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Biological management of plant diseases through bacterial bioagents

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Abstract : As the world population continues to upsurge while arable land and most other natural resources continue to decrease, and as our environment becomes further congested and stressed, the need for controlling plant diseases effectively and safely will become one of the most basic necessities for feeding the hungry billions of our increasingly overpopulated world and sustainable agriculture.In the last few decades, the control of plant diseases a has depended increasingly on the extensive use of pesticides. Controlling plant diseases often needs the application of pesticides not only on plants and plant products that we consume, but also into the soil, where many pathogenic microorganisms live and attack the plant roots. Many of these pesticides have been shown to be toxic to non-target microorganisms and animals and may be toxic to humans. The short- and long-term costs of environmental contamination on human health and welfare caused by our efforts to control plant diseases are difficult to estimate. The challenges for plant pathology are to reduce food losses while improving food quality and, at the same time, safeguarding our environment. Much of modern research in plant pathology aims at finding other environmentally friendly means of controlling plant diseases. The most promising approaches include enhancement of resistance in plants against pathogens, through conventional breeding, genetic engineering, cultural practices and use of biological agents antagonistic to the pathogens. The biological control aims at eradication and control of pathogens through the activity of other microorganisms. A number of antagonistic microorganisms have been found to increase in suppressive soils; most commonly, however, pathogen and disease suppression has been shown to be caused by bio-agents including bacteria of the genera *Pseudomonas*, *Bacillus*, and *Streptomyces*. The mechanisms by which antagonistic microorganisms affect pathogen populations are: (1) direct parasitism or lysis and death of the pathogen, (2) competition with the pathogen for food, (3) direct toxic effects on the pathogen by antibiotic substances released by the antagonist, and (4) indirect toxic effects on the pathogen by volatile substances, such as ethylene, released by the metabolic activities of the antagonist. The bacterial bioagents cand be applied through different means like seed treatment, soil amendment and foliar spray etc. for management of crop diseases.

Key words: Antibiosis, *Bacillus,* Bacterial bioagents, Competition, *Pseudomonas*, *Streptomyces*

Introduction : Plant diseases are the major constraint affecting the production and productivity of crops both in terms of quality and quantity. Globally, crop losses (27-42%) due to plant diseases are the major threat to the food production and this loss may be doubled if no disease management strategies applied (Singh, 2014 and Alizadeh *et al.,* 2020). Generally, pesticides are widely used for management of crop diseases.Pesticide application is effective, but their use is being discouraged because it is known to cause serious threat to environment, imbalance in the ecosystem and human health hazards. Eco-friendly approaches have attained importance in modern day agriculture to curtail the hazards of extensive use of toxic chemicals for disease control (Homer, 1988). However, as a modern and educated society, we should think about the effects and consequences of conventional (chemical) agriculture. Residues of pesticides and other chemicals used in conventional agriculture affect future generations and their health. The soil contaminated with pesticide residues on a larger scale, affecting the natural biological cycle.

Different agricultural practices, such as the use of disease resistant varieties, seed treatment, crop rotation, cover crops, good seed bed preparation and weed management practices have been applied to control plant diseases. However, such practices are not always sufficient

protection from crop losses. In recent times, diverse approaches are being used to manage and/or mitigate a variety of pathogens for control of plant diseases. The use of microbial pesticides is one of the best strategies available to combat the diseases in an eco-friendly manner. Biological control of plant diseases is not new; on the contrary, it was among the first attempts of plant protection in history. The Integrated Pest Management Regulation includes a preference for the use of biological protection over chemical plant treatments. Modern biological control agents are highly and long-term effects while being friendly to human health and the environment. They are low or non-toxic to non-target species. The bioagents used thus increase the richness, diversity and stability of natural systems in the agricultural landscape, enabling quality production. In addition to being non-toxic, bioagents are suitable for alternative cropping systems. Biological agents can also be used to treat seeds as effectively as pesticides. Agriculturalists have increased their efforts to take advantage of such natural biological antagonisms and to develop strategies by which biological control can be used effectively against several plant diseases. Biological antagonisms, although subject to numerous ecological limitations, are expected to become an important part of the control measures employed against many more diseases. Biological control practices for direct protection of plants from pathogens involve the deployment of antagonistic microorganisms at the infection court before or after infection takes place. The mechanisms employed by biocontrol organisms in weakening or destroying the plant pathogens they attack are primarily their ability to parasitize the pathogens directly, production of antibiotics (toxins) against the pathogens, their ability to compete for space and nutrients and to survive in the presence of other microorganisms, production of enzymes that attack the cell components of the pathogens, induction of defense responses in the plants they surround, metabolism of plant produced stimulants of pathogen spore germination, and possibly others. Although thousands of microorganisms have been shown to interfere with the growth of plant pathogens in the laboratory, greenhouse, or field and to provide some protection from the diseases caused by them, strains of relatively few microorganisms have been registered and are available commercially for use so far.

The most commonly used bacterial bioagents include *Agrobacterium radiobacter* K-84, for use against crown gall; *Pseudomonas fluorescens*, for use against Rhizoctonia and Pythium damping-off of cotton; and *Bacillus subtilis*, used for seed treatment. Although the actual use of these biological control products is rather limited, it is expected that these andother such products will find wide acceptance and will fill a real need in the not-too-distantfuture. A number of bacteria and fungus based biopesticides have been identified and developed but require effective adoption and further development of such agents. Moreover, consumers are becoming more and more concerned about pesticide-free safer foods which results in emergence of eco-friendly strategies for plant disease management.Nowadays, several beneficial bacterial based biopesticides are widely used in agriculture at commercial level. Bacterial bioagents *i.e., Pseudomonas fluorescens*, *Pseudomonas aureofaciens, Pseudomonas chlororaphis, Pseudomonas putida, Bacillus subtilis*, *Bacillus cereus*, *B. pasteurii*, *B. pumilus*, *B. mycoides*, *B. sphaericus*, *B. amyloliquefaciens*, *Burkholderia cepacia*, *Streptomyces lydicus, Arthrobacter* sp. and *Agrobacterium radiobacter* suppresses many plant pathogens on diverse hosts (Table 1) and commercial bacterial bioagents with manufacturers are also listed in Table 2. This article has been written for describing the details about bacterial bioagents including mode of action and application methods for management of crop diseases.

Mode of action: Crop diseases are the result of interactions among the components of disease triangle *i.e.,* host, pathogen and environment. Biological control agents are the organisms that interact with the components of disease triangle to manage the disease. Various unique and complex mechanisms of action (Junaid *et al.,* 2013) employed by the bacterial biocontrol agents in management of crop diseases are explained under:

Induced resistance : Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability. Plants also possess arrange of active defence apparatuses that are respond to a variety of chemical stimuli produced by soil and plant associated microbes (PGPR). Such stimuli induce through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens. If defence mechanisms are triggered by a stimulus prior to infection by a plant pathogen, disease can be reduced. Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance wherein plant defences are reconditioned by prior infection or treatment that results in resistance against subsequent challenge by a pathogen or parasite. Systemic acquired resistance (SAR) is a form

Table 1: List of crop diseases controlled by bacterial bio-agents

Crop name	Disease name	Target pathogens	Effective bacterial bioagents
Pulse crops			
Pigeon pea	Wilt	*Fusarium udum*	*Bacillus subtilis*
Chickpea	Wilt, seed rot, root rot	*Fusarium oxysporum* f. sp. *ciceris, R. bataticola, Pyhtium sp.*	*Bacillus subtilis*
	Grey mould	*Botrytis cineria*	*Brevibacillus brevis*
Oilseed crops			
Groundnut	Late leaf spot	*Phaeoisariopsis personata*	*Pseudomonas fluorescens*
Castor	Grey rot	*Botrytis cinerea*	*Pseudomonas fluorescens*
Rye	Vascular wilt	*Fusarium culmorum*	*Pseudomonas fluorescens*
Rapeseed	Stem rot	*Sclerotinia scletiorum*	*Bacillus subtilis* EDR4
Cereal crops			
Wheat	Root rot	*Sclerotium rolfsii, Fusarium oxysporum*	*Pseudomonas fluorescens*
	Loose smut	*Ustilago segatum tritici*	*Pseudomonas fluorescens*
	Take-all	*Gaeumanomyces graminis var. tritici*	*Pseudomonas fluorescens*
	Wilt	*Fusarium graminearum*	*Lysobacter enzymogenes*
Barley	Take-all	*Gaeumanomyces graminis var. tritici*	*Pseudomonas fluorescens*
Rice	Sheath blight	*Rhizoctonia solani*	*Pseudomonas fluorescens,P. putida*
	Sheath rot	*Sarocladium oryzae*	*Pseudomonas fluorescens*
	Blast	*Magnaporthe grisea*	*Bacillus* sp.
	Bacterial leaf blight	*Xanthomonas oryzae* pv. *oryzae*	*Pseudomonas fluorescens, B. subtilis*
	Leaf and Neck blast	*Pyricularia oryzae*	*Pseudomonas fluorescens*
	Leaf spot	*Helminthosporium oryzae*	*Pseudomonas fluorescens*
Pearl millet	Downy mildew	*Sclerospora graminicola*	*B. subtilis, B. pumilus*
Sorghum	Wilt	*Fusarium oxysporum*	*Paenibacillus sp.*
Maize	Damping off	*Pyhtium ultimum*	*Pseudomonas fluorescens*
	Wilt	*Fusarium oxysporum*	*B. amyloliquefaciens*
	Maize rot	*Fusarium verticillioides*	*Burkholderia* sp.
Vegetable crops			
Cucumber	Damping off/powdery mildew	*Pythium ultimum/ Sphaerotheca fuliginea*	*Pseudomonas fluorescens*
	Damping off	*Rhizoctonia solani*	*Bacillus pumilus*
	Bacterial wilt	*Erwinia tracheiphila*	*Bacillus pumilus*
	Grey mould	*Botrytis cinerea*	*Brevibacillus brevis*
	Root and crown rot	*Pythium aphanidermatum*	*L. enzymogenes*
	Wilt	*Fusarium oxysporum f. sp. cucumerinum*	*Bacillus subtilis*
Potato	Late blight	*Phytophthora infestans*	*Pseudomonas fluorescens*
	Soft rot	*Erwinia amylovora*	*Pseudomonas fluorescens*
	Bacterial wilt	*Ralstonia solanacearum*	*Bacillus cereus, B. subtilis*
Tomato	Foot and root rot	*Fusarium oxysporum* f. sp.*radicislycopersici*	*Pseudomonas chlororaphis*
	Mosaic disease	*Cucumber mosaic virus*	*Bacillus pumilus, B. amyloliquefaciens, B. subtilis*

Table 1 : Contd……

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	Tomato mottle disease	*Tomato mottle virus*	*B. amyloliquefaciens, B. subtilis, B. pumilus*
	Foliar diseases	*Corynespora cassiicola*	*B. cereus*
	Wilt	*Fusarium oxysporum*	*Collimonas fungivorans, Pseudomonas fluorescens*
	Root rot	*Rhizoctonia* spp.	*Pseudomonas fluorescens*
	Bacterial wilt	*Ralstonia solanacearum*	*B. amyloliquefaciens*
Bean	Seedling rot	*Pythium sp., S. sclerotiorum, R. solani, B. cineria*	*Pseudomonas fluorescens*
	Root rot	*R. solani*	*Pseudomonas fluorescens*
	Southern blight	*Sclerotium rolfsii*	*Aeromonas caviae*
Cauliflower	Damping off	*R. solani, P. aphanidermatum*	*Pseudomonas fluorescens*
Cabbage	Damping off	*R. solani*	*Pseudomonas fluorescens*
Brinjal	Wilt, damping off	*F. solani, P. aphanidermatum*	*Pseudomonas fluorescens*
Chilli	Damping off	*Pythium aphanidermatum*	*Pseudomonas fluorescens*
Radish	Wilt	*Fusarium* sp.	*Pseudomonas putida*
Okra	Wilt	*Fusarium oxysporum*	*Pseudomonas fluorescens*
Bottle gourd	Collar rot	*Sclerotinia sclerotiorum*	*B. subtilis*
Fenugreek	Root rot	*R. solani*	*Pseudomonas fluorescens*
Squash	Blight	*Phytophthora capsici*	*Bacillus* sp.
Pea	Damping off, root rot	*Pythium* sp.and *Aphanomyces* sp.	*Burkholderia cepacia*
	Wilt	*Fusarium oxysporum f. sp. pisi*	*Pseudomonas fluorescens*
Spices crops			
Pepper	Blight	*Botrytis cinerea*	*B. amyloliquefaciens*
Bell pepper	Blight	*Phytophthora capsici*	*Bacillus* sp.
Cash crops			
Cotton	Root rot	*Rhizoctonia solani*	*Bacillus cereus, Enterobacter agglomerans*
	Root rot	*R. solani and F. oxysporum* f. sp. *vasinfectum*	*Aeromonas caviae*
	Root rot	*Macrophomina phaseolina*	*Pseudomonas fluorescens*
	Damping off	*Pyhtium ultimum, Phomabetae*	*Pseudomonas fluorescens*
	Wilt	*Verticillium dehaliae*	*Bacillus subtilis*
Sugarcane	Red rot	*Colletotrichum falcatum*	*Pseudomonas fluorescens* VPT4, ARRIG, and EP1, *Pseudomonas putida* KKM1
Carrot	Root rot	*Athelia rolfsii*	*Pseudomonas fluorescens*
Sugar beet	Damping off	*Pythium ultimum*	*Pseudomonas fluorescens, Enterobacter cloacae, Stenotrophomonas maltophilia*
Fruit crops			
Banana	Bunchy top disease	*Banana bunchy top virus*	*Pseudomonas fluorescens*
	Sigatoka	*Mycosphaerella musicola*	*Bacillus subtilis*
	Wilt	*F. oxysporum f. sp.cubense*	*B. amyloliquefaciens*
Citrus	Post-harvest decay	*Penicillium* sp.	*Bacillus subtilis* PPCB001
Apple and Pear	Fire blight	*Erwinia amylovora*	*Bacillus subtilis* QST 713, *Pantoea agglomerans* C9-1, *Pantoea agglomerans*E325
Apple	Root rot	*Fusarium* sp.	*Bacillus subtilis* Y-1
Avacado	Root rot	*Dematophora necatrix*	*Pseudomonas fluorescens*
Stone fruit	Crown gall disease	*Agrobacterium tumefaciens*	*Agrobacterium radiobacter* K84
Plantation crops			
Arecanut palm	Fruit rot	*Phytophthora* spp.	*Pseudomonas fluorescens*
Tobacco	Blue mould	*Peronospora tabacina*	*Bacillus pumilus*
	Bacterial wilt	*Ralstonia solanacearum*	*Brevibacillus brevis, Streptomyces rochei*

Table 2: Bacterial bioagents and their commercial products used in crop disease management

Bacterial strains/species	Trade name	Manufacturer
Pseudomonas fluorescens	Conquer	Mauri Foods
Pseudomonas cepacia	Intercept	Soil Tech.
Pseudomonas + Azospirillum	Bio-jet	Eco-Soil
Pseudomonas fluorescens strain A 506	Frostban	Plant health technologies
Pseudomonas fluorescens strain TX-1	Bio-jet, Spot less	Eco-Soil Systems Inc.
Pseudomonas fluorescens	Bioshield	Anu Biotech International Ltd., Faridabad, India
Pseudomonas fluorescens	Plant Bio-control Agent-3	Department of Plant Pathology, G.B. Pant Uni. of Agric. And Tech., Pantnagar, Uttarakhand, India
Streptomycesgriseoviridis	Mycostop	Kemira Oy, Finland
Streptomyces lydicus	Actinovate	Natural Industries,Inc., USA
Pseudomonas syringae	Biosave 10LP, 110	Village Farms LLC
Agrobacterium radiobacter strain K1026	Nagol	Bio-care
Agrobacterium radiobacter	Nogall	New Bioproducts
Agrobacterium radiobacter strain 84	Galtrol	AgBioChem, Inc., USA
Agrobacterium radiobacter	Dygall	Agbioresearch Ltd.
Bacillus pumilus	Ballad Plus	Agraquest Inc.
Bacillus subtilis	Serenade	Agraquest Inc.
Bacillus subtilis strain FZB 24	Rhizo-plus	FZB Biotechnik, GmbH
Bacillus subtilis strain GB34	GB 34	Gustafon, Inc., USA
Bacillus subtilis strain GB03	Kodiac companion	Growth products, USA
*Bacillus pumilus*GB34	Yied Shield	Gustafson Inc., USA
B. amyloliquefaciens	Taegro	Novozymes, Denmark
B. pumilus	Sonata	AgraQuest, USA
B. subtilis	Larminar	Appliedchem,Thailand
B. subtilis	Rhapsody	AgraQuest, USA
B. subtilis	Subtilex	Becker Underwood, USA
Bacillus licheniformis	EcoGuard	Novozymes, Denmark
Bacillus subtilis	Avo Green	Ocean Agriculture, South Africa
Basillus subtilis	Bio-safe	LaboratoriodeBiocontrole Farroupilha, Brazil
Bacillus subtilis	Biotok	Tocklal Experimental Station, Tea Research Associantion, Jorhat, Assam, India
Bacillus subtilis	Ecoshot	Kumiai Chemical Industry, Japan
Bacillus subtilis	Biobest	Appliedchem,Thailand
Bacillus subtilis strain MB 1600	HiStick N/T, Subtilex	Becker Underwood, Ames, IA, USA
B. subtilis	Subtilex/Pro-Mix	Premier Horticulture Inc., Canada
B. subtilis + B. amyloliquefaciens	Bio Yield	Gustafson Inc.,Dallas, USA
Burkholderia cepacia	Deny	Stine Microbial Products

of induced resistance that is activated throughout a plant after being exposed to elicitors (cell wall component of the pathogen which are capable of inducing phytoalexins synthesis) from virulent, avirulent or non-pathogenic microbes or artificial chemical stimuli such as chitosan or salicylic acid (SA). Induced systemic resistance (ISR) is a resistance mechanism in plants that is activated by infection. Its mode of action does not depend on direct killing or inhibition of the invading pathogen, but rather on increasing physical or chemical barrier of the host plant. Selected strains of plant growth-promoting rhizobacteria (PGPR) suppress diseases by antagonism between the bacteria and soil-borne pathogens as well as by inducing a systemic resistance in plant against both root and foliar pathogens.SAR is triggered by plant pathogens and are mediated by SA-dependent pathway (Singh, 2014) which are activated by certain molecules secreted by microorganism (pathogens) referred as elicitors (cell wall polysaccharides, salicylic acid, cyclic lipopeptides, siderophores, antibiotics and the signal molecule N-acyl homoserine lactones reported by Perez-Montano *et al.* 2014) that leads to expression of defence responses (its functions as signal that spread "news" of the infection to nearby cells and also stimulate the cross-linking of molecules in the cell wall and the deposition of lignin, responses that set up a local barricade that slows the spread of the pathogen to other parts of the plant) like physical thickening of cell walls by lignification, deposition of callose, accumulation of phytoalexins (antimicrobial low-molecular-weight compounds formed by the plants in response to infection) and synthesis of various proteins (e.g., chitinases, glucanases, peroxidases and other pathogenesis related (PR) proteins (*i.e.*, PR-1, PR-2) produced in plant in the event of a pathogen attack. Infections activate genes that produce PR- proteins. These PR proteins are antimicrobial and cause lysis of invading cells, reinforcement of cell membranes to resist infections or induce localized cell death.ISR is triggered by beneficial microbes living in the rhizosphere which is generally mediated by salicylic acid (SA) independent pathway where jasmonic acid (JA) and ethylene (ET) are the central players (induced by non-pathogenic bacteria) and typically functions without PR protein activation. Combination of ISR and SAR can increase protection against pathogens that are resisted through both pathways besides extended protection to a broader spectrum of pathogens than ISR/SAR alone. Some strains of *Pseudomonas fluorescens, Pseudomonas putida* and several specific strains of species *Bacillus amyloliquifaciens*, *B. subtilis, B. pasteurii, B. cereus, B. pumilus, B. mycoides,* and *B. sphaericus* are produce significant reduction through induce resistance in the incidence or severity of various diseases on a diversity of hosts (Chaudhary *et al.,* 2007).

Competition : Both the bio-control agents and the pathogens compete with one another for the nutrients, oxygen, space and other requirements to get established in the environment. This process of competition is considered to be an indirect interaction between the pathogen and the bio-control agent whereby the pathogens are excluded by the depletion of nutrients base and by physical occupation of site. So far, as the competition for nutrients is concerned bio-control agents compete for the rare but essential micronutrients, such as iron and manganese especially in highly oxidized and aerated soils. Iron is required for growth and development of plants and microorganisms. In natural form, iron is present in ferric form, which is insoluble in water and is not utilized by both plants and micro-organisms (Junaid *et al.,* 2013).For example, different strains of *Pseudomonas fluorescens* synthesize siderophore (It is a microbial iron transport agent/act as chelating agents and extra cellular, low molecular weight compounds, it has micronutrients bindable capacity, specially, it binds the iron molecules and make insoluble form to soluble and also facilitate iron uptake to plants and microorganisms (bioagents), during this process, it is less available/unavailable to pathogens and ultimately disease controlled) *i.e*., Ferribactin, Ferrichrome, Ferroxamine B, Pseudobactin, Pyochelin, Pyoverdine (soluble fluorescent pigment)and *Bacillus subtilis* produce the catecholate siderophores-2 (bacillibactin), 3-dihydroxybenzoate and 2,3-dihydroxybenzoyl glycine.

Antibiosis : Production of low molecular weight compounds or an antibiotic like substances or other chemical metabolites by the microorganism that have a direct effect on the growth of plant pathogen. Bio-agents are known to produce three types of antibiotics *viz.,* nonpolar/volatile, polar/ non-volatile and water soluble. Among all of these the volatile antibiotics are more effective as they can act at the sites away from the site of production (Lo, 1998 and Pal and Gardener, 2006). For example, bacterial bioagents release following antibiotic *viz*., different strains of *Pseudomonas fluorescens* produces Phenazine-1-Carboxylic Acid (PCA), Pyrrolnitrin, Pyocinine, Pyoluteorin, Oomycin-A, 2,4-Diacetyl-pholoroglucinol (DAPG), Idionine, etc.; different *Bacillus subtilis*strains produces Bacillomycin D, Iturin A,surfactin, fengycin and Mycosubtilin, *Bacillus cereus*

produce Zwittermycin A; *Agrobacterium radiobacter* produce Agrocin 84, *B. amyloliquefaciens* produce Bacillomycin, fengycin and *Burkholderia cepacia* produce Pyrrolnitrin, Pseudane antibiotic like substances.

Lysis : It is one of the mechanisms used by biocontrol agents to control soil-borne pathogens involves the production of cell wall-degrading enzymes or other metabolites. Numerous microorganisms release lytic enzymes that can hydrolyse a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose and DNA. Expression and secretion of these enzymes by different microbes can sometimes result in the suppression of plant pathogen activities directly.Besides production of antibiotics and elicitation of systemic resistance in plant against a variety of plant pathogenic diseases, biocontrol strains of plant growth promoting rhizobacteria (PGPR) *viz.*, *Pseudomonas fluorescens* and *Bacillus* spp. are also capable of producing enzymes like chitinase, β-1, 3-glucanase, chitinase, cellulase and protease having a very strong lytic activity. It exerts a direct inhibitory effect on the hyphal growth of fungal pathogens. Cell wall-degrading enzymes of rhizobacteria affect the structural integrity of the walls of the target pathogen.The other microbial by-products *i.e.*, HCN production by certain *fluorescent pseudomonads* is believed to be involved in the suppression of root pathogens. *P. fluorescens* CHA0 produces antibiotics, siderophores and HCN, but suppression of black rot of tobacco caused by *Thielaviopsis basicola* appeared primarily to be due to HCN production (Junaid *et al.,* 2013).

Methods of application:

Seed treatment: Seed treatment is a process like vaccination applied in animal as well as human. In broad terms, it provides protection to seeds and plants and improve the establishment of healthy crops. Use for bigger seeds treatment @ 8-10gram bacterial formulation per kg seed while small seeds @ 6-8gram per kg seed before sowing. Mix the required quantity of seeds with bacterial formulation and ensure uniform coating. Shade dries the seeds for 20-30 minutes before sowing is essential. Seed treatment is highly effective against seed and soil borne diseases.

Soil application: 2-2.5 kg bacterial formulation (powder formulation) or 500-1000 ml (liquid formulation) is added in 25-50 kg farm yard manure (FYM). Mixed thoroughly, cover with jute bag/sugarcane leaves/paddy straw and kept for 2-3 week in shade for proper multiplication. Maintain moisture and mix the mixture in every 3-4 days intervals before broadcasting in the field. Maintain optimum moisture for better multiplication of bioagents. Apply well decomposed bacterial based FYM to the field before 15 days of sowing. This mixture can be applied in furrow/pit/pot and at the time of transplanting/sowing. This mixture is sufficient for one acre of land.

Cutting/Seedling's root dip application: Mix10 gram bacterial formulation (powder formulation) or 10 ml (liquid formulation) in one litre of water and dip the cuttings and roots of seedlings for about 30 minutes before transplanting. Root dipping is effective against soil borne diseases.

Nursery bed treatment: 500 gram bacterial bioagents (powder formulation) mix in 10-15 kg well decomposed FYM/compost/vermicompost and broadcast in a one-acre area at evening time and at proper moisture conditions.

Soil drenching : One-to-two-kilogram bacterial formulation mix in 200 litre of water and drench the soil in one acre area or 10 gram or 10 ml/litre of water bacterial bioagents is sufficient for soil drenching. Maintain optimum soil moisture while applying.

Foliar application: 10 gram/litre of water bacterial formulation (powder formulation) or 10 ml/litre of water (liquid formulation) spray uniformly after 35-40 days of transplanting (particularly in cereals, pulses and oilseeds)on diseased plants at cooler hours. 2-3 spray are required depending upon the disease incidence at 10-12 days intervals.

Seed bio-priming: It is a process of biological seed treatment. In this process, involves slurry treatment of seeds with bioagent in the presence of gum arabica, jaggery/or FYM powder. Dissolve 100gram jaggery in one litre of water and prepare solution there after add bacterial formulations @ 10 gram/kg seed in this solution and properly mix it. Next day required quantity of seeds is mix properly with culture medium. Use polythene bags for filling treated seed, heaped, covered with moist sack of jute and incubate at approximately 25-32 °C for 48 hours to maintain high humidity. Bioagent adhering to surface of seed grows and form a protective covering on seed coat during this period. This technique has potential advantages over simple coating of seeds as it results in rapid and uniform seedling emergence. Seed biopriming is beneficial for tomato, brinjal, chickpea and soybean crops.

Seed material treatment: Apply @ 10 gram or 10ml bacterial formulation with one litre of water for the treatment of seed material like sugarcane setts, banana suckers, turmeric, ginger rhizomes and potato tubers before sowing for about 30 minutes. Shade dries the seeds for

20-30 minutes before sowing is necessary.

Horticultural crops: 50-100 gram bacterial formulation mix in sufficient quantity of FYM/compost/vermicompost/ field soil and apply the mixture per plant in effective root zone of fruit tree. Doses will change in depending upon age of the plant.

Benefits:

– Bacterial bioagents do not cause any toxicity to the plants; rather these increase crop yields by enhancing the root and plant growth through the encouragement of beneficial microflora in rhizosphere. It also helps in the mobilization of plant nutrients and makes it available to the plant.

– Bacterial bioagentsavoid problems of resistance and also induce systemic resistance among the crop species that is responsible for protection of invading pathogens.

– It is safer both for the environment and the persons who are applying them and avoid environmental pollution (soil, air and water) by leaving no toxic residues.

– Bacterial bioagentseliminate the specific pathogens effectively from the site of infection and can be used in combination with biofertilizers.

– It is comparatively easier to manufacture biocontrol agents, sometimes less expensive than chemical agents.

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