.1992. *Mass production ,formulation and application of fungi as biocontrol agents.*In:Biocontrol Control of Locusts and Grasshoppers.CAB International Wallingford,UK,pp.219-229.

Bergis, H.D. and Jones, K.A.1998. Formulations of microbial biopesticides. New York press. pp. 33-45.

Bouse, L. F. and Morrison R. K. 1985. Transport, storage and release of *Trichogramma pretiosum*. *Southwest Entomologist Supplement*8:36–48.

Boyetchko, S.M. 1996. Impact of soil microorganisms on weed biology and ecology.*Phytoprotection*77: 41-56.

Brar, S.K., Verma, M., Tyagi, R.D. and Valero, J.R. 2006. Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. *Process Biochemistry*. 41(2): 323-342.

Bryant, J.E.1994.An industrial view of *Azospi rillum* inoculants: formulation of *Bacillus thuringiensis. Agric. Ecosystem. Environmental* 49:31-35.

Cao,C., Park,S. and Gardener, M.B.B. 2010.Biopesticide Controls of Plant Diseases: Resources and Products for Organic Farmers in Ohio.Department of Plant Pathology. The Ohio State University.

Chen, K.N., Chen, C.Y., Lyn, Y.C. and Chen, M.J. 2013. Formulation of a Novel Antagonistic Bacterium Based Biopesticide for Fungal Disease Control Using Microencapsulation Techniques. *Journal of Agricultural Science*5(3): 153-163.

Cook, R.J. and Baker, K.R.1983.The Nature and Practice of Biological Control of Plant Pathogens. *American Phytopathological Society*, pp.539-545.

Cory, J.S.and Bishop, D.H.L.1995.Use *of baculoviruses as biological insecticides*, In:Methods in Molecular Biology, Baculovirus Expression protocols (Eds. C.D. Richardson). Humana, Totowa, NJ,39:.277-294.

El-Sayed, W. 2005. Biological Control of Weeds with Pathogens: Current Status and Future Trends. *Journal of Plant Disease and Protection*, 112(3): 209-221.

Funk, L.M., He,D.N., Pedersen, E.A.and Reddy, M.S.1997.Optimization of product delivery for a microbial inoculant, *Burkholderia cepacia*, for commercial use in the forest industry. *Journal of Plant Pathology*.19:108.

Fuxa, J.R. 1995. Ecological factors critical to the exploitation of entomopathogens in pest control. *American chemical society Symposium Series*97(3): 556-589.

Gasic, S. and Tanovic, B. 2013. Biopesticide formulations, possibility of application and future trends. *Pesticides and Phytomedicine*28(2): 97-102

Gindrat, D. 1979. *Biological of plant diseases by inoculation of fresh wounds, seeds and soil with antagonists*. In:Soil-Borne Plant Pathogens (Eds. B. Schippers, and W.Grams). *Academic, New* York, pp.537-551.

Grewal, P.S.2005.Formulations and Quality control of entomopathogenic nematodes. Department of entomology, *Ohio-state-unverisity Wooster*.

Gupta,S. and Dikshit, A.K. 2010. Biopesticides:An eco-friendly approach for pest control. *Journal of*

Biopesticides. 3(1):186-188.

Harvey, L.T.1991.A Guide to Agricultural Spray Adjuvants Used in the United States. *Thompson Publication* 45: 345-356.

Kandpal, V.2014. Biopesticides. *International Journal of Environmental Research and Development*4(2): 191-196.

Knowles, A. 2005. New developments in crop protection product formulation. Agrow Reports UK: *T and F Informa UK Ltd*., pp.153-156.

Knowles, A. 2006. Adjuvants and additives. Agrow Reports: *T&F Informa UK Ltd*.pp.126-129.

Knowles, A. 2008. Recent developments of safer formulations of agrochemicals. *Environmentalist*. 28(1): 35-44.

Knowles, A.2001. Trends in Pesticide Formulations. Agrow Reports, UK: *PJB Publications Ltd*., pp 89-92.

Lewis, J.A.1991. *Formulation and delivery systems of biocontrol agents with emphasis scentonfung*, In:The Rhizosphere and Plant Growth (Eds.D.L. Keisterand P.B. Cregan). , Kluwer, Dordrecht, *The Netherlands*, pp.279-287.

Lumsden, R.D., Lewis, J.A., and Farvel, D.R.1995. *Formulation and delivery of biocontrol agents for use against soilborne plant pathogens* In: Biorational Pest Control Agents. Formulation and Delivery. (Eds. F. R.Hall, and J.W. Barry).*ACS symposium series*, Washington, 166-182.

Lyn, M.E., Burnett, D., Garcia, A.R. and Gray, R. 2010. Interaction of Water with Three Granular Biopesticide Formulations. *Journal of Agricultural and Food Chemistry*58(1):1804-1814

Marrone, P.G.2002. An effective biofungicide with novel modes of action. *Pesticide Outlook*13(5):193-194.

Mason, J. M., Matthews, G. A., Wright, D. J. 1999. Evaluation of spinning disc technology for the application of entomopathogenic nematodes against a foliar pest. *Journal of Invertebrate Pathology*73(3): 282–288.

Mason, J.M., Matthews, G. A., Wright, D. J. 1998. Appraisal of spinning disc technology for the application of entomopathogenic nematodes. *Crop Protection*17(5):453–461.

Matthews, G .A. 1999. Pesticides, IPM and training. *Phytoparasitica*27(4): 253–258.

Mazid,S., kalita,J.C. and Rajkhowa, R.C. 2011.A review on the use of biopesticides in insect pest management. *International Journal of Science and Advanced Technology*. 1(7): 2221-8386.

Mclntyre, J. L. and Press, L.S.1991. *Formulation, delivery systems and marketing of biocontrol agents and plant growth promoting rhizobacteria* (*PGPR*) In: The rhizosphere and plant growth(Eds. D.L.Keister and P.B. Creganeds.). *Betsville Symposia in Agricultural Research, Beltsville*, MD, pp. 289-295.

Moazami, N.2000.Biopesticide production. *Iranian research Organization for Science and Technology,Tehran,Iran.*

Mollet, H., & Grubenmann, A. 2001. Formulation technology. Weinheim, Germany. pp. 389-397.

Paau, A. S. 1988. Formulations useful in applying beneficial microorganisms to seed. *TibTech*6:276-279.

Perez, C. J., Shelton, A. M., Derksen, R. C. 1995. Effect of application technology and *Bacillus thuringiensis* subspecies on management of *B. thuringiensis* subsp. kurstaki-resistant diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*. 88(5): 1113–1119.

Piggott, S. J.,Wright, D. J., Matthews, G. A.

2000. Polymeric formulation for the application of entomopathogenic nematodes against foliar pests. *The BCPC Conference on Pests and Diseases, Brighton*, UK, 3: 1063–1068.

Piggott, S.J. 2000. Development of improved foliar application technology for entomopathogenic nematodes. PhD Thesis, University of London.

Quarles, W. 2005. Landscape IPM strategies for diseases of ornamentals. *IPM Practitioner*27(3):1-8

Quarles, W. 2011. Pesticides and honey bee death and decline. *IPM Practitioner*, 33(1/2):1-8.

Rishbeth, J.1975. Stump inoculation: *A biological control of Domes annosus.* In:Biology and Control of Soil-Borne Plant Patholgy (Ed. G.W.Bruehl). American Phytopathological Society, pp.158-162.

Rose, S., Yip,R. and Punja,Z.K.2004. Biological control of *Fusarium* and *Pythium* root rots on greenhouse cucumbers grown in rock wool. *Acta Horticulturae.* 635:73-78.

Seaman, D. 1990. Trends in the formulation of pesticides: An overview. *Pesticide Science.* 29(4): 437.

Shieh, T.R.1995. Biopesticide formulations and their applications, In:Proceedings of American Chemical Society (Eds.N.N. Ragsdale, P.C. Kearney and J.R. Plimmer). Eighth International Congress of Pesticide Chemistry options 2000, Washington, DC, pp.104-114.

Steinke, W. E. and Akesson, N.B.1993. *Atomization of biopesticide formulations*, In:Pesticide Formulations and Application Systems, Volume 12, ASM STP 1146 (Eds.B.N.Devisetty, D.G. Chasinand P.D.Berger).American Society for Testing and materials, Philadelphia, pp.257-271.

Tadros, F. 2005. Applied surfactants, principles and applications.*Wiley-VCH Verlag GmbH and Co. KGaA*, pp.187-256.

Thomashow, L.S. and Weller, D.M.1990.Application *of fluorescent pseudomonads* to control root diseases of wheat and some mechanisms of disease suppression, In:Biological control of Soil-Borne Plant Pathogens (Ed.D.Hornby). Redwood, Melkshan Wiltshire, UK, pp.109-122

Vernner, R., & Bauer, P. 2007. Q-TEO, a formulation concept that overcomes the incompability between water and oil. *Pfalzenschutz-Nachrichten Bayer*60(1): 7-26.

Weidemann, G.J., Boyette, C.D. and Templeton, G.E.1995. Utilization criteria in mycoherbicides. *American chemical society Symposium Series.* 88(6): 224-245.

Winder, R.S., Wheeler, J.J., Conder, N., Otvos, S.S.,Nevill, R. and Duan, L.2005. Microcapsulation: a strategy for Formulation of inoculation. *Biocontrol Science and Technology* 13(2):15-169.

Woods, T.S. 2003. Pesticide Formulations. *Encyclopedia of Agrochemicals*, New York, pp. 1-11.

How to cite this article:

Varsha Bharti and Shahida Ibrahim. 2020. Biopesticides: Production, Formulation and Application Systems. *Int.J.Curr.Microbiol.App.Sci.* 9(10): 3931-3946.
doi: https://doi.org/10.20546/ijcmas.2020.910.453