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BIOCONTROL AGENTS AND BIOPESTICIDES

(Liquid Formulations)

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CONTENTS

PREFACE

There is an increasing awareness about organic agricultural practices in the world. But, Indian farmers yet to be realize about ill effect of modern agriculture. Hence alternative farming particularly Organic Farming is hour of the need to get rid of chemical fertilizers, synthetic pesticides and growth regulators etc. To promote organic farming in India, Ministry of Agriculture, Govt. of India has launched a National Project on Organic Farming, during 1, October 2004. Under the National Project, Training Programmes for different level officials, farmers and input producers being implemented while educating the people there is demand of basic informations about the product available and their use in crop. There is also a section of educated field workers who are interested to know the mode of action of the different Bio-products. The authors has attempted the burning issue of liquid formulations. Although, it is still not disclosed by the selected producers, but it is a need of the hour to educate the proper liquid formulations to create the awareness among the users. So they may not be cheated by fake products sold in the market on the name of liquid formulations. The authors has described the specialities of liquid base carrier use. Author attempted to collect the practical informations disseminate the details in the form of booklet on Biocontrol agents and Biopesticides. This booklet gives good practical informations about Biocontrol agents and Biopesticides. We hope this booklet would be useful to trainees as well as farmers who are involved in promotion of organic farming or willing to convert their lands to organic cultivation. We acknowledge the support of Sri. B. Ramesh of Biotech International Ltd., Bangalore while preparing booklet.

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INTRODUCTION

Pests, Insects, disease causing Pathogens, weeds and nematodes are estimated to cause an annual loss of about 33-35 percent of the potential food production world wide. In India annual crop losses around Rs. 290,000 million. In order to increase agricultural production and "Green Revolution" have been launched in that the introduction of dwarf, high yielding cultivars, higher fertilizer doses, intensive cropping, monocropping, etc., inevitable. Due to introduction of new inputs leads to serious pest problems.

Worldwide an estimated 67000 different pest species attack agricultural crops, to protect them, many chemical pesticides have an unfavorable environmental impact, and there is pressure for decreased reliance on such agents and greater regulatory control of their use. Moreover, many pests have acquired resistance to widely used chemical pesticides. The trend is towards integrated Pest Management (IPM) programmes that decrease the frequency and intensity of the genetic selection of resistant insect mutants by employing in concert different means of insect population control. This strategy was very effective, but not sustainable and many small farmers were not able to take the advantages of the high yielding varieties. After realizing high yielding varieties the sustainability on environmental quality as the key components for growth of agriculture and the protection. A natural resource base for which the intensification of integrated pest management in crop protection is mandated. The IPM is a proven technology that is economical, socially accepted and environmentally sound. The integration of cultural practices, host plant resistance, mechanical methods and the application of biological suppression methods often avoids the need for pesticide application.

Estimated Crop losses due to Pests & Diseases

Crop	% Loss	Rs. (Crore)
Rice	18.6	5,512
Wheat	11.4	1,415
Jowar	10.0	173
Pulses	7.0	484
Oilseeds	25.0	4,180
Cotton	22.0	2,000
Sugarcane	15.0	1,336

2. BIOCONTROL AGENTS AND BIOPESTICIDES IN INDIAN AGRICULTURE

Inspite of increasing chemical consumption, the agricultural production in India is almost static since 1989 due to poor soil nutrition status and pests and diseases causing over Rs. 30,000 crore crop losses per annum. Indiscriminate use of chemical pesticides has resulted in resistance in pests, resurgence of minor pests and high level of pesticide residues, due to which, more than Rs. 4,000 crore worth of Indian agricultural exports get rejected every year. High pesticide residues in food chain cause pesticides poisoning cases and deaths through organ malfunctions, immuno suppression, neurotoxicity, impairment of reproductive functions, carcinogenicity, paralysis, etc. and harm to beneficial fauna and flora.

As we approach the 21st century, there is an enlarging stress on organically produced food, conservation of biodiversity, unpolluted environment and sustainable agriculture. To compound these challenges, Biocontrol agents and Biopesticides have emerged as an viable alternatives in pest and diseases control. Biopesticides are advantageous in view of their ecosafety, specificity, reduced number of applications, no resistance in pests, increased

yields and quality improvement of crops, higher acceptability and higher value of produce for exports and suitability for rural masses.

Biocontrol agents and Biopesticides take care of losses of crops, losses of exports, losses of man-hours and lives and losses of beneficials, natural parasites and predators. In spite of the thrust from Government of India and some State Governments, the adoption of Bio-control agents and Biopesticides by farmers is still in its infancy, only about 5,000 villages and around 100,000 farmers having reaped the knowledge and benefits.

The slow rate of adoptionof Bio-inputs is basically due to a preference for the procurement at lowest rates, giving no importance to quality. The lack of feedback on the effectiveness of Biopesticides supplied under the various schemes has also stifled progress. The literature provided is mainly concerned with chemical pesticides and does not emphasize Biopesticides, thus continuing with the old practices, because presumably a change in practices to biologicals would require considerable extension effort. Very little has been done to quality assess Biocontrol agents and Biopesticides and in the absence of adequate knowledge, the updating of the state pesticide testing laboratories for Biopesticides is still awaited.

The biopesticide industry has enlarged and a variety of products are now available across the country, but most of the entrepreneurs have set up units without much concern for quality. However, a few of them seriously manufacture quality products, follow regulatory procedures and contribute significantly to the proper extension down to end users. In absence of available local advice, the farmers depend totally on dealers, whose

demonstrations in local languages in a form that matches their level of understanding. However, they are not confident that Biocontrol agents and Biopesticides work because they are used to 'instant kill' with insecticides. They also believe the Biopesticides are costly, since they do not analyse the cost and benefits. The success of individual Biopesticides cannot be judged through the use as isolated products, because these have to be provided as part of a module of crop management operations, with proper surveillance and monitoring including pheromone and light traps and use Biopesticides as per advice, at the right time, right location and with appropriate equipment and procurement of biopesticide from reliable sources.

The biopesticide manufacturing units including botanical, microbial and bio-control insects and pheromone lures and traps are mainly concentrated in South India and few of them have recently come up in the North. Out of 410 such units, around 130 are in the private sector while around 280 are in the Government sectors. Not surprisingly, product availability from the 130 private sector units is timely, regular, extending into far stretched areas of the country and quantitatively much more than that from the 250 Government Sector Units. The most serious drawback of biopesticides industry (including the Government Sector Units) is that some of the units lack the required quality consciousness and some of them violate the Regulatory requirements to the extent of marketing products without Central Insecticide Board (CIB) registrations as is done by all Government units.

Around 1,000 tonnes of Azadirachtin formulations, 150 tonnes each of Bts and *Trichoderma* and around 50 tonnes of NPVs are being marketed in India per annum. The maximum utilization being in Vegetable crops, cotton, sugarcane and paddy, although these have found utility

BIOCONTROL AGENTS & BIOPESTICIDES
Chandra, Greep, Srivathsa

in fruit crops, pulses and plantation crops as well. Quite a bit of these products are used in the IPM programmes of various States, particularly Haryana, Rajasthan, Gujarat, Karnataka, Tamil Nadu, West Bengal, J&K and Kerala making these available to the farmers on 50% subsidy as well as in IPM demonstrations.

The real adoption of biopesticides however, will be evident only through adoption by farmers. In the market widespread acceptance of *Azadirachtin, B.t., NPV, Trichoderma*, *Pseudomonas*, traps and lures has seen accepted in seed dressing, soil applications and even as foliar sprays capable of taking care of fungal and bacterial leaf and fruit spots including both saprophytes and obligate parasites. The awareness being given by National & Regional Centre of Organic Farming and some of the States, APEDA, Tea Board, Coffee Board, Coconut Board, Spices Board etc. on organic farming as well as the interests of cotton, Basmati, Tea, Spices, Fruit and vegetable exporters on the organic produce has resulted in an increased use of biological inputs. The present consumption levels of biopesticides have increased from around 1% share in Indian pesticide market in 2001 to around 2.5% currently and it is expected to reach to 12-15% by the end of 2005, mainly through the "chorus of organic farming".

The biopesticide industry, however, is not in a very healthy state and only 8-10 players are really active, the Revenue Model being far from sound, in view of 15-20% expenditure on concept marketing compared to only 1-12% marketing expenses in case of conventional pesticides. Inspite of this, a lot of manufacturing capacities continue to lie idle for insufficient demand generation, resulting in increased overheads.

Table-1 shows the appropriate Biocontrol agent, target pathogen/disease, crop and application methods.

Biocontrol Agent and Biopesticides	**Diseases causing agent**	**Crop**	**Application method**
Agrobacterium radiobacter	Crown gall diseases, Agrobacterium tume faciens	Fruit, nut and ornamental nursery stock	Bacterial mass from one plate transferred to one gallon non-chlorinated water, suspension applied to seeds, seedlings, cuttings, roots, stems, and as soil drench.
Pseudomonas cepacia	Rhizoctonia solani, Fusarium spp., Pythium sp., Rhizoctonia solani	Maize, Vege-tables, cotton, legumes	Added to a slurry mix for seed treatment; hopper box treatment.
Bacillus subtillis	Fusarium spp., Alternaria spp., and Aspergillus spp.		
Streptomyces griseoviridis	Fusarium spp., Alternaria brassicicola, Phomopsis spp. Botrytis spp., Pythium spp. and Phytophthora spp.	Field, ornamental and vegetable crops	Drench, spray or through irrigation system.
Agrobacterium radiobacter	Agrobacterium tumefa-ciens	Trees	Root dips
Agrobacterium radiobacter	Crown gall disease, Agrobacterium tumefa-ciens	Fruit, nut and ornamental nursery stock	Root, stem, cutting, dip or spray
Bacteriophage phage	Pseudomonas tolassi	Agaricus spp., Pleurotus spp.	
Pythium oligandrum	Pythium ultimum	Sugarbeet	Seed treatment or soil incorporation

Pseudomonas solanacearum	Pseudomonas solanacearum	Vegetables	
Phlebia	*Heteroba-sidium*	Trees	Spray, chain saw oil
Trichoderma harzianum	*Pythium spp., Rhizoctonia solani, Fusarium spp.*	Trees, Shrubs, transplants, all ornamentals, cabbage, tomato, cucumber	Mix with soil or potting medium
Gliocladium virens	*Rhizoctonia solani* and *Pythium spp.*	Ornamental and food plants in green-houses, nurseries, homes and interior-scapes	Granules are incorporated in soil or soiliess growing media prior to seeding
Trichoderma harzianum	Various fungi		
Bacillus subtillis	Seeding pathogens	Barley, beans, cotton, peanut, pea, rice, soybean	Seed treatment in planter box
Trichoderma harzianum	Primarily *Botrytis cinerea,* also colletro-trichum spp., Fulvia fulve, Monilia laxa, Plasmopara viticola. Pseudo-peronospora cubensis, Rhizopus stolonifer, sclerotinia sclerotiorum	Cucumber, grape, nectarine, soybean strawberry, sunflower, tomato	Spray
Trichoderma harzianum and *T.viride*	Armillaria, Botryo-sphaeria, Chondrosterium Fusarium, Nectria, Phytophthora, Pythium, Rhizoctonia		

3. STATUS OF ADOPTION OF BIOCONTROL AGENTS AND BIOPESTICIDES AND IN INDIAN AGRICULTURE

- Use of Biocontrol agents and Biopesticides on the increase but not up to the desired level of growth.
- Only 1% of 143 million ha, Crop area and only 2500 villages out of over 6 lakh villages covered so far under IPM.
- Biopesticides and Biocontrol concept still in infancy in farming community.
- Basic I.P.M. modules designed to be refined for local needs.
- Many small Biocontrol agent and Biopesticide entrepreneurs coming up, but with little or no quality consciousness.
- State Biocontrol Labs and central Intergrated Pest Management (CIPMC) not able to provide timely supplies of biocontrol agents to farmers.
- Establishment of "ORGANIC COTTON", "ORGANIC TEA", "ORGANIC VEGETABLES", "ORGANIC BASMATI" and "ORGANIC FARMERS" units initiated recently.

4. IMPORTANT PREVENTIVE, CROP PROTECTION MEASURES

Some important preventive, crop protection measures are as follows :

Some of the important Pest and Disease Control measures in Agriculture ecosystem

- Optimum soil conditions (soil, climate and environment) Cultivations
- Organic manuring and crop nutrition
- Stalk and residue decomposition
- Soil moisture and irrigation
- Diversity over time
- Discontinuity of monocultures
- Crop rotations
- Use of short maturing varieties
- Use of crop-free periods
- Manipulation of sowing and harvesting dates
- Diversity in space
- Varietal mixtures
- Resistant cultivars
- Crop mixtures
- Strip / intercropping (companion cropping)
- Mixed cropping
- Undersowing
- Soil cover
- Management of wild plants in and around crops
- Altering pest behaviour
- Use of trap crops, Green manures
- Size, planting density and shape of crops
- Pheromones
- Biological control
- Augmentative releases of beneficial insects and pathogens

I. CULTURAL METHODS

By practicing the following methods we can regulate / modify the pest and disease incidence effectively.

a. Selection of adopted and resistant varieties

By choosing varieties which are well adopted to the local environmental conditions such as temperature, nutrient supply, pests and disease resistance, by which crop being allowed to grow healthy and makes them stronger against attack of pests and pathogen's entry. Table – 2 shows some of the resistant / tolerant varieties in different crops.

Table – 2 Pest and Disease resistant varieties

Crop	Insect / Disease	Resistant / tolerant variety
Chickpea	Pod borer	ICCV 7, Dulia
Pigeon Pea	Pod borer Pod fly	ICPL 332 ICP-10531, E1, ICP-7167-5
Ground nut	Leaf miner Thrips	ICGS-156 (M 13), ICGV 86031, DRS-10 M-13, Robut 33-1
Paddy	Stem borer Gall midge	Ratna, Sasyasree, Saket, MTU 5849 IR 36, Kakatiya, Danya Lakshmi, Surekha Phalguna, Kunti, Shakti, Shameli, Asha, Rajendradhan.
Sugarcane	Internode borer Stalk borer Top borer	CO-6806, CO-62175, CO-975, CO-77-1 , Co-7302, Cos 767 Co-7224, Co-67, Co-1158
Cotton	Leafhopper	Kandwa 2, Badnawar, MCU 5, Krishna, (Mahalaxmi), Sujay, Sanguineum, Eknath
	Pink bollworm	LD-135, Sanmguineu, Abadhita, Sujata, Lohit, Digvijay
Finger millet	Finger blast	GPU 28

Tomato	Bacterial wilt Leaf curl	Swaraksha, Arka abhijit, Ark shresta, Avinash-2, Mruthyunjaya-3, JK Asha,
	Leaf curl, bacterial Wilt Fruit borer	Alrounder T-32, T-27, BT-1
Brinjal	Bacterial wilt Fruit and shoot borer White fly Jassid	Arka nidhi Pusa purple, Chaklasi doli, Doli-5 Pusa purple Pusa purple, Chaklasi doli, Doli-5
Okra	Yellow vein mosaic Fruit and shoot borer	Arka anamika Parkings long green, Karnual special, AE-57, PMS-8
Potato	Potato tuber moth	QB 1A 21-29

(b) Adoption of suitable cropping systems

Cropping system in a particular agricultural ecosystem plays major role by adopting a suitable cropping system in right time and we could avoid most of the harmful pests and diseases. Some of the practical practices as follows.

(i) By adopting mixed cropping systems pest and disease pressure could be limited. Since pest has less host plants to feed on and more beneficial insects site in a diverse system.

(ii) By following crop rotation practices we could increase soil fertility and reduce the chances of soil born diseases.

(iii) By cultivating green manure and cover crops like Horse gram, Cow pea Sun hemp, Sesbania, Dhaincha, Glyrisidia could increase the biological activity in the soil and can enhance the presence of beneficial organisms. For instance plants colonized by arbuscular mycorrhizal fungi resist to other pathogenic organisms. This practice may increase pests also. So careful selection of proper green manures is essential.

(c) Selection of clean seed and planting materials

Seeds and planting materials are the primary sources of diseases so, selection and use of safe seeds after involving inspection for pathogens and weeds are very much essential. Further, it is advised to get seeds and planting materials from the reliable safe sources only.

(d) Selection of optimum planting / sowing time and spacing

Most of the pests or diseases attack the crops only in a certain life stages. Therefore it is crucial that this vulnerable life stage does not match with the period of high pest density and thus that the optimal planting time is chosen. Further, by adopting sufficient spacing between plants reduces the spread of a disease as well as allows good generation, sunlight to the plants which facilitates to less moisture on the leaves leads to hinders pathogen development and infection in the same way more sunlight allows plants to do more photosynthesis. This practices not only destracts or avoids disease and pests in cropping system but also increase the crop productivity also.

(e) Use of balanced organic nutrient management

Gradual and steady growth makes plants less vulnerable to infection. So this steady growth could be achieved by applying organic fertilizers timely and moderately because excess / indiscriminate use of fertilizers often results in damaging the roots, this damages facilitates to secondary infections. To overcome this problems we could adopt integrated nutrient management system with organic manures like FYM, compost, vermicompost etc., this organic manures releases nutrients slowly when plant needs. Further, by using liquid biofertilizers like Potash mobilizers namely *Frateuria aurentia* along with organic manures to provide balanced

potassium supply and contributes to the prevention of fungi and bacterial infections.

(f) Use of more organic matter

Organic content of the soil directly related to density and activities of microorganisms in the soil there by pathogenic and soil borne fungal population can be reduced. Besides this, organic matter provides

- All the nutrients that are required by plants.
- Correct C:N ratio in the soil
- Fertility and productivity of the soil
- Good physical chemical & biological support to soil
- More water holding capacity to the soil
- Cover from evaporation losses of moisture from the soil

Ultimately organic matter supplies substances which strengthens the plants own protection mechanisms.

(g) Application of suitable soil cultivation methods

Ploughing or hoeing helps to expose stages of soil inhabiting insects to sun or to the predatory birds. Besides it facilitates the decomposition of infected plant parts and regulates weeds which serve as hosts for pest and diseases.

(h) Conservation and promotion of natural enemies

The use of relatively safer pesticides, and their selective use could conserve the biotic agents and the following practices could conserve the natural enemies in agricultural ecosystems.

1) By providing artificial structures like nesting boxes for wasps and predatory birds.
2) By planting or retaining food and shelter plants on the bounds like pollen and nectar bearing flowering plants Euphorbia, wild clover etc.

3) By placing bundles of rice straw for attracting spiders, retention of crop stubble, grass weed heaps for maintaining predators populations.
4) By establishing perching sites and water pans.
5) By controlling ants
6) By avoiding complete trash burning in the field
7) By using trap crops
8) By using organic manures instead of chemical fertilizers

i) Use of good water management

By avoiding water logging in the field for the entire crop cycle plant's stress could be avoided otherwise pathogens take chance and infects the crop. Further, as much as possible the water on the foliage to be avoided otherwise water borne diseases will increase by giving chance to germinate pathogenic fungal spores.

j) Use of proper sanitation measures

"Pull and Burn" is the best method to control disease and removal of infected plant parts (leaves, fruits) from the ground to prevent the disease from spreading. Eliminate residues of infected plants after harvesting.

II. MECHANICAL METHODS

a. By hand picking

Hand picking of egg masses gregarious larvae and sluggish adults and their destruction helps in reducing pest population in certain situations. This is the best method, will be effective before reaching the loss beyond economic level. To save crop from fungal and bacterial diseases pull and burn method is most effective.

b. By installation of birds shelter

By installing dried twigs in the field above the crop facilitates birds population, while taking rest on the shelters birds predate larvae or moth available on the crop.

c. By installing Light traps

In this method a bulb (100 watts) is hung in front of a thin gunny bag or polyethylene sheet (3'x4') smeared with oil or grease. A bucket with water can also be kept below the bulb. Installing about 6-8 lights traps per hectare will be effective. Ultraviolet lamps are much more effective than ordinary electric bulbs. This traps should be used at appropriate time depending upon the site and cycle of the insects. After collecting insects to be destroyed.

d) By using pheromone traps

This method is effective about 7 times than light trap method. Administration of synthetic chemicals (sex pheromones) that are basically used to attract and trap insect pests.

Pheromone Traps used for trapping of the moths of pest of a various crops are made up of plastic material (resistant to sunlight, rain and wind), of bright mustard yellow colour (most attractive colour of the moths), which could outlive a period of one year.

Pheromone Trap is made up of canopy with provision of 3 slots on the lower side for attachment of trap funnel and one central slot for attachment of lure. The canopy protects the lure from rain and direct sunlight. The trap funnel with bottom hole. 'T' shaped handle for fixing to the support (strong enough to take care of dislodging due to strong winds) and 3 provisions (arms) for fixing into the 3 slots provided on the under side of the canopy. Polyethylene sleeve is to be fixed to the lower portion of the trap funnel and the moths get trapped in this and die after a few days. By removing the rubber band from the bottom of the sleeve, the trapped / dead moths should be removed to make space for new moths.

Pheromone Traps are to be installed in the fields at the rate of 5-7 traps per Ha. for monitoring and

15-20 traps per Ha. for mass trapping. The distance between the traps fitted with lures specific for a particular moth species may be around 30 meter. These traps should be positioned 6 to 9 inches above the crop canopy level by tying on the stick with the 'T' shaped handle provided on the funnel of the trap.

Pheromone Lures are sex attractants for specific male moth. The details are given below:

Pheromone Lures are based on sulfur free rubber dispenser / septa impregnated with pheromones (specific for various species of insects). Various chemicals (sex attractants) Pheromone Lures are species specific. Act as sex attractants for specific male moths. Effective for 3-4 weeks under field conditions because of presence of antioxidants. Biolures packed in original pouches can be stored in a cool dry place preferably in a refrigerator for 1 year without loss of efficacy. Safe for mankind, animals and beneficial insects. One lure each is to be used per Trap for monitoring of the specific pest. Pheromones are replaced in the traps after every 3 weeks and 3-4 lures per phenomone trap are required in a crop season starting from 30-40 days of crop age.

e) By using coloured plates

Various pests are attracted by different colours. For example leaf miner pest can be attracted by yellow plastic plates coated with insecticides were effective.

III. BIOLOGICAL METHODS

Nature has it own device to balance the ecosystem. Natural enemies like spiders, beneficial insects and parasites play a key role in maintaining the biotic balance and reducing levels of pest populations below those causing economic injury. Natural as well as applied biological control agents are important in pest and disease

control. The natural enemies of pests viz. spiders and beneficial insects as predators, Entomo phagons Parasitoids and the insect pathogens play a vital role in keeping the pest population at low level.

By encouraging spiders like long jawed spider (*Tetragnatha marillosa*), Orb spider (*Argiope Sp*), Wolf Spider (*Lycosa pseudoannualata*), Lynoc spider (*Oxyopes javanus*), Jumping Spider (*Phidippus Sp.*) Dwarf Spider (*Steypena Sp.*) and beneficial insects like lady beetle (*Micraspsis Sp. Harmonia Sp. & Menochilus*), Ground beetle (*Ophionea Sp.)* Mirid bug (*Cytrorhinus Sp.*), Assasin bug (*Polytoxux tuscovitatus*), Ant (*Solenopsis germinate)*. Ear wing (*Euborellia Sp.)* could be controlled a good number of harmful pests incidence through out the season. This spiders and beneficial enemies are highly specific and safe to non target species. Once established they survive in nature till the pest is prevalent as self perpetuating in nature.

a) Parasitoids

Parasitoids can be effectively used for the management of pest of various agricultural and horticultural crops. These are host specific when a parasite egg hatches on pest it will stop feeding or die. Parasites able to attack the eggs, larvae, hymphs, pupae or adult. Depending upon the attacking stages parasites can be divided as egg parasites, larvae parasites.

i) Trichogramma

Trichogramma is a biocontrol agent against lepidopterian insect pest the details of Trichogramma chrysopericarnea given below :

Trichogramma is based on minute wasps belonging to *Trichogramma* species, parasitic on eggs of lepidopteran insect pests. Trichogramma lays its eggs in the host insect

eggs, multiplies therein, thus preventing hatching of host insect larvae.

Trichogramma is available in the form of cards containing 20,000 live parasitised eggs which have 90-96% hatching within 7-10 days of parasitization. These are applied @ 3-5 cards per Ha., 3-4 times at 10-15 days intervals in various crops as follows :

Trichogramma chilonis	:	Sugarcane, Cotton, Pulses, Vegetables
Trichogramma japonicum	:	Sugarcane, Paddy, Pulses
Trichogramma acheae	:	Castor, Cotton
Trichogramma brasiliensis	:	Cotton, Tomato
Trichogrammatoidea bactrae	:	Sugarcane, Pulses, Groundnut, Cabbage, Cauliflower

ii) Chrysoperia

Chrysoperia is a biocontrol agent against most of the insects. The Details of *Chrysoperia carnea* given below **:**

Chrysoperia carnea (commonly known as Green Lace Wing) is a voracious predator of sucking pests including whiteflies, aphids, thrips, mealy bugs and eggs and young larvae of a group of insects including Helicoverpa. It's larvae with sickle shaped mouth parts feed on pests in large numbers and during 8-10 days life cycle span each larvae can eliminate over 500 aphids / 500 thrips / 800 – 1000 eggs of Helicoverpa and scores of its I – II stage larvae.

Chrysoperia is available in form of vials containing 1000 – 5000 live eggs / larvae mixed with its food, inert

carrier & paper strips to avoid cannibalism. It is applied to crops like cotton, sunflower, tobacco, groundnut, mustard and vegetables @ 5000 – 10000 eggs / larvae per ha., 3 – 4 times at 15 days intervals coinciding with egg laying young stages of pests.

Microbial organisms can be used for the management of pests and plant pathogen which are part of the ecosystem and creates infection and kill the pest and plant pathogens. Microbial organisms may be belonging to fungal, bacterial and viruses.

b) Fungal Biopesticides

i) *Trichoderma viride*

Trichoderma viride is available in wettable Powder and liquid formulations.

Mycelian fragments, Conidia & clamydospore of *Trichoderma viride*. Highly active on root and stem rots caused by Schlerotinia and Rhizoctonia, wilts caused by *Fusarium* and *Verticillium* and blights / leaf spots caused by Alternaria, Ascochyta, Cercospora, Macrophomina, Myrothecium, Ramularia, Downy mildews & Powdery mildews, Fungal diseases of Cotton, Cereals, Pulses, Vegetables, Oilseeds, Fruit plants and Floriculture. *Trichoderma viride* acts on the plant pathogens through competition for space & nutrients, parasitization, disintegration of pathogen hyphae by enzymes and antogonism by *Trichoderma viride*. Apart from this, *Trichoderma viride* metabolites stimulate seed germination, plant growth and early flower formation. Extremely safe to mammals, man, non – target organisms, pollinators, fishes & birds etc. Classified as Class – IV, low hazardous (green label) pesticide. No phytoxicity and exempt from residue analysis. It can be used along with Biofertilizers.

Trichoderma viride is applied as paste or slurry on seeds @ 4 ml / kg seed / seedings as coating or as soil application @ 250 ml/ ha., after mixing with farmyard manure. It can be used as foliar spray @ 250 ml/ ha. Do not use chemical fungicides before or after the used of *Trichoderma viride*. Do not apply to seeds treated with chemical fungicides.

Physical Features of Liquid *Trichoderma*

- Very thin mate or no mate formation at surface of liquid formulation.
- Only spore appears in solution.
- Light green colour apperance
- Count ensures more than 10^7 spores/ml with Nil Contamination
- Enzyme activity very high
- Tolerates upto 70^0c temperature

ii) *Beauveria*

Beauveria Spp. is a water dispersible powder / emulsion / liquid formulation

a) Conidiospores
(b) Blastopores

Highly active on Lepidopteran caterpillars including Helicoverpa, Spodoptera, Plutella, Borers, Hairy caterpillars, Pests of vegetables & Fruit plants, and sucking pests like mites & spidermites of vegetables & ornamentals, whiteflies on cotton & vegetables, aphids & scale insects, locusts, Colorado beetles of potato and Coffee pod – borer. *Beauveria Spp.* infects all stages of insects including eggs, larvae, pupae, grubs, nymphs, hoppers and adults. The spores attached to insect cuticle / integuments, germinate and penetrate insect body. The hyphae, through enzyme action, proliferate in insect body and cause

mortality through a combination of chemical, mechanical, water loss and nutrient loss effects. The insect body ultimately gets covered with mycelium and spores, which may get dispersed and cause subsequent infections. Certain toxins produced by this fungus also cause the mortality of insects shelflife is 6 months, if stored in dark, well ventilated rooms in original packings at cool dry places one year for liquid. Extremely safe to mammals, man, natural parasites & predators (acts in harmony with them), non – target insects, fishes & birds etc. Classified as Class – IV, low hazardous (green label) pesticide. No phytoxicity and exempt from residue analysis. Spray @ 200ml/ ha., by knapsack or any other conventional sprayer at 12 – 15 days intervals based on pest populations. *Beauveria* should not be mixed with fungicides at any stage. Spray preferably in evenings and on young larval stages or on sighting of egg – laying. *Beauveria Spp. r*esults in instant stoppage of feeding & damage, death of insects within 24 – 72 hrs. of application. This results in decline of insect populations as well as decline of crop damage.

Physical Features of *Beauveria*

- Appearance white
- No mycellium formation only spores appeared in solution.
- Very high count up to 10^{10} depends upon strain.
- Very high enzymetic activity thus the action on target is quick.
- Contamination Nil.

c) *Metarhizium anisopliae*

Metarhizium anisopliae is available in wettable powder liquid formulation. Mycelial fragments & spores of

Metarhizium anisopliae. Highly active on Whitegrubs, Beetle Grubs, Caterpillars, Semiloopers, Cutworms & Sucking pests like Pyrilla, BPH, Mealybugs and Aphids on crops like Sugarcane, Groundnut, Rice, Tobacco, Potato, Maize, Sorghum, Barley, Cotton, Soyabean, Oranges and other Horticultural crops. *Metarhizium anisopliae* infects all stages of insects including eggs, larvae, pupae, nymphs and adults. The spores attached to insect cuticle / integuments, germinate and penetrate insect body, and cause mortality through a combination of chemical, mechanical, water loss and nutrient loss effect. The insect body ultimately gets covered with mycelium and spores, which may get dispersed and cause subsequent infections. Certain toxins called destruxin produced by this fungus also cause the mortality of insects shelflife is 6 months, if stored in well ventilated rooms in original packings at cool dry place at temperatures below 40^{0}C. One year for liquid. Extremely safe to mammals, man, natural parasites and predators (acts in harmony with them), non-target insects, pollinators, fishes & birds, etc. Classified as Class-IV, low hazardous (green label) pesticide. No phytotoxicity and exempt form residue analysis. For Soil application, mix 200ml of *Metarhizium anisopliae* in 50 Kg FYM and broadcast in one acre, especially in the root zone. For Potato and sugarcane, apply before planting and mix thoroughly with the soil by ploughing.

iv) *Verticillium lecanii*

Verticillium lecanii is a wettable powder and liquid formulation Mycelial fragments & spores of *Verticillium lecanii*. Highly active on sucking pests like Scale Insects, Coccus viridis, Aphids, Thrips, Mites, Bugs, Jassids, Hoppers, etc. on crops like Mustard, Sugarcane, Mango, Sorghum, Rice, Cotton, Tomato, Chillies, Brinjal, Onion,

Vegetables, Coffee, Fruits, and Flower Crops. *Verticillium lecanii* infects all stages of insects including eggs, larvae, pupae, nymphs and adults. The spores attached to insect cuticle / integuments, germinate and penetrate insect body. The hyphae through enzyme action, proliferate in insect body and cause mortality through a combination of chemical, mechanical, water loss and nutrient loss effects. The insect body ultimately gets covered with mycelium and spores, which may get dispersed an cause subsequent infections. Certain toxins (dipicolinic acid & bassianolide) produced by this fungus also cause the mortality of insects shelflife is 6 Months, if stored in well ventilated rooms in original packings at cool dry place at temperatures below 40^{0}C. One year for liquid formulations. It is extremely safe to mammals, man, natural parasites & predators (acts in harmony with them), non-target insects, pollinators, fishes & birds etc. Classified as Class – IV, low hazardous (green label) pesticide. No phytotoxicity and exempt from residue analysis. *Verticillium lecanii* is applied as sprays @ 5 ml/ L. of water. Addition of jaggery or soap solution to the spray solution before spraying on the standing crop improves the results.

Physical Features of *Verticillium*

- Appearance slightly reddish.
- No mycellium formation only spores in solution.
- Releases much quantity of enzymes which ensure quick action.
- Adoptable to extreme environments

v) Chaetomium globosum

This has demonstrated potential for control of soilborne plant pathogens and seedborne *Alternaria*

raphani and *Alternaria brassicicola* when applied as a seed treatment. Vannacci and Harman (1987) formulated the fungus by suspending ascopores in a solution of methyl cellulose to a concentration of 10^7-10^8 spores/ml. This was applied to crucifer seeds at the rate of 1ml suspension to 220 seeds. Gordon-Lennox et al. (1987) inoculated sugarbeet seeds by coating them in methyl cellulose and dusting with ascospores.

vi) Gliocladium virens

This is used in a soil-applied granular formulation for control of damping-off and root-rot pathogens, including Pythium and Rhizoctonia. Ristaino et al.(1994) formulated the fungus in both bran prills and a vermiculture-bran formulation. Its use as a seed treatment has also been investigated. Mukhopadhyay et.al (1992) coated seeds of chickpea, soybean and lentil in conidial suspensions of the fungus.

Burgess and Hepworth (1996) treated sunflower seeds with an isolate of G. virens by soaking them in a conidial suspension of the fungus for at least 2 h. The crude seed treatment was effective in controlling Sclerotinia minor in both glasshouse and field experiments.

vii) *Pythium oligandrum*

This occurs in soil and gives promising biological control for a range of damping-off plant pathogens (Al-Hamdani et.al., 1983; Vesely and Hejdanek, 1984; Martin and Hancock, 1987; Walther and Gindrat, 1987; McQuilken et.al. 1990b; Whipps et. al., 1993). The mycoparasite produces bulk-produced for application to seeds. The inherent stability of the oospores is an exceptionally valuable commercial attribute.

Penicillium oxalicum Kaiser and Hannan (1984) reported that a seed treatment of chickpea with P. oxalicum

consistently enhanced emergence and increased yields under field and glasshouse conditions.

viii) *Talaromyces flavus*

This is a potential biological control agent of a range of plant pathogens including R. solani, Sclerotinia sclerotiorum and Verticillium dahliae (Marois et.al. 1982; Fravel et.al. 1986: Adams, 1990). Approaches to formulation include application as a dust to potato seed pieces and encapsulation in alginate with a bran nutrient base (Papavizas et.al. 1987; methods. Ground soil-oatmeal preparations of T. flavus have also been incorporated into seed pellets of Chinese aster and tomato for control of damping-off pathogens, using a commercial split-pill process (Nagtzaam and Bollen, 1994).

e) *Arbuscular mycorrhizae*

Arbuscular mycorrhizae is available in soil based formulations. Needs 1200 infective propagules / gram of inoculum containing mycelliam fragments and spores to colonise effectively.

Arbuscular mycorrhizae colonize all most all crops in all stages and creates resistance to entering pathogens and nematodes in plants. On the other hand this fungus help to plants to absorb nutrients altering root anatomy, modifying root exudations and root system morphology by which plant gets good health as well as less disease incidence. Extremely safe in all respects to environment. In nursery 750g to 1000gm for $1m^2$ bed, 2-3 cm below the soil in nursery poly bag 10g / poly bag apply below soil. For established plants 10g / plant, applying in planting hole. For establishment perennial crops – 100g / tree at the root region.

Details of AM Fungi in disease control given in Table-3.

Table - 3 Effect of AM association on soil borne diseases by fungi and nematodes

Pathogen	Host	Effect of AM association
Olpidium brassicae	*Tobacco* *Lettuce*	*Reduction of infection*
Pythium ultimum	Soybean Poinsettia	None Reduced stunting
Phytophthora palmivora	Papaya	None
Phytophthora parasiuca	Citrus	Reduction of damage
Rhizoctonia solani	Poinsettia	Reduced stunting
Theilaviopsis basicola	Tobacco Alfalfa Cotton	Less stunting Inhibition of chlamydospore Production
Fusarium oxysporum	Tomato Cucumber	Less stunting Reduction of infection
Fusarium terrestris	Onion	Less stunting Reduction of infection
Meloidogyne incognita	Soybean Cotton Tomato Tobacco Cucumber	Fewer galls, increased yield
Heterodera solanacearum	Tobacco	Fewer nematodes
Bratylenchus brachyurus	Cotton	Fewer nematode larvae on roots

Source : Schonbeck 1979

C) Bacterial Biopesticides

i) *Bacillus subtilis* : Seed treatment with B.subtilis to stimulate plant growth and to protect against soilborne pathogens has been a subject of research interest for over 20 years as an inoculant for cotton, peanut and beans to suppress the *Rhizoctonia* and *Fusarium* root disease complex. Applied as a seed treatment, the bacteria colonize the root system, competing with root-invading pathogens (Tomlin, 1994). The bacterium produces resistant endospores tolerant to heat and desiccation and whose production is controlled by fermentation conditions. Spores are concentrated and dried into a powder. The products may be applied as a water-based slurry or a hopper-box treatment in several alternative formulations at the rate of 10^4-10^5 spores per seed, and are compatible with a number of chemical seed treatments.

ii) *Enterobacter cloacae* : This enteric bacterium has potential for control of *Pythium* seed root. It does not form spores, or other structures able to survive dehydration. Studies have used simple inoculation techniques successfully e.g. application to cotton seeds with methyl cellulose sticker.

iii) *Serratia entomophila*: This entomopathogenic bacterium is supplied to order and applied as a soil treatment for control of the grass grub (*Costerlytra zealandica.* Preliminary investigations suggest that it have potential for application as a seed treatment on ryegrass. Seeds were coated with a lime-based formulation at a rate of $1.5x10^6$ to 7.5x10 c.f.u./g (Sadler *et al, 1992).*

iii) *Pseudomonas fluorescens*

Pseudomonas fluorescens is available in wettable powder and liquid formulations. Spores of *Pseudomonas fluorescens* highly active on root and stem rots caused

by Schlerotinia and Rhizoctonia, damping off caused by Pythium, blights / leaf spots caused by Alternaria, Ascochyta, Cercospora, Macrophomina, Myrothecium, Ramularia, Downy mildews and Powdery mildews, Fungal diseases of Cotton, Cereals, Pulses, Vegetables, Oilseeds, Fruit plants and Floriculture. *Pseudomonas fluorescens* acts on the plant pathogens hyphae by enzymes and antagonism. Seed treatment with *Pseudomonos fluorescens* provides a protective zone around seeds. It also acts as a plant growth promoter to stimulate seed germination and early flowering and fruiting shelflife is 6 Months, if stored in well ventilated rooms in original packings at cool dry place at temperatures below 40^0 C. 2 years in liquid bottle pack at room temperature. Extremely safe to mammals, man, non – target organisms, pollinators, fishes and birds etc. Classified as Class – IV, low hazardous (green label) pesticide. No phytoxicity and exempt from residue analysis. It can be used along with Biofertilizers. Pseudomonas *fluorescens* is applied as paste or slurry on seeds @ 4-5 ml / Kg seed /seedings as coating or as soil application @ 250 ml / Ha., after mixing with farmyard manure. It can also be used as foliar spray @ 200ml/ Ha. Do not use bactericidal chemical before or after the used of *Pseudomonas fluorescens*. Do not apply to seeds treated with chemical bactericides.

Physical Features as Liquid *Pseudomonas fluorescens*

- Slightly greenish solution
- Once the formulation mixed with water for use it release large amount of Flurorescens which act quickly to control diseases.
- Nil Contamination high count upto 10^{10}.

v) *Bacillus thuringiensis Var. Kurstaki*

Thuringiensis var. kurstaki Parasporal crystal containing Delta – endotoxin (b) Spores high active ingredient content : (Potency 50000 IU / mg min. on *Helicoverpa armigera*, 55000 IU / mg min. on *Spodoptera exigua* 32000 IU / mg min. on *Trichoplusia ni* & 3000 IU / mg min. on Gypsy moth) Highly active against 1st / IInd / IIIrd instar Lepidopteran caterpillars including Helicoverpa, Spodoptera, Plutella, Borers, Hairy caterpillars, Cut worms, Army worms, Leaf-rollers & miners, Skelotonizers & Defoliaters, Pests of cereals, Pulses, Vegetables, Oil-crops, Fruit trees and Forest trees. Bacillus *thuringiensis* Var. Kurstaki acts on host larvae through its parasporal crystal delta endotoxin and bacterial spores causing starvation, paralysis and septicemia of larval bodies ultimately resulting death of larval bodies. The endotoxin acts on midgut epithelium causing disintegration of gut wall, cessation of feeding activity, starvation and leakage of gut contents into haemolymph and body cavity of the larvae leading to general paralysis of larval body. The spores germinate to form bacterial cells that multiply in the larval gut and body causing septicemia. Surviving larvae, if any, because of consuming sub-lethal doses, lead to under developed / deformed pupae / adults unable to form the progenies.

Shelflife is minimum 6 months, if stored in dark, well ventilated rooms in original packings at temperatures upto 40^0C. For liquid, more than 2 years at room temperature. Extremely safe to mammals, man, natural parasites and predators (acts in harmony with them), non – target insects, fishes & birds etc. and classified as Class – IV, low hazardous (green label) pesticide.

No phytotoxicity and exempt from residue analysis. Make homogenous thick paste of Bt in small quantity of water. Dilute the paste in desired volume of water under intermittent stirring. Spray at the rate of 200ml / Ha., by knapsack or any other conventional sprayer at 12 – 15 days intervals based on pest populations. Spray preferably in evenings and on young larval stages or on sighting of egg – laying. *Bacillus thuringiensis Var. Kurstaki* results in instant stopage of feeding and damage, death of caterpillars within 48 – 72 hrs. of application. This results in decline of adult insects as well as decline of crop damage.

d) Viral Biopesticides

i) Nuclear Polyhedrosis viurs (NPV) of Spodopteralitura

Nuclear Polyhedrosis Virus of *Spodoptera litura* is a liquid formulation of living Nuclear Polyhedrosis Virus (NPV) of *Spodoptera litura* in liquid form. Polyhedral Inclusion Bodies (PIB) of Nuclear Polyhedrosis Virus (NPV) of Spodoptera litura highly active on *Spodoptera litura* caterpillar pest of cotton, groundnut, pulses, cabbage, chillies, tobacco, oil crops and roses. Nuclear Polyhedrosis virus ingested by larvae cause severe infections of larval bodies causing darkening, disintegration of tissues, larval death and release of whitish fluid within 2-4 days of applications at young larval stages. The NPV infection is at times carried inside the infected pupae and adults. The caterpillars emerging out of the eggs layed by such adults also carry the PIBs of NPV shelflife is 6 months, if stored in well ventilated rooms in original packings at cool dry places. **Chandra et al has developed a new liquid formulation for NPV which renders minimum 18 months shelflife and contains upto level**

of 10^{10}. The formulation contains sunscreen which protect the NPV from ultra-violet rays etc. Extremely safe to mammals, man, natural parasites and predators (acts in harmony with them), non – target insects, pollinators, fishes and birds etc. Classified as Class - IV, low hazardous (green label) pesticide). No phytoxicity and exempt room residue analysis. NPV *Spodoptera litura* results in stoppage of feeding and damage, death of insects within 72 – 96 hrs. of application. This results in decline of insect populations as well as decline of crop damage.

ii) Nuclear Polyhedrosis virus of Helicoverpa armigera

Nuclear Polyhedrosis Virus o*f Helicoverpa armigera* is a liquid formulation of living Nuclear Polyhedrosis Virus (NPV) of Helicoverpa armigera. Polyhedral Inclusion Bodies (PIB) of Nuclear Polyhedrosis Virus (NPV) of *Helicovarpa armigera. Highly* active on Helicovarpa armigera caterpillar pest of cotton, pigeon pea, gram, tomato, cabbage, pea, groundnut, tobacco, millets, oil crops and roses. NPV of Helicoverpa armigera ingested by larvae cause severe infections of larval bodies causing darkening, disintegration of tissues, larval death and release of whitish fluid within 2-4 days of applications at young larval stages. The NPV infection is at times carried inside the infected pupae and adults. The caterpillars emerging out of the eggs layed by such adults also carry the PIBs of NPV shelflife is 6 months, if stored in well ventilated rooms in original packings at cool dry places. **Chandra et al has developed the new liquid formulation for NPV which renders minimum 18 months shelflife and contains upto level of 10^{10}.** The formulation contains sunscreen which protect

the NPV from ultra-violet rays etc.Extremely safe to mammals, man, natural parasites and predators (acts in harmony with them), non – target insects, pollinators, fishes and birds etc. Classified as Class – IV, low hazardous (green label) pesticide). No phytoxicity and exempt from residue analysis. Shake the bottle well and dilute 1 ml in 1 L. water. Spray at the rate of 250 – 500 ml formulation / Ha., 2-3 times at 10-15 days intervals, by knapsack or any other conventional sprayer at 12-15 days intervals based on pest populations. Spray preferably in evenings and on young larval stages or on sighting of egg – laying. Nuclear Polyhedrosis Virus of *Helicoverpa armigera* results in stoppage of feeding and damage, death of insects within 72 – 96 hrs. of application. This results in decline of insect populations as well as decline of crop damage.

5. BOTANICAL PESTICIDES

Plants during their long evolution, have synthesized a diverse array of chemicals to prevent the colorization by insects and other herbivores. They produce secondary metobosites like terpenoids alkaloids, flavonoids, phenolic compounds. These secondary metabolites having insecticidal properties. In Indian flora several plants having insecticidal properties such as nicotinoids, natural pyrethrins, rotenoids, Neem products have been used in the past for suppression of pest species. Among botanical pesticides Neem occupies very important place in pest control. Different parts of Neem tree can affect more than 200 insects and some nematode fungi, bacteria and viruses. Table - 4 shows details of plants used for disease and pest control.

Table – 4 List of some important plants with important pests managed and their preparation.

Plant	Botanical Name	Part used and Pests controlled	Method of preparation
Tobacco	*Nicotiana tobaccum and N.rustica*	Leaf extract and oil acts as good repellent	Dried leaf extract can be made by crushing leaves in water and boiling for few minutes
Tulsi	*Oscimum sanctum*	Plant itself is good repellent for insects, mosquitoes and snakes. Aqueous extract of leaves can prevent attact of leaf minor and leaf curl in various crops like potato, brinjal, tomato, chilli, crucifers and onion.	Crush leaves in water and take out the extract by squeezing in a cloth.
Karanj	*Pongamia pinnata*	Karanj oil has insecticidal and bactericidal properties and effective against wide range of pests on almost all crops. Oil emulsion can also be used as disinfectant and insecticide is domestic applications.	Karanj oil directly or as water emulsion can be used as spray.

Genda or marigold	*Tagetes sp.*	Excellent nematode repellent. Crushed roots or root extract can control root rot, nematodes and brown rust in paddy, wheat and ornamental plants.	Crush roots and leaves in water to obtain aqueous extract.
Mahua	*Madhuca longifolia.*	Mahua oil can prevent stem root and root rots of various crops like jute, groundnut and potato. Aqueous leaf extract is also equally effective.	2.5-3% of oil emulsion in water is used as spray.
Methi	*Trigonella foenumgraceum*	Fresh leaf extract is effective against wide range of agricultural pests	Crush fresh leaves in water and boil to take out extract. Filtrate is used as pesticide.
Onion	*Allium cepa*	Dust is effective Against many fungal Diseases and pests of Rice, wheat, fruit trees, And pea. Dust or extract is sprayed to prevent leaf curl, powdery mildew and other fungal growth.	the bulbs and ground to fine powder. For extract crush the bulbs in water and filter.

Sitaphal	Custard Apple Anona squamosa.	Seed and leaf extract spray can prevent diamond-back moth, Pod borer and some other insects in brinjal, rice and wheat.	25g of seed or leaf extract in one lit of water used as foliar spray.
Chaul-moogra	Hydnocarpus curzii H. laurifolia H. wightiana and H.alpine.	Oil prevents and cures stem rot and fruit rot citurs, Oil cakes are deterrent against ants and other insects.	2.5-3% oil in water and emulsifier at 0.1% with little detergent is used as foliar spray.
Datura	Datura metal	Leaf extract is excellent Antifungal agent and effective Against brown rust in paddy and wheat. Also effective as Storage pesticide in grains.	Powdered dry leaf can be used as dust to prevent rust attack. Leaf extract in water can be used as spray. 10 gm leaf dust per kg of grain, effectively check the Incidence of storage pests in grains.
Tea	Camellia Sinensis C. indica	Dried spent tea leaves used as manure-cum-insecticide for rose plants.	Grind dry spent leaves to a fine powder and mix with soil.
Castor	Recinus communis	Castor oil is effective repellent of weevil, aphids and caterpillars. In maize and other crops. Oil Cake is good manure and insect Repellent.	Mix castor oil with water to make emulsion and apply as spray
Drum - stick	Moringa oleifera & M. Petrygo-sperma	Root and leaf extract is effective bactericide and control root rot in vegetables, fruits and ornamental plants.	Crush fresh roots and leaves in water. To make the extract stronger boil for 5-10 minutes. Filter and apply.

Saunf or mauri	Foeniculum vulgare	Leaf extract is effective as repellent against many Agricultural pests on Vege-tables and ornamental plants.	Crush partly dried leaves and plants in water and boil to make a strong tea. Filtrate is used as pesticide.
Ipomea or Kalmisag	Ipomea raptons	Leaf extract or leaf dust are effective against white fly, Sheath rot, ear had, bug, brown, rust and burn spot in case of Paddy, wheat and cotton.	For dust grind the dried leaves to fine dust. 5-10% fresh leaf extract in water is to be applied as fine spray or as atomized spray.
Ratanjyot or Jatropha	Jatropha curcas J. glandulifera	Extract of both the species is effective against wide range of pests in all crops. Oil cake is excellent manure and insect repellent.	Fresh green biomass is crushed in water, boiled and extracted.
Chrysan-themum	Chrysanthemum cinerariaefolium	Aphids, white fly, spider mite, mealy bugs etc.	500g of fresh flowers in 4 lit. of water to make a strong tea. Cool and filter. Mix with equal Amount of water and add 30ml of Liquid soap. Apply as foliar spray.
		Termites and ticks of animals	Mix 500 gm of dry flower powder with 500 ml of ginger oil and 3 lit of used cooking oil and boil. After cooling pour 10-20 ml of this oil in termite hills daily for 7-10 days. All termites will migrate, rub this oil on the skin of animals to prevent the attack of ticks.

Mustard	Brassica nigra and B. juncea	Leaf extract of black mustard is effective against stem rot of jute. Leaf extract of brown mustard Prevents burn spot of paddy.	Crush leaves in water and boil for 10 minutes. Filtred extract is used for spraying.
Bael patra	Aeglo marmelos	Leaf and fruit extract act as repellent to wide range of leaf Eating insects and sucking pests.	Crush fresh leaves and fruits with some water and boil. Filtered extract can be used as foliar Spray.
Cahulai	Amaranth Amaranthus blitum & A.tricolour	Leaf dust and leaf extract can prevent brown rust of wheat and paddy	Crush 25-50 gm of leaves in 1 lit. of water and boil. Filtrate is used as spray.
Lantana	Lantana camara	Leaf extract is effective against Leaf minor of various crops like Brinjal, Tomato, Chilli, crucifers and onions.	Crush fresh leaves in water and take out the extract by squeezing in a cloth.
Turmeric	Curcuma longa	Turmeric power is good storage pesticide. Its rhizome dust prevents root not in paddy, wheat and vegetable crops. Can also be used as seed dresser.	Dried rhizomes and seeds can be grounded to fine powder.

i) Neem oil

Neem oil is a well known botanical pesticide based on neem seed kernel ingredients emulsifiable concentrate formulation. Azadirachtin 0.15 % along with triterpenoids and limenoids and excellent broad spectrum control of Aphids, Jassids, White flies, Beetles, Caterpillars, Cutworms, Shoot and Fruit Borers including Bollworms etc. on cotton, paddy, vegetables, pulses, oil crops, fruit trees, sugarcane, millets and tea plantations.

Neem oil acts on the insects through multiple actions as repellent, Anitfeedant, Insect growth regulator and Oviposition deterrents shelflife is 2 years, if stored in well ventilated rooms in original packings at temperatures below 40^0 C. Extremely safe to mammals, man, non – target organisms, pollinators, beneficial insects, fishes and birds etc. Classified as Class – IV, low hazardous (green label) pesticide.

1 L. of Neem oil 1500 is diluted in 200 – 300 L. water and sprayed in 1 Ha. at the time of hatching of eggs and appearance of young stages of insect pests. Repeat the spray after after 15 days intervals depending on pest populations. Besides neem the various plants as whole or part of plant as it is or after some preparations are being used for control of pest and disease (Table 5 - 9).

Table - 5 Pests controlled by Neem

Common Name of pest	Scientific Name of pest
Asian cornborer	*Ostrinia furnacalis*
Black carpet beetle	*Attaqenus piceus*
Brown aphid	*Lipaphis erysimi*
Brown planthopper	*Nilaparvata lugens*
Cabbage aphid	*Brevicoryne brassicae*
Cabbage white butterfly	*Pieris brassicae*

Castor bean caterpillar	*Spodoptera litoralis*
Citrus leaf miner	*Phyllocnistis citrella*
Citrus psyllid	*Diaphorina citri*
Citrus red mite	*Panonychus citri*
Cotton bollworm	*Heliothis zea*
Fall Armyworm	*Spodoptera fruqiperda*
German roach	*Blatella germanica*
Green peach aphid	*Myzus persicae*
Imported cabbage worm	*Pieris rapae*
June bag	*Anomala cupripes*
Migratory locust	*Locusta migratoria*
Milkweed bugbeetle	*Oncopeltus fasciatus*
Pink bollworm	*Pectinophora gossypiella*
Rice green leafhopper	*Nephotettix virescens*
Rice noctuid	*Spodoptera abyssina*
Nephotettix virescens	
Rice noctuid	*Spodoptera abyssina*

Table - 6 Pests controlled by (Pongamia glabra)

Common Name	Scientific Name
Angoumois grain moth	*Sitotroga cerealella*
Army worm	*Spodoptera litura*
Brown Plant Hopper	*Nilaparvata lugens*
Citrus aphid	*Toxoptera citricidus*
Citrus leaf miner	*Phyllocnistics citrella*
Coffee green scale	*Coccus viridis*
Lesser grain borer	*Rhizopertha dominica*
Maize stem borer	*Chilo partellus*
Nemotode	*Meloidogyne incognita*
Potato tuber mothe	*Phthorimaea operculella*
Pulse beetle	*Callasobruchus chinensis*
Red ant	*Oecophylla smaragdina*
Red flour weevil	*Tribolium castaneum*

Rice case worm	*Nymphula depunctalis*
Rice weevil	*Sitophilus oryzae*
Saw-toothed grain beetle	*Oryzaephilus surinamensis*
White fly	*Bemesia tabaci*

Table - 7 Pests controlled by Garlic

Common Name of pest	Scientific Name of pest
Angoumois grain moth	*Sitotroga cerealella*
American Boll Worm	*Helicoverpa armigera*
Aphids	—
Armyworms	*Spodoptera litura*
Bacteria	*Xanthomonas sp.*
Brown leaf spot of rice	*Helminthosporium oryzae*
Colorado beetle	*Leptinotarsa decemlineata*
Cotton Stainer	*Dysdercus cingulatas*
False Codling moth	*Cryptophlebia leucotreta*
Housefly	*Musca domestica*
Imported Cabbage Worm	*Pieris rapae*
Japanese root-knot nematode	*Meloidogyne javanica*
Khapra beetle	*Trogoderma granarium*
Mexican Bean Beetle	*Epilachna varivestis*
Mites	—
Mosquitoes	—
Onion Thrips	*Thrips tabaci*
Pests of fruits	—
Pulse beetle	*Callasobruchus chinensis*
Riceblast fungi	*Pyricularia oryzae*
Root Knot nematode	*Meloidogyne incognita*
Ticks	—
Vermins	—
Whitefly	—
Wireworms	—

Table - 8 Pests controlled by Aloe

Common Name of pest	Scientific Name of pest
American bollworm	*Helicoverpa armigers*
Aphids	
Army worm	*Spodoptera litura*
Bacterial diseases	
Fungal diseases	
Japanese beetle	*Poppilla japonica*
Pink bollworm	*Pectinophora gossypiella*

Pest controlled by Tulsi

The range of Pests controlled by Tulasi is provided. Symptoms of major pests controlled by Tulasi and the damage caused by them and described. Some pests have already been described under Pongam.

Table - 9 Pests controlled by Tulasi

Common Name of pest	Scientific Name of pest
Aphid	*Aphis gossypii*
Caster Semi looper	*Spodoptera litura*
Cockroach	*Pericallia ricini*
Mites	*Tetranychns sp.*
Nemotode	*Meloidogyne incognita*
Recl Cotton bug	*Dysdercus cingulaths*

Table - 10 Biocontrol agents and Biopesticides and IPM products for various crops

Crop	Pest / Disease	Biopesticides & I.P.M. Products of choice
Cotton	Bollworms	Traps, Lures, B.T., N.P.V., Trichogramma
	Whitefly, Jassids, Thrips	Neem 1500 PPM,
	Mites	Chrysoperia, Verticillum, Baeuveria
	Wilts & Leaf Spots	Trichoderma, Pseudomonas
Paddy	Yellow stem borer, Leaf folder	Traps, Lures, B.T., Trichogramma
	Hoppers	Neem 1500 PPM, Baeuveria
	Sheath blight & Leaf spots	Trichoderma, Pseudomonas
Gram, Arhar Moong, Urad	Bollworms / Cutworms	Traps, Lures, B.T., N.P.V., Trichogramma
	Wilts	Trichoderma, Pseudomonas
Sugarcane	Borers	Trichogramma
Soyabean	Caterpillars	Traps, Lures, B.T., N.P.V., Trichogramma
Mustard	Aphids	Neem 1500 PPM, Chrysoperia
	White rust & Leaf spots	Trichoderma, Pseudomonas
Cauliflower	Diamond Back Moth, Heliothis	Traps, Lures, B.T., N.P.V.,
Cabbage	Wilts	Trichogramma
Tomato	Heliothis	Traps, Lures, B.T., N.P.V.
Capsicum	Wilts	Trichogramma
Brinjal, Bhindi	Fruit Borers Mites	Traps Lures, B.T., Trichogramma Neem 1500 PPM, Verticillium
Potato	Cut Worms Early Blight, Late Blight	Traps, Lures, B.T. Trichoderma, Pseudomonas
Citrus	Caterpillars Whitefly	B.T. Neem 1500 PPM
Guava	Wilt	Trichoderma, Pseudomonas

Table - 11 shows the biological inputs used for various pests / diseases.

Table - 11 Biocontrol agents and Biopesticides and IPM products for various pests and diseases

Pests / Diseases	Biological Inputs
Bollworms / cutworms / Borers	*Bacillus Thuringiensis* (BT), Beauveria, Trichogramma, Nuclear Polyhedrosis virus (NPV / GV), Chrysoperla, Traps & lures
Whitefly Beauveria, Sticky Traps	Neem 1500 PPM & Above, Chrysoperia,
Aphids & Jassids Beauveria, Sticky Traps	Neem 1500 PPM & Above, Chrysoperia,
Thrips	Neem 1500 PPM & Above, Verticillium, Sticky traps
Mites Verticillium, Beauveria	Neem 1500 PPM & Above, Chrysoperla,
Wilts, Rootrots, Leaf spots, Blights, Anthrachose, Mildews	Trichoderma, Pseudomonas
Bacterial Wilts / Blights	Pseudomonas
Nematodes	Trichoderma

APPLICATION SCHEDULE TO BE DECIDED ONLY ON THE BASIS OF FIELD SITUATIONS *BASED ON SAU TRIALS

Table - 12 shows the permissibility of some of pests / disease control inputs in organic farming practices.

Table - 12 Products for Plant Pest and Disease Control and its permissibility in Organic Farming Practices

Name of the Input	Conditions for use
Synthetic chemical pesticides	Banned
Pheromones	Permitted in traps and dispensers only
Mechanical traps	Permitted
Chromatic traps	Permitted
Algal Preparations	Permitted
Bacterial preparations (e.g. *Bacillus thuringensis)*	Permitted
Beeswax	Permitted
Bio-dynamic preparations	Permitted
Calcium Hydroxide	Permitted
Carbon dioxide	Permitted
Chitin Nematicides (natural origin)	Permitted
Chloride of lime	Permitted
Clay (bentonite, perlite, vermiculite, zeolite)	Permitted
Coffee grounds	Permitted
Corn gluten meals (weed control)	Permitted
Chromatic traps	Permitted
Dairy products (e.g. milk, casein)	Permitted
Diatomaceous earth	Restricted – Risk of nonselective effects
Ethyl Alcohol	Permitted
Sabadilla (unprocessed)	Permitted
Silicates (e.g. sodium silicates, quartz)	Permitted
Sodium bicarbonate	Permitted
Lecithin	Permitted
Lime sulfur (Calcium polysulfide)	Permitted

Mulches from natural sources	Permitted
Natural acids (e.g. vinegar)	Permitted
Neem (Azadirachta indiaca)	Restricted - Risk of nonselective effects
Plant based repellents	Permitted
Propolis	Permitted
Plant and animal oils	Restricted - of non selective effects
Plastic mulches	Restricted
Potassium bicarbonate	Permitted
Quicklime	Restricted
Soft soap	Permitted
Soda	Permitted
Seasalt and salty water	Permitted
Sulphur dioxide	Restricted
Gelatine	Permitted
Release of predators of parasites of insect and pests	Restricted - Risk of influencing the local ecosystem
Viral, fungal and bacterial preparations, sterilized insects	Restricted - Risk on influencing the local ecosystem irreversibly
Sulphur	Restricted - Risk of non-selective effects
Copper salts (copper sulphate might be good to control)	Restricted – Risk of non-selective effects and contamination with heavy metals
Permanganate of potash	Restricted – Risk of non-selective effects
Chloride of lime soda imbalances	Restricted – Risk of physiological
Light mineral oils	Restricted – Risk of non-selective effects
Deris root (Rontenone)	Restricted – Risk of non-selective effects
Diatomaceous earth	Restricted – Risk of non-selective effects
Pyrethrum cinerafolium	Restricted – Risk of non-selective effects
Ryania spectosa	Restricted – Risk of non-selective effects
Tobacco tea	Restricted – Risk of non-selective effects
Cassia amara	Restricted – Risk of non-selective effects
Nomiticides from natural sources e.g.Tayestus	Restricted – Risk of contamination
Mulches from natural sources	Permitted
Common (unprocessed) salt	Permitted

6. CONSTRAINTS IN WIDE ADOPTION OF BIOCONTROL AGENTS AND BIOPESTICIDES

- Lack of knowledge and suspicion in farmers.
- Lack of simple illustrated IPM informations.
- Absence of really well executed integrated Pest Management (IPM)
- Degradation of information during knowledge transmission.

  Extension system of Centre as well as States is not able to perform the desired quality and quantity of knowledge transmission for IPM.
- Non adoption of IPM practices in large areas.

  Very few farmers adopt IPM strategies whereas its adoption in entire village will provide much more efficacious results.
- Un-consistent/Un-planned demands.

  Planning by State Governments for IPM inputs is not timely and if the inputs are procured timely, their distribution to farmers is not in time.
- **Short Shelf Life**

  Biocontrol insects in general have very short and limited shelf life and therefore, have to be supplied fresh. To overcome this grim problem liquid formulations should be encouraged, which has more than one year shelflife.
- **Absence of Well equipped quality testing laboratories**

  State / Regional Pesticide Testing Laboratories lack infrastructure and trained human resource for analysis of biopesticides. At times the availability of reference standards and test organisms is also problematic.
- Inappropriate application technologies and equipments.

  Not much work has been done at SAUs / Institutions to standardize the application equipment design for optimum application of pesticides particularly biologicals.
- Dealers interested in chemical pesticide rather than in Biocontrol agents.

  In view of higher sale margins and higher acceptance of chemical pesticides, the dealer concentrate on them rather than on biologicals, where the margins are less and the efforts are more.
- State Biocontrol Laboratories & C.I.P.M.C. centers not able to make timely supplies of Biocontrol agents according to the designed production capacities and Govt. investments. It should be promoted through private agencies.
- Major Indian Pesticide companies not yet interested in Biopesticides.

  In view of higher sale margins and higher acceptance of chemical pesticides, the companies concentrate on them rather than on biologicals where the margins are less and the efforts are more.

7. SOME SUGGESTED MEASURES FOR INCREASED ADOPTION OF BIOCONTROL AGENTS AND BIOPESTICIDES

a) Earthmarking of 50% of the State Plant Protection budget for eco-friendly Biocontrol agents, Biopesticides, Botanical Pesticides and Pheromones.

b) Waiver of custom duty, Reduction / Waiver of Excise Duty and abolition of Octroi on Biocontrol agents, Biopesticides, Botanical Pesticides & Pheromones.

c) Exemption of sales tax (S.T.) on Biocontrol agents, Biopesticides, Botanical Pesticides and Pheromones in various states.

d) In the Harmonious Classification System, the classification of Biopesticides should be separate from chemical pesticides (Chapter 38) for all purposes including duties.

e) Pollution Control Boards should consider eco-safe Biopesticides under a category separate from hazardous conventional pesticides.

f) All State Agriculture Universities should include Biopesticides, Biocontrol Insects in the "Package of Practices". Details of various Biopesticide products and their utility in different crops along with methodology for use by farmers should be provided.

g) Purchase of bio-inputs should be on the basis of efficacies and product quality, irrespective of prices. It is suggested that the inputs from various reputed CIB registered manufactures should be evaluated by the Department of Agriculture and, based on the good efficacies, list of manufacturers of good quality biopesticides should be circulated to all States as "Accredited / Preferred Manufacturers".

h) Nodal Biopesticide quality testing GLP Laboratories should be set up.

i) State Pesticide Testing Laboratories for Biopesticide Testing should be updated and each and every batch of the product should be tested randomly for quality control.

j) Prescription based sales of pesticides, as in case of medicines, should be introduced. Prescriptions could be issued by Plant Protection Officials / Research Scientists / Industry Staff.

k) Training programmes of Existing Distributors / Dealers on Biological control and Biopesticides should be designed in collaboration with industry and training

should be imparted to them as a refresher course. Qualifying tests should be conducted every two years thereafter and refreshers courses should be continued.

l) Farmer education programs on the utility of biopesticides and biocontrol agents should be on television channels including Krishi – Darshan.

m) Incentives should be provided to the Biopesticides and Biocontrol Agent Industries to ensure increased availability and improved quality.

n) Incentives should be provided to Exporting Farmers to adopt Biopesticides and Biocontrol Agents.

No doubt that in spite of good progress on the biopesticides front in the country, a lot more effort is desirable to implement several suggested tariff and non-tariff measures and to provide appropriate extension through inter-organizational synergies. Central Government, State Governments, NGOs and Industry need to synchronize their planned efforts with sincerity and accountability for improving the availability of high-quality biocontrol inputs, use the available trained expertise, execute extensive good demonstrations to enhance the confidence of farmers in eco-safe biopesticide and prevent the damage to the economy, the environment and the mankind by reducing the consumption of hazardous chemical pesticides.

8. IPM MATERIALS COVERED UNDER SCHEDULE OF INSECTICIDES ACT. 1968 OF GOVERNMENT OF INDIA

Bacillus thuringiensis (Bt)
Bacillus sphaericus
Azadirachtin & other botanicals
Verticillium

Baeuveria
Metarrihizium
Nomuraea
Nuclear Polyhedrosis viruses (NPV)
Granulosis Viruses
Trichoderma
Gliocladium
Pseudomonas
Pheromones for Mating Disruption

9. IPM MATERIALS NOT COVERED UNDER SCHEDULE OF INSECTICIDES ACT, 1968 OF GOVERNMENT OF INDIA

Pheromone Traps

Pheromone lures for Monitoring

Trichogramma, Chrysoperla & other Natural Parasitic and Predator insects

Nematodes

Organic Nitrogen sources / Protein Hydrolysates (Like bioboost)

10. IPM MATERIALS REGISTERED WITH CENTRAL INSECTICIDES BOARD AND REGISTRATION COMMITTEE OF GOVERNMENT OF INDIA

UNDER SECTIONS : 9 (3B) / 9 (3)

Bacillus Thuringiensis var. Galleriae
Bacillus Thuringiensis var. Kurstaki
Bacillus Thuringiensis var. Israelensis
Bacillus Sphaericus
Trichoderma viride
Pb Rope

UNDER SECTIONS : 9 (3B) / 9 (3) & 9 (4)

Azadirachtin 300 ppm
Azadirachtin 1500 ppm
Azadirachtin 3000 ppm
Azadirachtin 5000 ppm
Azadirachtin 10000 ppm

11. LIQUID BIOPESTICIDES

Some pesticidal and other beneficial organisms have been very effective in the laboratory only, fail at some stage in the field, even after development of a product for marketing. Common causes of this demise are poor stability of the product during storage prior to application, too little active material actually reaching the field target, and rapid degradation of the active material on the target. Formulation plays a vital role in helping to solve these problems and in making an organism effective in practice. However, this must be achieved in a cost-effective manner if the final product is to survive commercially.

What is formulation? Defined collectively, formulation comprises aids to preserving organisms, to delivering them to their targets and – once there - to improving their activities. A technical concentrate of an organism that has been formulated is termed a formulation, or a product, which may be stored and put on sale commercially. A product often does not fully serve all the requirements of use on all crops. Formulations are being made for the following commercially important microbes : 1) microbial insecticides; 2) microbes that destroy, inactivate or compete with plants pathogens; 3) microbial herbicides; 4) beneficial organisms that improve plant nutrition; and 5) microbes applied to seeds.

Table - 13 shows the main types of formulated organisms and environment to which they are applied to cater the crop disease.

Table - 13 Main Types of formulated organism and environments to which they are applied.

Organisms	Distributed life stage	Mode of action	Main environment
Spore-forming bacterial insecticide	Crystal toxin, durable spore	Stomach poison, infection	Plant surfaces, water, soil
Protozoan	Durable spore	Infects via gut	Plant surfaces
Insect viruses	Durable inclusion body	Infects via gut	Plant surfaces
Mycoinsectide	Relatively delicate or durable spore	Infects on contact	Soil, Plant surfaces, water, insect cuticle
Mycoherbicide	Relatively delicate or durable spore	Infects on contact	Plant surfaces, soil
Bacterial herbicide	Delicate bacterial cell, tough spore	Infects on contact	Plant surfaces, soil
Fungi bacteria combating	Durable or delicate spore, bacterial or actinomycetes cell	Infects or inhibits on contact	Plant surfaces, soil
Pathogens Bacterial, Fungal Symbionts	Bacterium, delicate	Infects on contact	Soil

(Burge and Jone, 1998)

a) Types of Inoculum

There are a wide variety of formulation types, both liquid and solid. The main types currently used for organisms have been classified into dry products (dusts, granules and briquettes) and suspensions (oil or water-based and emulsions). A wider range of formulation types, together with additive types, are available in market.

i) Dry inoculum

These comprise dusts, granules and briquettes, a classification based on particle or aggregate size. Also included in this group are wettable powders, which are formulated as dry powder designed to be added to a liquid carrier, normally water, just before application.

Dusts based on inert diluents or carriers, normally of low absorbent capacity, these have particle sizes ranging from 5-20 mm. Particles of <10mm are abrasive and insecticidal, also an inhalation hazard, but the smaller particles adhere best. Minerals such as clays are often the first choice of diluent, but silica minerals are also used, varying the proportions to obtain the desired bulk density. Diluents with high surface acidity or alkalinity are usually avoided as they tend to form an unstable product.

Dusts typically contain <10% of an organism by weight. They are normally prepared by feeding the organism into an air stream for mixing with the mineral diluent in a blender. Particle size, bulk density and flow ability are extremely important. The proportion of components is varied to form a free-flowing, fluffy powder which does not stick to machinery or allow separation of the organisms from the diluent during transport, storage and application. At application, dust particles are carried on air currents to penetrate plant canopies. Smaller particles collect on target surfaces, larger ones fall through. Usually only 10% of the particles adhere to the surface. Stickers can be included, such as milk powder (which is hygroscopic so the air must be dry during mixing), or a desiccant such as sodium sulphate (added to prevent caking). However, most of the products available in the market are using marble powder, talc powder, kaolin powder some are using lignite without any stickers.

ii) Granules, pellets, capsules and briquettes inoculum

Granules are discrete masses 5-10mm^3 in size, pellets are >10mm^3, and briquettes are large blocks up to several cubic centimeters; like dusts, these products contain an inert carrier holding the organisms. Carriers include clay minerals, starch polymers, dry fertilizers and ground plant residues. Choice of carrier depends on sorption (more important for formulating slurries of organisms), hardness, bulk density and product disintegration rate in water. Soft carriers, eg. Bentonite, disintegrate quickly to release the organism. The product can be coated with various materials to slow and control the rate of release, which also depends on unit size.

Typically, the concentration of organisms is 5-20%, usually <15%. There are three types of granules: 1) the organisms are attached to the outer surface of a granular carrier in a rotating drum by a sticker. 2) the organisms are sprayed onto a rotating granular carrier without a sticker. 3) the organisms are incorporated into a carrier paste or powder which sets as a matrix size being controlled by passing the product through a sieve. Type-3 is the most common with nitrifying microorganisms. When the carrier forms a protective coat around a core aggregate of organisms, the unit is termed a capsule very few products are available in India.

Although, these products present no inhalation hazard, do not readily drift in the wind and can be measured easily or weighed out, in contrast to dusts. Even the smaller products can be applied to surfaces such as foliage and leaf axils, much material falls to the ground.

iii) Wettable powder inoculum and their limitations

These predominated among all commercial products and comprise technical powders blended with additives to make them stable during storage on the shelf and readily miscible with water, to which the powder is added shortly before spraying. As with other formulations using water as carrier, chlorinated water must be avoided (or the water allowed to stand to evaporate the chlorine) for fear of damaging the organisms.

Most wettable powders contain 50-80% technical powder, 15-45% filler, 1-10% dispersant and 3-5% surfactant by weight. The filler should be insert and hydrophilic to mix well with water. Normally a mineral such as silica is added to prevent clumping and fusing during grinding (grinding may not be possible with some organisms and aids flowability by minimizing caking during storage, all these are the good quality of products but in India very rare companies are using it.

A dry powder, put into a liquid, must penetrate the surface and overcome surface tension at the liquid-solid interface. The surfactant helps to reduce this tension and allows liquid to displace air around particles. Too much surfactant, however, can cause excessive foaming, preventable to some degree by silicone antifoams and by using low foam surfactants. As a general rule no more than 10ml of foam should remain in a 100ml cylinder 5 min after mixing. Foam should be avoided not only because it is a nuisance, but also because some organisms, such as spores, separate differentially into foam. When rain re-wets sprays dried on foliage, surfactants also act as detergents, increasing the vulnerability of organisms, to wash off. Often one additive acts as both dispersant and surfactant.

Usually, wettable powders tend to mix slowly into water and separate mixers may be needed before filling spray tanks, since tank agitators are often not forceful enough. Powders form unwetted balls, a few of which may persist even after protracted mixing. A filter, preferably of nylon mesh, is essential in the spray line to prevent nozzle blocking. Mixing problems can largely be solved by dry blending a powder with a binder and forming the mix into water-dispersible granules. These break surface tension more easily than powders. They allow high concentrations of organisms, flowing freely with little dust and can be accurately measured by volume like a liquid. However, production costs are high, more agitation is needed for dispersion in cold water in the spray tank, and small particle sizes may be difficult to achieve.

Problems of solid application to water

Initially a product must readily penetrate through the water surface. This has tended to lead to the use of larger droplet sizes with the sprays. Further requirements depend on the type of water body and the habits of the target within. For example, target larvae of mosquitoes and simulate black flies feed by filtering particulate food from the water. Black fly larvae attach themselves by silken threads to a substrate in rapidly flowing water and trap particles moving past. In contrast, mosquito larvae are mobile, living mainly in static or slow flowing waters. These range from large rivers to small streams and from swamps to small bodies like water butts, car tyres and leaf axils. Logistics range from application across large areas without dense population of organism in carrier is very difficult to achieve the target. The response is almost nil in water bodies. To achieve the proper response a large quantity of carrier base inoculum is required.

iv) Liquid Formulations

Many researchers have still doubts about the liquid formulations. Although, in commercial all the fungus and bacteria being well adopted by the users. The survival of fungus in liquid form still questioned by number of field workers but it was noted by the authors, all the fungus like *Beauveria , Verticillium, Trichoderma spp.* etc. survives in liquid formulation more than one year without losing their efficiency. The bacteria like *Bacillus subtilis, Pseudomonas fluorescens, Bacillus thuringiensis*, etc. have more than 2 years shelf life. The main features of the liquid formulation is devoid contaminations.

This range of formulations uses a liquid as carrier, usually water or oil, but solvents are also possible. The commonest are suspension concentrates and emulsions.

The liquid suspension prepared by the authors contains particular organisms about 10-40% , suspender ingredient 1-3%, dispersant 1-5%, surfactant 3-8% and carrier liquid (oil or water) 35-65% by weight. Viscosity should roughly equal the setting rate of the particles. This is achieved by the use of colloidal clays, polysaccharide gums, starch, cellulose or synthetic polymers.

b) Basic Concept of Liquid Formulations

There are four basic functions of formulation. They are:

- ☞ to stabilize the organism during production, 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.

A wide variety of approaches are available to the formulator to achieve these basic functions, ranging of type of liquid suspensions for production , and even incorporation of the agent in a living organism after or before production. The final product developed depends on the following ten features :

i) Stabilization

The longest period of time in the life of a product elapses during storage, i.e. the time between manufacture and eventual use in the field. This period can range from several weeks to years. Organisms are required to remain viable during storage, with minimum loss of potency/ activity and without loss or breakdown of the desired formulation properties.

Couch and Ignoffo (1981) state that a shelf-life of 18 months is the minimum practical for microbial pesticides. It was recorded by authors that Biopesticides and beneficial microorganisms are usually live, albeit often in a dormant stage, so are generally less stable cannot easily be altered chemically to improve stability. The authors treat the liquid formulations for improving stability by appropriate growth conditions during production by appropriate storage prior to formulation by appropriate processing after production. In addition, or alternatively, additives are included to improve stability.

Depending on the organisms involved, different problems must be addressed to improve stability, such as fungi, may be stored as a resting stage, and additives that prevent premature growth needed or those with a nutritive value avoided, also moisture a critical factor. Some

additives that inhibit germination of the organisms after application must be avoided during the preparation of liquid formulations.

ii) Effect of pH on formulations

The pH of a product plays a vital role in liquid inoculum preparations it must be stabilized within certain ranges. Very high and low pH conditions will normally inactivate agents. A buffer therefore is maintained by adding some additives which render the better shelf life in liquid. Maintenance of optimal pH improve shelf-life of some of microorganisms like *Bacillus subtilis, Pseudomonas florescence, Bacillus thuringiensis* and fungus *Trichoderma Viride, Trichoderma harzianium, Verticillium lacanii etc .*

Many commercial products currently available avoid the shelf life problem by recommending immediate use or storage in controlled conditions, e.g. refrigeration. However, this is not practical or possible in our situations. It was noted mostly such type of products are simply broth not proper liquid formulations.

iii) Handling and Application of Liquid inoculum

Liquid ensure that the product is easy to handle and apply. For example, in suspensions thickeners or suspenders added by the authors which help in maintaining even distribution of the organism. The liquid prevent clumping of the organism and ensure its ready resuspension after prolonged storage. Dusts and wettable powders not able to maintain uniformity.

Effective and economic use of a product requires the active ingredient to reach the target; no matter how good the product, if it does not reach the target it will not perform the required function. With liquid insecticides

this problem was partly solved through the development of active ingredients or formulations able to move within the plant-translaminar or systemic action. Whereas in dust powder it restrict entry. It was also reported in the case of solid base carrier that a Biopesticide is broadcasted in the region where the target is located; some will impart on or near the target; much will miss completely. For example, Himel *et.al.*(1990) estimate that as little as 5% of the total active ingredient applied reaches the target site, and Graham-Bryce (1977) estimates that >97% of applied materials are lost to the general environment. Much can be lost through being blown or drifting off target.

iv) Application of Liquid Formulations

As the organism is carried in a liquid, normally oil or water in the case of authors formulation it is water base. Addition of surfactant or oil and emulsifier to water, or use of pure oil, forms drops of more even size than those of water alone, with consequently better controlled spray. Oil is preferred for ultra low-volume (ULV) sprays, water is normally used as the diluent at higher volumes. Liquid formulations contain high count ranges from 10^7 to 10^{10} cells or spores per ml which optimize spray droplet size to maximum coverage of the target and ensures maximum number of effective bacteria/spores for action.

v) Effect of Environment on liquid inoculum

Effect of environment has been discussed by the many workers. It is also true a negative impact was observed in different strains, but all the research was done on the carrier base. The carrier base itself has negative impact on the formulation, but it is least true with the liquid. It was noted that liquid efficiency is almost same in all environment, but efficiency may reduce 10-15% in

different climatic conditions whereas, in the case of carrier is opposite. Normally in RCOF liquid formulation an organism remain active for a time after application, ideally throughout the period that a pest is likely to attack the crop, or in soil throughout the crop cycle. Microbes are inactivated by several environmental factors, including sun, high temperature, humidity, leaf surface exudates and competitors. Also they may be lost physically from the target location by the action of wind, rain or leaching. The relative importance of each of these factors depends on why and where a product is used. For example, inactivation by sunlight is the most important factor reducing persistence of microbes applied to foliage, whereas field temperatures and humidity have relatively little effect except on fungi. In contrast, sunlight is not important when microbial insecticides are applied to pests in stores or for control of soil dwelling pests; here temperature, humidity and soil biota are more likely to affect persistence. Liquid formulations contain additives to protect agents from ravages of the environment.

vi) Effect of sunlight on liquid inoculum

It was always mentioned on the packet of microbial inoculum. It must be kept away from direct sunlight. It is because of the most harmful wavelengths reaching the Earth's surface are between 280 and 320 nm (UVB); 320-400nm (UVA) some are less damaging, but some greater in quantity. However, there may be sensitivities of some organism to wave-lengths outside this range. To counter harmful effects, sunscreens added to a formulation. Sunscreens act by physically reflecting and scattering, or by selectively absorbing radiation, converting short wavelengths to harmless longer ones. However, there is no such type of material is present in the solid base to avoid the effect of sunlight.

viii) Effect of Temperature on liquid inoculum

Temperature is important for the shelf-life of microbial products and it can affect their activity once applied. Temperature optima and limits vary with the microorganism. For example, insect pathogenic viruses are unaffected by 1-10^{0}C and <0^{0}C has little effect. At the other end of the scale, exposure to 60^{0}C for more than 10 min inactivates these viruses. Infection proceeds at field temperatures in the plant growing season, but slows at lower temperatures. Insect pathogenic fungi can be frozen; survival is improved by additives. Although inactivated by high temperatures fungi, if protected by additives, can survive spray drying. Strains used in liquid pest control normally will not grow at 37^{0}c, and able to tolerate temperature upto 45^{0}C for one year or more. Whereas, solid base shelf life is hardly cross 4 months as raise in temperature beyond 40^{0}C start rapid death of organisms.

viii) Effect of humidity/water availability on liquid inoculum

The effect of moisture content on storage stability, some organisms may also have certain moisture needs for activity, which is fulfilled by liquid inoculum but in case of carrier base inoculum bacteria get stressed, when carrier become dry during transport and storage. Antagonistic bacteria used against plant pathogens need the plant surface to be wet in order to establish themselves. Fungal spores normally need high humidity to germinate. These needs be overcome by only liquid formulation as product contains humectant. In general, there is little direct effect of relative humidity on the activity of viruses and spore forming bacteria in liquid form.

ix) Effect of Chemicals at the target substrate on liquid inoculum

Chemicals on or in the target substrate may influence organisms in various ways. These include chemicals present in the soil, as well as plant secretions and chemicals on the phylloplane. For example, on cotton and many other xerophytic plants, high levels of magnesium, calcium and manganese as carbonates and bicarbonates are exuded onto the leaf surface. This can raise the pH of the leaf surface to values as high as 10 or 11. The presence of these ions or the associated pH value, when wetted by dew or water spray, can inhibit or inactivate some organisms. To overcome with these problems only liquid proper formulations take care as it has a buffering property which neutralize the effect of temporary pH effect.

Sometimes, Plant extracts can also harm organisms. Some extracts depress the growth of *B. thuringiensis* (Morris and Moore, 1975). However, since *B. thuringiensis* acts more by poisoning larvae with the crystal toxin than by infection, the bactericidal effect of leaves probably has a relatively minor effect on pest control. Plant extracts have also directly or indirectly inactivated baculo viruses, or at least affected activity. Similarly, chemicals on the insect cuticle may suppress the growth of fungal spores. Added additives overcome from antimicrobial activity. The activity of products on these substrates.

x) Effect of Physical Loss on liquid inoculum

Microorganisms can be lost from target areas through action of wind and rain, physical abrasion or flowing water. Loss varies with type and properties of the formulation. The effects of drop size and liquid properties on spray retention are discussed. Similarly, particle size in

dry solid formulations also influences retention. Fine dusts readily adhere to leaf surfaces. Larger and heavy solid formulations will not easily be washed away in moving water. Effectiveness varies, from the delaying action of water soluble materials such as molasses, to the fastness of materials such as resins, which dry to become insoluble. But it is difficult to spray and retain on targeted diseases. However, liquid formulation contains Stickers which improve adherence of organisms to foliage and persistence during wind and rain. Stickers may double up as thickeners i.e. additives such as gums and molasses to increase spray viscosity and reduce evaporation from drops, or they may double up as phagostimulants such as molasses.

It was evidence from continuous observation made by author that, liquid formulation has no comparison with solid base. However, if solid base inoculum used within one month of manufacture, the results may be at par to the liquid. But as the time period increases in end use, the results of liquid inoculum always superior.

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BIOCONTROL AGENTS & BIOPESTICIDES
Chandra, Greep, Srivathsa

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4:47 PM

LIQUID BIOFERTILIZERS

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CONTENTS

PREFACE

Intensive commercial farming involves excessive use of agricultural land area through multiple cropping and uses of Chemical fertilizers and Pesticides as well. In the recent past good quantity of chemical fertilizers and pesticides has been employed in farmlands. It is feared that practice of uses of Chemical fertilizers and Pesticides continually would result in gradual decrease in quality of soils especially in terms of fertility. Use of agriculturally important microorganisms in different combinations is the only solution for restoration of soils.

Even though for the last three decades biofertilizers are being continuously produced and distributed by private agencies, NGO's, State and Central Government production units. To cope with the rising demands serious efforts are being made for sufficient production and availability of biofertilizers to promote and popularize amongst the farmers by the State as well as Central Government under the National Project. Inspite of these efforts, the consumption of Biofertilizers is not satisfactory. The reason for this may be attributed to non-availability of good carrier materials which brings contamination problems and shorter shelf life.

To cope with this alarming situation the authors initially developed Liquid formulations which ensures quality product in liquid over conventional carrier based Biofertilizers in 1998 giving rise to the new era in Biological input technology. This liquid formulations which facilitates long shelf life up to 2 years, zero contamination, no need of carriers, convenience of handling, storage and transportation, easy quality control, more export potentials and also being preferred by the community of farmers as well as manufacturers.

Since liquid formulations is a growing baby and has very specific characteristics and uniqueness in its production methods so as to disseminate the knowledge and experience by the authors in the form of a booklet on liquid Biofertilizers. Recently without understanding liquid formulations some of the existing biofertilizers production units started to fill the regular broth in bottles and sell to farmers.

We firmly believe that this is the first genuine attempt to bring out a booklet with complete information on Liquid Biofertilizers. The chapters presented in this booklet to understand the concepts and advantages of research data of liquid formulations not only to scientists but also to the producers as well as persons who are dealing with the subject. We are fully aware that there may be some errors at presenting or printing level and on clarity of ideas at specific point which we would like the readers to suggest improvements and suggestions, if any.

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INTRODUCTION

India is a agricultural based country. In order to feed the ever growing populations, India has to increase the per unit area productivity. Further, in order to increase the unit area productivity of agricultural land, the role of different crop nutrients in contributing increased crop yield play a vital role. Among the crop nutrient, nitrogen as well as phosphorus play an important role in increasing the crop productivity. Further, the nitrogenous chemical fertilizers are manufactured industrially using non renewable petroleum products under high temperature and high pressure. Increase in petroleum cost day by day effects the cost of the chemical fertilizers. On the other hand when increased doses of chemical fertilizers are used to increase crop production it causes environmental pollution and toxic to soil as it kills the beneficial microorganisms and so on. However, plant nutrients like N.P. and K are highly essential for plant growth and metabolism. It is also evidence that plant remove much nutrient from the soil in modern intensive cultivation and needs replenishment. Under such conditions Microbial organisms offer good alternative technology to replenish crop nutrients.

In agricultural eco-system, microorganisms are having vital role in fixing / solubilizing / mobilizing / recycling nutrients. These microorganisms occur in soils naturally, but their populations are often scanty. The beneficial microbial population occurs in soils always may not be supporting for higher crop yield. In order to increase the crop yield, the desired microbes from rhizosphere are isolated and artificially cultured in adequate count and mixed with suitable carriers or as it is with some changes in artificial culturing. These are known as Biofertilizers or Microbial inoculants. It includes *Rhizobium, Azotobacter,*

Acetobacter, Azospirillum, Phosphate solubilizing microorganisms, Phosphate mobilizing Microorganisms (VAM), Potash mobilizing bacteria, and Plant growth promoting Rhizomicroorganisms (PGPRM) like *Pseudomonas* and micro-nutrient mobilizing bacteria (MNMB) like *Thiobacillus spp.*

These Biofertilizers are microbial inoculants, which contain living cells of efficient nitrogen fixing microorganisms, which fix atmospheric nitrogen either symbiotically with host plant or free livingly. Phosphate Solubilizing/Mobilizing Microorganisms as well as potassium mobilizers / release or mobilize $P_2 0_5$ and $K_2 0$ from soil. These Biofertilizers are available in markets in carrier based as well as liquid base.

Biofertilizers are inexpensive and eco-friendly. Many State Agricultural Universities, Govt. Agriculture/Forest Departments and a good number of commercial units in private and public sectors are producing and distributing Biofertilizers. In addition to that, Govt. of India has established a Regional Centre of Organic Farming (RCOF) to promote Organic Farming, give technical support and quality control of organic inputs in Karnataka, Tamil Nadu, Lakshadweep, Kerala, Pondicherry. It is observed that, the technology of production of good quality Biofertilizers has been available for several decades but, till date poor quality Biofertilizers are not uncommon in southern India. Therefore, Regional Centre of Organic Farming (RCOF) tried to solve the different problems with Biofertilizer at every stages in production units. Although, primarily the quality of the Biofertilizer produced is the responsibility of the manufacturer, but often, the manufacturers do not follow the proper quality control protocols at various stages of production. In order to acquaint the personnels involved in Biofertilizers production or in quality control

LIQUID BIOFERTILIZERS
Chandra, Greep, Nath, Srivatsa

of Biofertilizers, RCOF, Bangalore, Govt. of India is organizing different types of training course on biofertilzers as well as other organic inputs usage and its quality control aspects.

2. LIQUID BIOFERTILIZERS - A NEW SOLUTION FOR CARRIER BASED BIOFERTILIZERS

Liquid Biofertilizers are the microbial preparations containing specific beneficial microorganisms which are capable of fixing or solubilizing or mobilizing plant nutrients by their biological activity. They are broadly classified into three groups.

1. Nitrogen Fixing Microbes (NFM)
2. Phosphorus Solubilizing Microbes (PSM) and Phosphate Mobilizing Microbes (VAM) and
3. Potash Mobilizing Microbe *(Frateuria aurentia)*

Among the three groups Phosphorus Solubilizing Microbes are used to a larger extent compared to other Biofertilizer. Potash mobilizing bacteria has been recently developed commercially. The authors presently worked on Solubilization or mobilization of Micronutrients by exploiting the biological activity of microbes like

1. Zinc and Sulphur Solubilizing Bacteria (*Thiobacillus* spp.)
2. Manganese solubilizer (*Pencillium citrinum*)

The biggest challenge in the Biofertilizer production units is survival of organisms up to field application. Some beneficial organisms are very effective in the laboratory only but, fail at some stage in the field, even after development of a product for market. Common causes of this demise are poor stability of the product from storage prior to application, too little active material actually reaching to the field and rapid degradation

occurs in the field. Liquid formulation of Biofertilizers plays a vital role in helping to solve these problems and in making an organism effective in the field. However, this must be achieved in a cost-effective manner so that product has to survive commercially.

What is formulation ? Defined collectively, formulation comprises aids to preserving organisms and to delivering them to their target fields and – once there-to improving their activities. A technical concentrate of an organism that has been achieved by a particular process is called as a formulation, or a product, which may be stored and put on sale commercially.

A product often does not fully serve all the requirements of organisms up to the field.

3. TYPES OF INOCULUM

There are varieties of formulation both in liquid and solid. The main types currently used for Biofertilizers have been classified into dry products (dusts, granules and briquettes) and suspensions (oil or water-based and emulsions). A wider range of formulations with additives are available in market

i) Dry inoculum Products

These formulations comprise dusts, granules and briquettes, based on particle or aggregate size. Wettable powders also included in this group, which are formulated as dry powder mixed in water as a carrier, just before use. Dusts based on inert diluents or carriers, normally with low absorbent capacity, these have different particle sizes ranging from 5-20 mm. Particles of <10mm are hazardous when inhaled, but the smaller particles adhere best on the particles. Dusts mainly contain 30% of an organism's in suspension by weight. They are normally

prepared by feeding the organism into an air stream for mixing with a blender. Particle size, bulk density and flow ability are extremely important.

ii) Granules, pellets, capsules and briquettes Inoculum

Granules are discrete masses 5-10mm^3 in size, pellets are >10mm^3, and briquettes are large blocks up to several cubic centimeters; like dusts, these products contain an inert carrier holding the organisms. Carriers include clay minerals, starch polymers and ground plant residues. Choice of carrier depends on absorption (more important for formulating slurries of organisms), hardness, bulk density and product disburse rate in water. Soft carriers, eg. Bentonite, disburse quickly to release the organism. The product can be coated with various materials to slow down or control the rate of release, which also depends on unit size.

The concentration of organisms in granules is 20-30%. There are three types of granular formulations: 1) the organisms are attached to the outer surface of a granular carrier in a rotating drum by a sticker. 2) the organisms are incorporated into a carrier paste or powder which sets as a matrix size being controlled by passing the product through a sieve. 3) the organisms are sprayed onto a rotating granular carrier without a sticker. When the carrier forms a protective coat around a core aggregate of organisms, the unit is termed a capsule.

iii) Wettable powders inoculum

This formulations are predominated among all commercial products and comprise charcoal, lignite, vermiculite powders blended with 3% gum to make them stable during storage on the shelf and readily stick with seeds.

4. LIMITATIONS OF CARRIER-BASED BIOFERTILIZERS

- Shorter Shelf life (3 months).
- Sensitivity to temperature.
- More chance of contamination.
- Lack of identifiable character.
- Lack of instant visual effects on application.
- Restriction on use as a measure of conservation (wood charcoal).
- Unavailability of good carrier in local area.
- Quality inconsistency of carriers.
- Bulk sterilization problem in terms of economic and facilities.
- Poor moisture retention capacity.
- Labour intensive.
- Poor cell protection.
- Even less contamination in packets the enzymatic activity of beneficial bacteria disturbed.
- Problem of proper packing
- High transport cost.
- Takes more time for quality control.
- Dosage contradictory to farmers.
- Very slow adoption by farmers.
- No commercial value.
- Less scope for export.

5. LIQUID FORMULATIONS

Although, India boasts to be the largest Biofertilizers producer in the globe the creditability of Biofertilizers is slow among farmers. Apart from the carrier based inoculums, considering above said limitations in carrier based Biofertilizers Dr. Krishan Chandra, Regional

Director, Regional Centre of Organic Farming (RCOF), Bangalore formulated the liquid based biofertilzers in year 1998 for all types of Biofertilizers and Biopesticides. This technology is an alternative solution to carrier based Biofertilizers, Biopesticides and Biocontrol agents and this is not the usual broth culture from the fermentor or a water suspension of the carrier based Biofertilizers.

Then What is Liquid Biofertilizers ?

Liquid Biofertilizers are special liquid formulation containing not only the desired microorganisms and their nutrients, but also special cell protectants or substances that encourage formation of resting spores or cysts for longer shelf life and tolerance to adverse conditions.

(i) Dormant Aqueous Suspensions

Several current commercial products available in market following the dormant technology. Generally, they are using growth suppressants, contaminant suppressant like Sodium azide, Sodium benzoate, Butanol, Acetone, Fungicides, Insecticides etc. for the long term viability. It is the author's experience, that these formulation in particular is not suitable for organisms whose fast actions are required immediately for short duration crops. It was noted the Rhizobium dormant liquid formulations when used after 8 months reduce the size of nodules and effect nitrogen fixation process. Due to the need of long duration of activation time. It was noted that the indigenous strains nodulate the plants.

In case of Azospirillum, Azotobacter, PSM, it was observed that these bacteria crossed extreme dormant stage. Therefore, when they applied in crops takes prolonged reactivation time. This long time is not desirable for short duration crops. Therefore, the authors adopted a new technique in liquid formulation a process involves arresting of bacteria without preservatives.

(ii) Dormant Oil Suspension

Microorganisms can be suspended in oil at high concentration in various degrees of dehydration and remain viable. This formulation delivers organisms in a physiologically dormant state and does not encourage the growth of contaminants during storage. The bacteria / fungus has been successfully dried by continuous aeration as a suspension in oil to provide inoculants with shelf lives of several years.

The authors noted that oil suspension also have the limitation in reactivating the bacteria / fungus. It generally takes 10 to 20 days to regenerate and the initial requirement of the plant is not fulfilled by them.

The liquid suspension prepared by the authors contains particular microorganisms about 40%, suspender ingredient 1-3%, dispersant 1-5%, surfactant 3-8% and carrier liquid (water) 35-65% by weight. Viscosity roughly equal the setting rate of the particles. This is achieved by the use of colloidal clays, polysaccharide gums, starch, cellulose or synthetic polymers.

6. BASIC CONCEPT OF LIQUID FORMULATIONS

There are four basic characteristics in formulation. They are :

- To stabilize the organism during production, distribution and storage.
- To easily delivered to the field in the most appropriate manner.
- To protect the microorganism from harmful environmental factors at the target site (field), thereby increasing persistence.
- To enhance activity of the organism at the target site by increasing its activity, reproduction, contact and interaction with the target crops.

A different of approaches is available in the liquid formulation to achieve these basic characteristics. Chandra et.al. 1999 have developed the liquid formulation to explain the above said concept. However, the final product developed highly influenced the following factors :-

i) Effect of Temperature on liquid inoculum

Temperature is important for the shelf life of microbial products and it can affect their activity before or after application. Temperature optima and limits vary with the microorganism. Colonization proceeds at field temperatures in the cropping season, but slows at lower temperature. Strains used in liquid formulation normally grow at 37^0c, (Chandra et.al. 1999) and able to tolerate temperature up to 45^0C for two year or more. Whereas, solid base shelf life is hardly up to 3 months as raise in temperature beyond 35^0C and start rapid decline of organisms.

ii) Environmental effect on liquid inoculum

Effect of environment on Biofertilizers has been studied by the many workers. It is also true a negative impact was observed in different strains, but all the research was done on the carrier base Biofertilizers. As per our view the carrier base itself has negative impact on the formulation, but it is least true with the liquid. It was noted that liquid efficiency is almost same in all environment, but efficiency may reduce 20-25% in different climatic conditions in case of solid base. Normally in RCOF liquid formulation of an organism remain active for a time after application, ideally throughout the period of the crop, or in soil throughout the crop cycle. Microbes are inactivated by several environmental factors, like high temperature, humidity, leaf surface exudates and competitors. Also they may be lost physically from the target location by the action

of wind, rain or leaching. The relative importance of each of these factors depends on why and where a product is used. For example, inactivation by sunlight is the most important factor reducing persistence of microbes applied to foliage, whereas field temperatures and humidity have relatively little effect except on fungi. Liquid formulations contain organic additives to protect against from adverse of the environmental conditions.

iii) Effect of humidity/water availability on liquid inoculum

The effect of moisture content on storage stability, some organisms may need moisture for its activity this need is fulfilled by liquid inoculum but in case of carrier base inoculum bacteria get stressed, when carrier become dry during transport and storage. Bacteria used for plant growth need the plant surface to be wet in order to establish them. These needs can be overcome by only liquid formulation as product contains humectant. In general, there is little direct effect of relative humidity on the spore forming bacteria in liquid form.

iv) Effect of sunlight on liquid inoculum

It was always mentioned on the packet of microbial inoculum that it must be kept away from direct sunlight. It is because of the most harmful wavelengths reaching the Earth's surface are between 280 and 320 nm (UVB); 320-400nm (UVA) some are less damaging, but some greater in quantity. However, there may be sensitivities of some organism to wave-lengths outside this range. To counter harmful effects, sunscreens added to a formulation. Sunscreens act by physically reflecting and scattering, or by selectively absorbing radiation, converting short wavelengths to harmless longer ones. However, there is no such type of material is present in the solid base to avoid the effect of sunlight.

v) Effect of pH on liquid inoculum

The pH of a product plays a vital role in liquid inoculum preparations. It must be stabilized within certain ranges. Very high and low pH conditions will normally inactivate agents. A buffer therefore is maintained by adding some additives which render the better shelf life in liquid. Maintenance of optimal pH improve shelf-life of some of microorganisms like *Azospirillum, Azotobacter,* Phosphorus Solubilizing bacteria (PSM), Potash Mobilizing bacteria (KMB) *Frateuria aurentia* (Chandra K. 1998)

vi) Effect of plant exudates on liquid inoculum

The exudates released by the plants may influence liquid biofertilzer's efficiency in various ways. These include chemicals present in the soil, as well as plant secretions and chemicals on the phylloplane. For example, on cotton and many other xerophytic plants, high levels of magnesium, calcium and manganese as carbonates and bicarbonates are exuded onto the leaf surface; this can raise the pH of the leaf surface to values as high as 10 or 11. The presence of these ions or the associated pH value, when wetted by dew or water spray, can inhibit or inactivate some organisms. To overcome with these problems only liquid proper formulations take care as it has a buffering property which neutralize the effect of temporary pH effect, however, Potash Mobilizing bacteria (KMB) *Frateuria aurentia* has no effect as it can tolerate even at pH 11.

vii) Stabilization of liquid inoculum

The longest period of time in the life of a product elapses during storage, i.e. the time between manufacture and eventual use in the field. This period can range from several weeks to years. Organisms are required to remain viable during storage, with minimum loss of potency/

activity and without loss or breakdown of the desired formulation properties. The authors treat the liquid formulations for improving stability by appropriate growth conditions during production by appropriate storage prior to formulation by appropriate processing after production. In addition, or alternatively, additives are included to improve stability. Depending on the organisms involved, different problems must be addressed to improve stability. It is suggested during preparation of base of liquid some additives that inhibit germination of the organisms after application must be avoided.

viii) Application of Liquid Inoculum

As the organism is carried in a liquid, normally oil or water in the case of authors formulation it is water base. Addition of surfactant or oil and emulsifier to water, or use of pure oil, forms drops of more even size than those of water alone, with consequently better controlled spray. Oil is preferred for ultra low-volume (ULV) sprays, water is normally used as the diluent at higher volumes. Liquid formulations contain high count ranges from 10^9 to 10^{10} cells per ml which optimize spray droplet size to maximum coverage of the target and ensures maximum number of effective bacteria for action.

ix) Handling and Application of Liquid Inoculums

Liquid inoculum ensure that the product is easy to handle and apply. For example, in suspensions thickeners or suspenders added by the authors which help in maintaining even distribution of the organism. The liquid prevent clumping of the organism and ensure its ready resuspension after prolonged storage. Dusts and wettable powders not able to maintain uniformity.

It is shown that in the case of Azospirillum the population come down up to 10^5 at six months duration

at room temperature where as in liquid survives up to 2 years and population maintain up to 10^8/ml. Similarly in Azotobacter, KMB, Rhizobium solid maintain the shelf life up to 6 months except PSM which services up to 8 months but in the case of liquid formulations in Azotobacter, PSM and KMB survives up to 2 years followed by Rhizobium only 14 months. This shows the superiority of liquid formulation over carrier base formulations. (Table -1)

Table 1. Shelf life of Liquid Inoculam Vs carrier based Inoculam

Inoculam	Months											
	0	2	4	6	8	10	12	14	16	18	20	24
Azosp(L)	10.0	10.0	10.0	10.0	9.0	9.0	9.0	9.0	8.0	8.0	8.0	8.0
Azosp(S)	9.0	8.0	7.0	5.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Azoto(L)	9.0	9.0	9.0	9.0	9.0	8.0	8.0	8.0	8.0	8.0	7.0	7.0
Azoto(S)	8.0	8.0	7.0	6.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
P.S.M(L)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
P.S.M(S)	9.0	8.0	8.0	8.0	7.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil
K.M.B(L)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	8.0	8.0	8.0	8.0	8.0
K.M.B(S)	8.0	8.0	8.0	7.0	5.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Rhizo(L)	9.0	9.0	9.0	9.0	9.0	9.0	8.0	7.0	5.0	Nil	Nil	Nil
Rhizo(S)	9.0	8.0	8.0	7.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

(Source : chandra et. al. 2004, Biofertilizer vision 2004)

L- Liquid (Liquid formulation without preservative was used)

S-Solid (lignite carrier mesh size 150 micron, presterilized in autoclave used)

7. ADVANTAGES OF LIQUID BIOFERTILIZERS

The advantages of Liquid Biofertilizer over conventional carrier based Biofertilizers are listed below:

Number of advantages noted by the authors from last six years, some are mentioned here :

- Longer shelf life – 12- 24 months.
- No contamination.
- No effect of high temperature.
- No loss of properties due to storage upto 45^0C.
- Greater potentials to fight with native population.
- High populations can be maintained more than 10^9 cells / ml up to 12 months to 24 months.
- Identified by typical fermented smell.
- Cost saving on carrier material, pulverization, neutralization, sterilization, packing and transport.
- Quality control protocols are easy and quick.
- Better survival on seeds and soil.
- No need of running Biofertilizer production units through out the year.
- Very much easy to use by the farmer.
- Dosages is 10 times less than carrier biofertilizers.
- High commercial revenues.
- High export potential.
- Very high enzymatic activity since contamination is nil.

Further, it is very necessary to understand that, "liquid biofertilizers are not merely usual broth cultures from fermenters which are specially prepared with desired agriculturally important micro organisms along with proper nutritional base to facilitate protection of organisms from adverse conditions and resting spore formation or cysts etc". Many researchers still have doubts about the liquid formulations. Although, in commercial all bacterial inoculum being well adopted by the users. It is well proved from multi location trails (Table 18 - 24) and laboratory tests that all the biofertilizers have more then 2 years shelf life.

8. DESCRIPTION AND CHARACTERISTICS OF DIFFERENT LIQUID BIOFERTILIZERS

a) Rhizobium

This belongs to bacteria group and the classical example of symbiotic nitrogen fixation. The bacteria intact the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is readily utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. The site of symbiosis is within the root nodules. It has been estimated that 40 - 250kg N/ha/year could be fixed by different legume crops by the microbial activities of *Rhizobium.*

It was reported by Chandra et al. 1995. The percentage of nodules occupied, nodules dry weight, plant dry weight and the grain yield per plant by the multistrain inoculant was highly promising Table- 2 shows the N Fixation rates.

Table- 2 Quantity of biological N fixed by Liquid *Rhizobium* in different crops

Sl. No.	Host Group	*Rhizobium* Species	Crops	N fix kg/ha kg/ha
1	Pea group	*Rhizobium leguminosarum*	Green pea, Lentil	62-132
2	Soybean group	*R. japonicum*	Soybean	57-105
3	Lupini grp. group	*R. lupini orinthopus*	Lupinus,	70-90
4	Alfalfa grp. group	*R. meliloti Medicago Trigonella*	Melilotus,	100-150
5	Beans group	*R. phaseoli*	Phaseoli	80-110
6	Clover group	*R. trifolii*	Trifolium	130
7	Cowpea group	*R. species*	Moong, Redgram, Cowpea, Groundnut	57-105
8	Cicer group	*R. species*	Bengal gram	75-117

(i) Physical features of Liquid *Rhizobium*.

- ☞ Dull white in colour
- ☞ No bad smell
- ☞ No foam formation, pH 6.8 to 7.5

(ii) Use of Fungicides / Pesticides / Herbicides with *Liquid Rhizobium inoculum*

Spray of any of these chemicals do not have any effect on *Rhizobium* nodulation and nitrogen fixation. It was reported by Chandra and Karmakar, 1996 that Seed dressing fungicides such as Thiram and mercury containing fungicides do have some adverse effect on *Rhizobium* survivability, this can be overcome by doubling the doze of *Rhizobium* culture. But other fungicides such

as Dithane M-45 and Bevistin do not have any adverse effect on *Rhizobium.* The minimum tolerance doses of five different fungicides, Corbandazium, Dithane M-45, Dithane Z-78, Topsin and ceresin were tested for *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* locally isolated from *Glycine max* and *Pisum sativum.* Amongst these fungicides, ceresin was found to be more toxic to *Bradyrhizobium japonicum* and *Rhizobium leguminosarum*, since the minimum tolerance dilution was 10^7, whereas minimum dilution of Dithane Z-78 for soybean 10^3; pea 10^4; Dithane M-45 for soybean 10^4; pea 10^5; Topsin for pea 10^6; soybean 10^7 and Corbandazium for pea and soybean was 10^6. Efficiency of nodulation of minimum dilution of these fungicides were also tested on host plants. Maximum number of effective nodules were recorded in pea for Dithane M-45 at 10^5 dilution whereas in soybean of Topsin at 10^7 (Table 3-4).

Table - 3 Effect of some pesticide on the biofertilizers when treated together and showed within 4 hours in the field.

COMMON NAMES	FORMULATION	TRADE NAME	EFFECT ON BIOFERTILIZERS
A. CHLORINATED HYDROCARBONS			
Aldrin	Dust 2-4%	Aldrin, Addred	
	WDP 12-40%	Hexamar Aldrin	M
	EC 30%	Aldrin sandoz	
Chlordane	EC 75%	Hexamar, Chlorodane	
	Dust 5-10%	Mico Chlorodane lethal	M
		Rock chloridane	
Dieldrin	Dust 2%	Dieldrin, Dieldrex	
	WD 50%	Hexamar Dieldrin, Mico	
	Solution 20-30%	Dieldrin, Diethol rock	M
Heptachlor	EC 20%	Micro Heptachlor	
	WDP 25%	Hexamas Heptachlor	
	Dust 3-10%		

Lindane	WDP 6.5-25%	Lindane Hexamar	
	EC 20%	Lindane Krishi	M
	Granule 6-10%	Lindane	
Toxophene	Dust 5-10%	Anatox Helitox	
	EC 20%		M
	WDP 25%		
Endosulfan	EC 35%	Thiodan, Hexasulfan	
		Hildan, Coroban	M
Dicofal	EC 18%	Kelthane	N
ORGANO PHOSPHOROUS			
Diazinon	WDP 10%	Banzann 20 EC	N
	EC 80%		
	Granule 10%		
Dimethoate	EC 40%	Rogor, Cygos, Hexagor	N
Parathion	Dust 1-2%	Famax, Folidol, Ekatox	M
	Thiophos		
	EC 50%	Mico Parthion, Paratex	
Fenitrothion	EC 50-100%	Sumithion, Accothion	M
	Dust 2-5%	Folithion	
Malathion	EC 100%	Mico Malathion	
	Dust 2-5%	Malathion Sandox	
	Solution 50-100%	Meltex, Malmar	
Phosphamidor	EC 100%		
	Dust 3%	Dimecron	N
	Dust 6%		
Phorate	Granule 10%		
	EC 100%	Thimet	M
	Dust 6%		
Carbofuran	Granule 3%	Furadon	N
Menzon	EC 40%	Sofos	M
	WDP 75%		
Thiometon	EC 25%	Ekatin, Hexatin	M
Methyl demiton	EC 5%	Metazytox	N
Trichfon	Dust 5%	Diptered	M
Monocrotophs	EC 40%	Murvacron, Azodrin	N
Quinalphos	EC 25%		
	Granule 5%	Ekalux	M
Carbaryl	Dust 5%	Sevin, Hexavin	
	Gr 6-19%		
	WDP 50%	Carboryl Sandox	
Phosalone	Dust%	Zolone	M
	EC 35%		
Chloropyriphos	EC 20%	Dursban, Coroban	M
Methyl Parathion	-	Matacid	N

M-Medium, N- No effect, T.H, - Highly Toxic

Table - 4 Effect on Biofertilizers on different fungicides

COMMON NAMES	FORMULATION	TRADE NAME	EFFECT ON BIOFERTILIZERS
COPPER GROUP			
Copper Oxychloride	WDP 50%	Blitox, Fytolan, Cupramar Copex Cupravit, Blimix Arysycom.	M
Cuprous Oxide	-	Copper sandox dust Copesan, Fungimar Fytomix	M
Captain	WDP 35%	Captan	N
ORGANIC FUNGICIDES			
Femam	WDP 50-80%	Ferbam, Hexafem	
Manccozeba	WDP 50%	Dithane M-45	M
Thiram	WDP 75%	Thiram, Hexathion Thiride	N
Zineb	WDP 75%	Dithane Z-78, Hexathane	N
Ziram	WDP 80%	Zirade, Cuman Hexazir, Ziriate	N
Ediphe-nophos	EC 50%	Hinosan	M
Difoloton	WDP 50%	Difoloton	N
Carbendazim	WDP 505	Bavistin	N
PCNB	WDP 75%	Brassicol	N
SULPHUR GROUP			
Wettable sulphur	WDP 75-80%	Thiovit micrusul Sulkul spitox, Sultaf Hexasal, Solfar	M
Sulphur dust	Dust 70%	Sulphur dust	M
ORGANO MERCURIALS			
Phenyl Mercuricatate	WDP 25%	Cereran dry	H.T
Methozy Ethylchloride	WDP 25%	Ceresan wet	M
Phenymercuric Accutate plus Ethyl mercury	Dust 1%	Agrosan G.N. hexason	H.T
Mercury Chloride	WDP 3-6%	Tafsan, Agallol, Tilex	H.T
Caroxin	75% WP	Vitavax 75 WP	N

M-Medium, N- No effect, T.H, - Highly Toxic

iii) Use of Chemical Fertilizer along with Liquid *Rhizobium*

Most pulses and legume oil seed crops require 30 - 40 kg N and 40 Kg, Phosphorus/ha. for better growth and yield. If crop is provided chemical N_2 only 5 - 10 kg /ha as a basal dose; is essential to fulfill the initial nitrogen requirement of crop up to 20 - 25 day i.e. till the *Rhizobium* nodules start their functioning.

Phosphorus is essential for better *Rhizobium* inoculation so 30 - 40 kg of phosphorus/ha is required as basal dose, if PSM is used the dose of chemical Phosphorus can be 15 - 20 Kg/ha. Potassium is also essential for better *Rhizobium* inoculation so 30 - 40 kg of Potassium /ha is required as basal dose, if KMB is used the dose of Potassium chemical can be 10 - 15 Kg/ha.

(iv) Use of sticky materials for seed treatment

The sticky materials is required to increase the no of bacteria on the seeds for maximum nitrogen fixation. The germination and nodulation tests were performed on the pellated seeds by Chandra, et. al.1995. and results showed that although the sugar and molasses initially bound less inoculant and lime to the seed, but the number of surviving rhizobia was similar to that obtained by the gum arabic treatment after storage at 27.5^0 C for five days for fast growing rhizobia. The inoculated pellated seeds of slow growing rhizobia were also tested for germination and nodulation in acidic soil which showed promising results. Table- 5 shows the amount of Inoculant, sticker solution and lime to be used in acidic soil when pH is below 6.0.

Table - 5 Amount of Inoculant, sticker solution and lime to pellet legume seeds in acidic soil

Legume	Sticker solution (ml/kg of seed)	Inoculant (g/kg of seed)	Lime g/kg of seed	No. of Rhizobia/ seed
White clover	25	10	40	10^2
Alfalfa	20	10	40	10^3
Stylo	20	10	40	10^3
Crown vetch	20	10	40	10^3
Sirafro	20	10	35	10^3
Greengram	20	10	35	10^4
Blackgram	20	10	35	10^4
Pea	20	10	25	10^4
Pigeon pea	20	10	25	10^4
Winged bean	20	10	25	10^4
Cowpea	15	10	25	10^5
Ricebean	15	10	25	10^5
Soyabean	15	10	25	10^5
Seem	15	10	25	10^5
French bean	15	10	25	10^5
Chickpea	15	10	25	10^5
Peanut	15	10	25	10^5
Broadbean	15	10	25	10^5
Limabean	15	10	20	10^6
Jackbean	15	10	20	10^6
Velvetbean	15	10	20	10^6
Swordbean	15	10	20	10^6

Note: i) legume seeds are arranged in ascending order of size
ii) Inoculant of $1x10^8$ cells/gram as per ISI specification.
(Source: Chandra *et. al.* 1995)

As per new BIS the inoculants are allowed $1x10^7$ cells per gram, so the required inoculum is 100 gm/kg of seeds.

In the case of liquid inoculant, the sticker solution is not required, lime can also be omitted if the acid tolerant strains were used. The recommended dose of liquid *Rhizobium*, PSM and KMB as all the bacteria should be mixed properly before use. (Table 6).

Table - 6 Amount of Liquid Inoculant and lime to pellet legume seeds

Legume	Inoculant((ml/kg of seed)	Lime g/kg of seed	No. of Rhizobia / seed
White clover	1	40	10^3
Alfalfa	1	40	10^4
Intortum	1	40	10^4
Stylo	1	40	10^4
Crown vetch	1	40	10^4
Sirafro	1	35	10^4
Greengram	1	35	10^5
Blackgram	1	35	10^5
Pea	1	25	10^5
Pigeon pea	1	25	10^5
Winged bean	1	25	10^5
Cowpea	1	25	10^6
Ricebean	1	25	10^6
Soyabean	1	25	10^6
Seem	1	25	10^6
French bean	1	25	10^6
Chickpea	1	25	10^6
Peanut	1	25	10^6
Broadbean	1	25	10^6
Limabean		20	10^7
Jackbean	1	20	10^7
Velvetbean	1	20	10^7
Swordbean	1	20	10^7

Note: i) legume seeds are arranged in ascending order of size.

ii) Inoculant of $1x10^9$ cells/ml as per concentration.

(b) *Azospirillum*

It belongs to bacteria and is known to fix considerable quantity of nitrogen in range of 20-40 Kg N/ha in the rhizosphere in non-leguminous plants such as cereals, millets, oilseeds, cotton etc. The efficiency of *Azospirillum* as a biofertilizers has increased because of its ability of inducing abundant roots in several plants like rice, millets and oilseeds even in upland conditions. Considerable quantity of nitrogen fertilizer 25-30% can be saved by the use of *Azospirillum* inoculant. The genus *Azospirillum* has three species viz. *A. lipoferum*, *A. brasilense* and *A. amazonense*. These species have been commercially exploited for the use of nitrogen supplying biofertilizer. One of the characteristics of *Azospirillum* is its ability to reduce nitrate and denitrify. Both *A. lipoferum* and *A. barasilense* may comprise of strains which can actively or weakly denitrify or reduce nitrate to nitrite and therefore, for inoculation preparation, it would be necessary to select strains which do not possess these characteristics. *Azospirillum lipoferum* present in the roots of some tropical forage grasses such as Digitaria, Panicum, Brachiaria, Maize, Sorghum, Wheat and rye.

(i) Physical Features of Liquid *Azospirillum*

- The colour of the liquid may be blue or dull white.
- Bad odors confirms improper liquid may be broth.
- Production of yellow gummy colour materials confirms the quality product.
- Acidic pH always confirms no *Azospirillum* bacteria present in liquid.

Table - 7 shows the occurrence of *Azospirillum* and N_2 fixing capacity bacteria in the roots of several plants and the amount of N_2 fixed by them.

Table - 7 Occurrence of *Azospirillum* and N_2 fixing capacity bacteria in the roots of several plants and the amount of N_2 fixed by them

Plant	mgN_2 fixed/g of substrate
Oryza sativa (Paddy)	28
Sorghum bicolor (Sorghum)	20
Zea mays (Maize)	20
Panicum Sp.	24
Cynodon dactylon	36
Setaria sp.	12
Amarnanthus spinosa	16

(ii) Production of Growth hormones:

Azospirillum cultures synthesize considerable amount of biologically active substances like vitamins, nicotinic acid, inodle acetic acid gibberellins. All these hormones/ chemicals helps plants in better germination, early emergence, better root development.

(iii) Role of Liquid *Azospirillum* under field conditions

- Stimulates growth and produces green colour characteristics of a healthy plant.
- Aids utilization of potash, phosphorus and other nutrients.
- Encourages plumpness and succulence of fruits and grains and increases protein percentage.

(iv) Signs of non functioning of *Azospirillum* in the field

- No growth Promotion activity.

- Yellowish green colour of leaves. Indicate no Fixation of Nitrogen.

(v) Effect of liquid *Azospirillum* when used as Foliar spray

Recently, Dr. S. Ramarethinam, T. Stanes and Co. Ltd, Coimbatore has conducted the experiment on chilly crop in the year 2004. The liquid inoculum provided by RCOF, Bangalore used as a foliar spray and findings as follows :

"It is now felt that it may not be improper to conclude that the entry of *Azospirillum* in this study may be effected through the process of preferential permeability by the cell wall of the leaf tissues like paranchymatus spongy mesophyll cells. The existence of *Azospirilum* in the entire plant system indicate that having got an entry into the laminar cells without any resistance, *Azospirillum* might have spread in to the plant system either through the process of preferential permeability or through the continuity of the protoplasm that exist between the cells in the plant system. This study reflect the other face of *Azospirillum* has only been used as a soil application and that too for Nitrogen fixation". The table - 8 shows the efficiency of *Azospirillum* spray.

Table - 8 Performance of Liquid Azospirillum on Chillies

Treatment	Plant height (cm)	Photosynthetic Rate (PR) (μ mol m-2S-1)	Stomatal Frequency	Leaf Area cm^2	Yield in dry weight
Control	63.7	13.87	13.87	28.3	138.24
Foliar Spray(10ml)	73.4	12.77	12.77	39.7	172.56

(Source: Dr. S. Ramarethinam et.al. 2004, details publication elsewhere)

(c) *Azotobacter*

It is the most important and well known free living nitrogen fixing aerobic bacterium. It is used as a Biofertilizer for all non leguminous plants especially rice, cotton, vegetables etc. Azotobacter cells are not present on the rhizoplane but are abundant in the rhizosphere region. The lack of organic matter in the soil is a limiting factor in the proliferation of *Azotobacter* in the soil.

Field experiments were conducted in 1992, 1993 and 1994 during the pre-kharif wet seasons to find out the influence on rice grain yield by the combined use of N-fixing organisms and inorganic nitrogen fertilizer (Chandra et.al 1995) recorded increase in yield.

(i) Physical Features of Liquid *Azotobacter*

The pigmentation is produced by *Azotobacter* in older culture is melanin due to oxidation of tyrosine by tyrosinase an enzyme which has copper. The colour can be noted only in liquid forms. Some of the pigmentation are described below-

A. chroococcum **:** Produces brown-black pigmentation in liquid inoculum.

A. beijerinchii **:** Produces yellow-light brown pigmentation in liquid inoculum.

A. vinelandii **:** Produces green fluorescent pigmentation in liquid inoculum.

A. paspali **:** Produces green fluorescent pigmentation in liquid inoculum.

A. macrocytogenes **:** Produces ,pink pigmentation in liquid inoculum.

A. insignis **:** Produces less, gum less ,grayish-blue pigmentation in liquid inoculum.

A. agilies **:** Produces green-fluorescent pigmentation in liquid inoculum.

(ii) Role of Liquid Azotobacter in Tissue Culture

The study was conducted by Dr. Senthil *et.al.*(2004) on sugarcane variety CO 86032 in Tissue Culture Laboratories of Rajashree Sugars & Chemicals Ltd, Varadaraj Nagar, Theni, Tamil Nadu. The liquid bioinoculants were provided by Dr. Krishan Chandra, Regional Director, RCOF, Bangalore to evaluate their growth promoting effects on sugarcane micropropagation. He recorded Biometric Observations like Plant height, leaf length, width, root length, no. of roots. Chemical Parameters-Protein, Carbohydrates, N, P, K, total biomass and concluded as follows :

1. The performance of *Azotobacter* liquid inoculant was comparatively better than all the treatments in 10% MS medium followed by *Azospirillum.*
2. the performance of *Azotobacter* liquid inoculant was comparatively better than all the treatments followed by *Azospirillum* for the growth of polybag sugarcane seedlings.

(iii) Role of Liquid *Azotobacter* as a biocontrol agents

Azotobacter have been found to produce some antifungal substance which inhibits the growth of some soil fungi like *Aspergillus, Fusarium, Curvularia, Alternaria, Helminthosporium, Fusarium* etc.

(d) *Acetobacter* **:** This is a sacharophilic bacteria and will associate with sugarcane, sweet potato and sweet sorghum and fixes 30 kgs / N/ha / year. Mainly this bacterium is being commercialised for sugarcane crop. It is known to increase cane yield 10-20 t/acre and sugar content by 10-15 percent.

(i) Effect of Liquid *Actobacter diazotrophicus* for sugarcane

In South India use of *Azospirillum* and Phospho-bacterium for the cash crop sugarcane has been a regular practice for the past few years with a saving of nearly 20% Chemical nitrogen and Phosphate applications. Now, it has been reported that a bacteria *Acetobacter diazotrophicus* present in sugarcane stem, leaves, soils have a capacity to fix up to 300 kgs of nitrogen. This bacteria first reported in Brazil where the farmers cultivate sugarcane in very poor sub-soil fertilized with Phosphate, Potassium and micro elements alone, could produce yield for three consecutive harvests, without any nitrogen fertilizer. They have recorded yield 182 to 244 tones per ha. This leads to the assumption that active nitrogen fixing bacteria must be associated within the plant. The Scientists of Brazil & Australia revealed after their extensive work, the presence of bacteria like *Acetobacter diazotrophicus, Herbaspirillum seropedicae* and *Herbaspirillum rubisubalbicans* in leaves, stems and roots in large numbers are responsible for N-fixation. In Orissa, a bacterium was isolated by Chandra (1997) and nitrogen fixation recorded up to 100kg. N/ha. The specialty of this bacterium is that it tolerate Inorganic nitrogen up to 200 kgs N/ha and the pH increased above 6.0 the N-fixation reduced tremendously.. The other features of this bacterium is that it increases the sugar content by 2 – 5%, depends upon the varieties. The varieties of sugarcane are recommended CB 47-89, CB 45-3, IA 56-79, IAC 52-50, SP 70-1142, SP 71-799 Korajatae. The maximum nitrogen fixation up to 200 kg N/ha was recorded in variety CB 45-3 and Krajatau.

An field trial conducted by R. Muthukumaraswamy et al at Main Biocontrol Research Laboratory, Chengalpattu,

Tamilnadu in sugarcane varity Co. V. 92102 at Thiruttani shows 25% increase of cane yield in *Frateuria aurentia* applied field over control.

(ii) Role of *Acetobacter pasteurianus* for sulfur

Sulfur is important in plant synthesis like nitrogen, sulfur is a constituent of several amino acids, including methionine and cystine, which are essential components of plant and animal proteins. If methionine can be considered as standard, essential amino acid that is present in plants and animals, it is also evident that nitrogen-to-sulfur ratio in good quality protein should be around 15:1. Sulfur acts more like nitrogen than any other essential plant nutrients. Indeed, sulfur deficiency symptoms in plants closely resemble nitrogen deficiency symptoms.

Moreover, leaching losses of sulfur from the soil are very much like those of nitrogen caused by percolating waters through light-textured sandy and salty soils. Thus there is need for annual supplies of sulfur to meet the requirements of growing plants. Although, we are accustomed to use of sulfur in the form of fertilizers but number of such farmers are very less due to its cost factors etc.

To cope with this grim problem Chandra (1998) has isolated/developed the bacteria named *Acetobacter pasturianus* at R.B.D.C., Bhubaneswar which secretes the sulfur and make it available to the plants in usable form. It was estimated that about 70-90% of total surface soil sulfur is found in organic matters. The remainder of the sulfur is present as sulfides and as soluble and insoluble sulfates. Usually, only 5 - 10% of the sulfates are found in the surface furrow slice of humid region, cultivated soils. In humid region soils most of the sulfates are formed

12 - 14 inches deep and are associated with Oxides of Iron and aluminum, especially in acid soils having Kalononitic type clay. These bacteria helps in converting this non-usable form to usable form. It was experienced that use of 200ml/acre bacteria influenced in level of sulfur in crops like vegetable, cabbage, turnip, onion, cotton, fruits, oilseeds, spices etc.

(e) Phosphorus Solubilizing Microorganisms (PSM)

The most important aspects of the phosphorus cycle are microbial mineralization, solubilization and mobilization, besides chemical fixation of phosphorus in soil. The mineralization of organic phosphorus which is left over in the soil after harvesting, or added as plant or animal residues to soil, takes place through enzymatic activity of microorganisms.

Phosphorus solubilizing bacteria and fungi play an important role in converting insoluble phosphatic compound such as rock phosphate, bone meal and basic slag and particularly the chemically fixed soil phosphorus into available form. These special types of microorganisms are termed phosphate solubilizing microorganisms (PSM).

PSM include different groups of microorganisms such as bacteria and fungi which convert insoluble phosphatic compounds and fixed chemical fertilizers into soluble form. The species of

1. *Pseudomonas*
2. *Micrococcus*
3. *Bacillus*
4. *Flavobacterium*
5. *Penicillium*
6. *Fusarium*

7. *Sclerotium*
8. *Aspergillus*

And others have been reported to be active in bio-conversion.

Such bacteria and fungi can grow in media where $Ca_3(PO_4)_2$, $FePO_4$, $AlPO_4$, apatite, bonemeal, rock phosphate or similar insoluble phosphate compounds are the sole source of phosphate. Such organisms not only assimilate phosphorus but also cause a large amount of soluble phosphate to be released in excess of their own requirements.

Several rock phosphate dissolving bacteria, fungi, yeasts and actinomycetes were isolated from soil samples collected from rock phosphate deposits and rhizosphere soils of different legume crops. Some of the isolates solubilized and made available phosphorus to crop from rock phosphate and many isolates solubilized a very high quantity of tricalcium phosphate.

The most efficient bacterial isolates were identified as

Pseudomonas striata
Pseudomonas rathonis
Bacillus polymyxa
B. megatherium

And fungal isolates such as

Aspergillus awamori
Penicillium digitatum
Aspergillus niger
Schwanniomyces occidentalis

These efficient microorganisms have shown consistency in their capability to solubilize chemically fixed soil phosphorus and rock phosphate from different sources. PSM were found to mineralize organic phosphorus into soluble form due to enzymatic activity. The bacteria saves P_2O_5 up to 30-50 kg/ha.

(i) Physical Features of Liquid PSM

- Solution appears always turbid.
- Colour may be creamy depends upon strain.
- Pleasant but some time mild smell.
- pH 4.5 or buffer but buffer 6.5-7.0 ensures more than 2 years expiry.

(ii) Effect of Liquid PSM under field conditions

- Encourages early root development.
- PSM produced organic acids like malic, succinic, fumaric, citric, tartaric and alpha ketoglutaric acid which hastens the maturity and thereby increases the ratio of grain to straw as well as the total yield.
- Increases the compatibility of other beneficial microbes with plants.
- Stimulates formation of fats, convertible starches and healthy seeds.
- Helps rapid cell development in plants and consequently increases resistance towards diseases.
- Increase micro nutrients in soil like Mn, Mg, Fe, Mo, B, Zn, Cu etc. depends upon the nutrient presents in non available form.

(f) PHOSPHATE MOBILIZING MICROORGANISMS (PMM)

Some of the soil borne fungi are capable to mobilize / make it available from the unmobile form of phosphorous

by its hyphal structures called as phosphate mobilizing microorganisms. This soil microbes has mutualistic association with plants. It can associate with all the crop plants except family Brasicaceae. This fungus mobilize besides phosphorus zinc and sulphur.

The term mycorrhizae literally meaning "fungus root' to denote the association between certain soil fungi and plant roots where the relationship not over pathogenic. There are two primary types of mycorrhizal fungal associations with plant roots the ectomycorrhiza, endomycorrhiza. and ectendomycorrhiza. Mycorrhizal plants increases the surface area of the root system for better absorption of nutrients from soil especially when the soil are deficient in phosphorus.

The later one endomycorrhizae are known as Arbuscular mycorrhiza which possess special structures known as vesicles and arbuscules, the latter helping in the transfer of nutrients from soil into root system. These fungi are classified on the basis of their spore morphology in seven genera namely *Glomus, Gigaspora, Acaulospora, Sclerocystis, Scutellospora, Entrophospara, Archeospora as para Glomus. The* role of mycorrhizas in improving phosphate uptake and plant growth is now widely recognized, Arbuscular mycorrhizas (AM), the most common type, are formed in all agricultural crops except sugarbeet and brassicas. The symbiotic fungi improve plant productivity under certain conditions, especially where soil phosphate is a limiting factor, and they may have potential of assuring plant production with a minimum input of fertilizers. Advances in biotechnology have promoted experimentation in artificial inoculation of plants with AM. Trials have mainly involved broadcasting and raking-in inocula over plant-growing substrates rather than application of inocula to seed before sowing.

Since large-scale production of AM in axenic culture is not yet attained, inocula have been produced in pot cultures or in small field plots on plants grown under carefully controlled conditions, to avoid contamination by plant pathogens. Such inocula have comprised infected roots or spores and hyphae trapped in soil, peat and clay carriers.

Introduction of AM into field on a large scale has tended to use inoculum at excessively high and impractical rates in order to guarantee rapid infection by the introduced fungi. Application of inocula directly to seed may reduce the amounts of inocula required. Experimental inoculation has involved coating spores, mycelium and infected root fragments, wet-sieved from soil, onto seeds using methyl cellulose as sticker.

(i) Mechanism of improved plant growth due to AMF application

- Nutrient uptake
- Production of growth promoting substances
- Beneficial interactions between soil microorganisms
- Drought tolerance
- Disease resistance

(ii) Advantages of AMF Application in Microprobagated plants

- Corrects the physiological defects of tissue culture plantlets
- Enhances the absorption and transport of water and nutrients
- Hormonal effects on shoot and root development
- Resistance to pathogenic infection

- Better survival and establishment
- Reduces the hardening period
- Healthy and vigorous plantlets

(iii) Constraints in AM Fungi Efficacy

- Obligate symbionts – needs host for multiplication
- Cannot be cultured on synthetic media in laboratory
- Problems in mass cultures
- Difficult to apply
- Variation between plant genotypes in mycorrhizal
- Affected by pesticides especially fungicide
- Variation in soil nutrient status
- Competition with indigenous population of mycorrhizal fungi
- Phosphorus inhibition of mycorrhizal development
- Slow growth of mycorrhizal fungi and slow development of mycorrhizal association and
- Incompatibilities with established cultural practices

(iv) Benefits of AM Fungi

- Improves plant growth is attributed to better uptake of nutrients like P, Zn, Cu etc.
- This is mainly because of increased surface area of absorption of nutrients by the hyphae. Mycorrhizal plant roots can extent up to 8 cm.
- Because of the difficulty in mass producing AM fungi, the best way to utilize AM fungi for crop

production would be to concentrate on crops which are normally grown on nursery beds or root trainers or poly bags, where they could easily be inoculated with desired AM fungi and transplanted to the field.

- ☞ Nearly 25 to 50% of phosphatic fertilizers can be saved through inoculation with efficient AM fungi.

(g) A New Biofertilizer to reduce Potassium application

(i) Potash Mobilizing bacteria (KMB)

Recently, apart from regular biofertilizers like *Rhizobium, Azospirillum, Azotobacter, Acetobacter* and Phosphate Solubilizing / Mobilizing and Phosphorus to meet N&P nutrition. Potassium mobilizing microorganisms was isolated and authenticated/developed from Banana rhizosphere by Dr. Krishan Chandra, 1998 which is having the ability to mobilize the elementary or mixture of potassium (Chandra and Singh 1999; Chandra et.al 2001; 2002). It can be easily absorbed by plants. By using this new Biofertilizer, 50 to 60% of potash chemical fertilizers cost could be reduced. The bacteria known as ***"Frateuria aurentia"*** *(species conformation by IMTECH Chandigarh)* belonging to the family Pseudomonaceae. This bacteria having the extra ability to mobilize K in all types of soils especially, low K content soils, pH 5-11 and survival temperature is 42^{0}c. There was no antagonistic/inhibiter effect noticed (Chandra 1998, 2000, 2004).

Table : 9 shows the physical parameters and K_20 release.

Table - 9 Performance of Potash Mobilizing Bacteria in different soil conditions at different locations in Orissa State.

Name of place from where soil was collected	Initial			10 days inoculation			20 days inoculation		
	pH (1:2)	Ecc (ds/m)	AV, K_2O Kg/ha	pH (1:2)	Ecc (ds/m)	AV, K_2O Kg/ha	pH (1:2)	Ecc (ds/m)	AV, K_2O
Phulbani (Red soil)	6.94	0.04	504.0	7.0	0.09	524.7	6.94	0.07	510.7
Aska (Alluvial soil)	6.71	0.06	73.9	7.48	0.07	140.4	7.33	0.07	120.9
Bhavanipatna (Black soil)	5.50	0.04	208.3	5.53	0.08	215.0	6.66	0.08	275.5
Keonjhar (Black soil)	8.09	0.03	215.0	8.07	0.07	295.7	8.10	0.09	288.9

(Source - M.Rath, N.K.Pradhan, A. Mishra and K.Chandra (2002) in International Seminar. On Traditinal knowledge – Health and Environment).

This potash mobilizing biofertilizers can be applied along with *Rhizobium, Azospirilum, Azotobacter, Acetobacter, PSM* etc. Field result, reveals that by applying this K mobilizers, 15-25% yield can be enhanced. Bacteria can be applied for all types of crops at the rate of 200 ml per acre for agriculture crops for perennial crop 400 ml / acre after mixing in 200-500 kgs of matured FYM before sowing or in furrows (Chandra et.al.2004). Dr. D.Clarson, 2004 published the details about Potash Mobilizing bacteria (KMB). Table – 10 shows the yield increase in yam & Tapioca by potassium mobilizer when it applied along with other Biofertilizers and chemical fertilizers.

Table - 10 Effect of Potassium Mobilizer on the growth and yield of Yam and Tapioca.

Crops	Treatment	Treatment	Yield/ha (q)
Yam	C1T1	BF	257
	C1T2	PC	255
	C1T3	BF+PC	248
	C1T4	CF+BF+PC	352
	C1T5	CF	349
	C1T6	CF+PC	343
	C1T7	FYM	245
TAPIOCA	C2T1	BF	242
	C2T2	PC	246
	C2T3	BF+PC	248
	C2T4	CF+BF+PC	302
	C2T5	CF	283
	C2T6	CF+PC	289
	C2T7	FYM	238

BF – Biofertilizer, *Azospirillum* + *Azotobacter*

PC – Potash culture CF – Chemical fertilizer (N:P:K – 80:60:100 /ha)

FYM – Farm yard manure (1kg per pit common to all)

(Source- Dr. Sabyasachi Rath, ADR, RRTTS, Semiliguda, Koraput, Orrisa).

Uptake of Potash by different plants with the use of potash mobilizing bacteria *(Frateuria aurentia)* was studied by Bismitha et. al. Department of Microbiology, O.U.A.T., Bhubaneswar in 2001.

(ii) Effect of Liquid Potassium mobilizer with AM

A experiment was conducted by Greep et.al. (2005) to evaluate the effect of different liquid *Azotobacter,* Phosphate solubilizing organisms. Arbuscular mycorrhae (soil base) and Potassium mobilizing bacteria as individual as well as in combinations along with compost and phosphocompost in capsicum, Hangarian yellow verity shows the better results when compared to the controls. (Plain soil). Further the phospho compost applied field shows pronouncing results when compared to

compost applied field in all the parameters studied. In growth parameters Potassium mobilizing bacteria *(Frateuria aurentia)* enriched Phosphocompost shows the highest results next to all the biofertilizers applied together. This may be due to the mobilization of K as well as release of growth hormones by the K-mobilizer and uptake of potassium also significantly increased in the plants. (Table 11-12).

Table 11 - Effect of Liquid biofertilizers along with compost from Ayurvedic Herbal waste in Capsicum at 30 days.

Treatments	Length. (cm)		Dry weight (g)		No of branches	No of flowers	No of fruits
	Shoot	Root	Shoot	Root			
Control	20	31	0.5	0.2	2	1	0
Compost	27	33	2.1	0.5	3	2	1
Compost + Azotobacter	29	34.5	2.4	0.5	3	2	1
Compost + PSM	32	35	3	0.7	3	2	0
Compost + AM fungi	31	41.6	2	0.7	2	2	2
Compost + KMB	35	45	2.9	0.7	3	3	2
Compost + Azotobacter + PSM + AM + KMB	37.5	47	2.9	1.1	4	2	2

Compost = Compost prepared from Ayurvedic Herbal wastes

Azotobacter = Liquid *Azotobacter chroccocumm*

PSM = *Bacillus polymixa*

AM Fungi = consortium *of endomycorrhizal spores*

KMB = *Frateuria aurentia* (Potassium mobilizing bacterium)

Table 12 - Effect of Liquid biofertilizers along with Phospho compost from Ayurvedic Herbal waste in Capsicum at 30 days.

Treatments	Length. (cm)		Dry weight (g)		No of branches	No of flowers	No of fruits
	Shoot	Root	Shoot	Root			
Control	20	31	0.5	0.2	2	1	0
Phospho Compost	27.9	33.6	2.6	0.5	3	3	1
Phospho compost + Azotobacter	30.6	34.8	2.9	0.6	3	3	2
Phospho compost + PSM	34.1	37	3.6	0.7	3	3	1
Phospho compost + AM fungi	34.8	42.2	3	0.8	2	2	3
Phospho compost + KMB	35	49	3.1	0.9	3	3	3
Phospho compost + Azotobacter + PSM + AM fungi + KMB	40.7	53	3.8	1.3	4	5	3

Compost = Compost prepared from Ayurvedic Herbal wastes

Azotobacter = Liquid *Azotobacter chroccocumm*

PSM = *Bacillus polymixa*

AM Fungi = consortium *of endomycorrhizal spores*

KMB = *Frateuria aurentia* (Potassium mobilizing bacterium)

A pot experiment conducted by Nayak indicates results that application of Potash Mobilizers can save 50% chemical potash and additional around 51% uptake of K increased than control pots and 28.5% of biomass increased than control (Table - 13).

Table - 13 Potassium uptake by plants & K status in soil and Biomass in Brinjal at different treatments

Treatments	K Uptake in K/ha	K in soil kg/ha	Biomass generated g/pof
Control (No. Biofertilizer +No. Chem. Fertilizer)	2.935	89.4	23.50
Potash Mobilizing Bacteria (KMB)	3.076	139.6	29.02
100:60:60kg $N_1P_2O_5$ K_2O ha^{-1} (General recommended dose)	3.930	134.4	48.91
100:60kg $N_1P_2O_5$ ha^{-1} + KMB	3.762	129.0	38.19
100:60:60kg $N_1P_2O_5$ K_2O ha^{-1} (General recommended dose)	3.682	155.8	34.74
100:60kg $N_1P_2O_5$ ha^{-1} +45 kg K_20 + KMB	4.109	131.6	31.37
100:60kg $N_1P_2O_5$ ha^{-1} +30 kg K_20 + KMB	4.438	134.4	36.23

(Source Nayak. B. 2001)

(iii) *Frateuria aurentia* as a K-mobilizer from ores-

The study was conducted by Dash in O.U.A.T with the help of Dr. L.B. Sukla, Regional Research Laboratory, Bhubaneswar recorded use of ***"Frateuria aurentia"*** is capable of releasing elementary potassium from ores.

Table - 14 shows the 5% KMB inoculum mobilized K_20 maximum quantity (60.58 kg / ha)

Table - 14 Available K_2O after 20 days incubation with the KMB culture from Manganese 0re.

Ore	% of Inoculum	Available K_2O of dry ore without any incubation (control) (Kg/ha)	Available K_20 of ore inoculated with culture (Kg/ha)
Mn	1%	23.3	27.96
	2%	23.3	27.96
	3%	23.3	37.28
	4%	23.3	46.6
	5%	23.3	60.58
	10%	23.3	55.92
	15%	23.3	55.92
	20%	23.3	46.6

(Source Dash D.M. 2002)

It is evident from Table - 15 that the availability of K_2O in soil after application of KMB in Rhizosphere of Paddy increased 83% in the same way in Okra Rhizosphere the availability of K_2O is 215%.

Table - 15 Soil characterstics before and after inoculation of KMB in Paddy and Okra Rhizosphere

Soil Samples	Before Amendment				After Amendment			
	pH	N (Kg/ha)	P_2O_2 (Kg/ha)	K_2O (Kg/ha)	pH	N (Kg/ha)	P_2O_2 (Kg/ha)	K_2O (Kg/ha)
Paddy Rhizo-sphere	5.0	299.6	232.9	131.7	7.0	341.6	232.2	241.9
Okra Rhizo-sphere	5.2	190.5	40.8	108.0	7.2	375.2	392.8	341.2

(Source Biswas. S. 2001)

Table - 16 shows that the uptake of K_20 and biomass production due to inoculation of Potash and *Rhizobium* inoculated Groundnut is on far with recommended chemical fertilizers application. So it is evident that the chemical fertilizers could be replaced when we use nitrogenous biofertilizers along with KMB culture. The same trend of results has been recorded in paddy crop also (Table - 17).

Table - 16 Potassium and biomass uptake of Groundnut (g/pot) as influenced by different treatments at 45 days of growth

Treatments	K uptake g/pot	Dry weight (pods) (g/pot)	Dry weight (plant) g/pot
Control	0.232	4.915	16.907
Potash mobilizing bacteria	0.270	7.021	17.338
Potash mobilizing bacteria + *Rhizobium*	0.364	8.584	28.845
20:40:40 kg $N_2P_2O_5$ K_2O ha^{-1} (General recommended dose)	0.347	8.359	26.086
20:40:40 kg $N_2P_2O_5$, K_2O ha^{-1}+ (General recommended dose) + Potash mobilizing bacteria	0.516	9.106	31.736
20:40:40 kg $N_2P_2O_5$, K_2O ha^{-1}+ (General recommended dose) + Rhizobium + Potash mobilizing bacteria	0.570	10.079	37.651

(Source Mishra. M. 2001)

Table - 17 Potassium uptake Biomass of Paddy as affected by different treatments at 45 days harvest

	Treatment g/pot	K uptake (g/pot)	Dry weight
T1	Control (No fertilizer + No biofertilizer	0.297	22.235
T2	Potash mobilizing bacteria	0.372	28.238
T3	Potash mobilizing bacteria + *Azospirillum*	0.569	30.466
T4	Potash mobilizing bacteria + *Azospirillum* + Phosphorus solubilizing bacteria	0.632	34.175
T5	T_4+ 80 : 40 : 40 kg $N_1P_2O_5$, K_2O ha^{-1} (General recommended dose for rice)	0.527	35.175
T6	T_4 + 56 : 28 : 28 kg $N_1P_2O_5$, K_2O ha^{-1} (30% less than the recommended dose)	0.631	30.502
T7	80 : 40 kg $N_1P_2O_5$,ha^{-1} (Recommended dose) + Potash mobilizing bacteria	0.771	38.559
T8	80 : 40 kg N_1 P_2O_5,ha^{-1} (Recommended dose) + Potash mobilizing bacteria + 30 Kg K_2O ha^{-1} (25% less than recommeded dose)	1.001	50.089

(Source Biswal.S. 2001)

(iii) Physical Features of Liquid KMB

- Solution always brown in colour.
- Smell of H_2S or chocolate.
- pH-4.5 but buffer 6.5-7.0 ensures more than 2 years expiry.

(iv) Combined effects of Liquid Biofertilizers

(a) The field demonstration was conducted in year 2004 under the supervision of Agrl. Dev. Officer, Annaimalai on the paddy crop in the field of Aravindam, S/o Ramadors, Annamalai. The liquid Biofertilizers like *Azospirillum,* Potash Mobilizers in liquid form @ 200ml per acre was used. The observation recorded by the Agrl. Officer is as follows.

1. Germination was increased up to 85-90%.
2. Dark green color in leaves.
3. More no. of tillers ranged up to 29 to 32.

(b) The Field Demonstration was conducted in year 2004 under the supervision of Agrl. Dev. Officer, Dharmapuri on paddy crop in the field of Smt. C. Chitra, W/o Cheniappan, Nallanpatti, Dharmapuri DT, T.N. The liquid Biofertilizers *Azospirillum,* Potash Mobilizers in liquid form @ 200ml per acre was used. The observation recorded by the Agrl. Officer is as follows.

1. More no. of tillers
2. panicle length is higher than control plot
3. synchronization of flowering
4. leaf folder, BPH attack prevailed and high rainfall during flowering stage, eventhough trial plot given 7.2 quintal high yield than control

(c) Field experiment on rice variety Prasad was conducted during 1993 and 1994 at Manipur to study the effect of inoculations with *Azotobacter chroococcum* and *Bacillus megatarium* in combination at graded doses of nitrogen and phosphorus on the grain yield and dry matter weight. (Chandra et.al.1996) results indicates better yield.

(h) Zinc and Sulphur Solubilizing Bacteria

(Thiobacillus spp)

Thiobacilus ferrooxidans also known as *Acidithiobacillus ferrooxidans* and *Thiobacillus thiooxidan*s and *Thiobacilus*

acidophilus is an acidophilic bacteria present in water / soil. The bacteria produces number of organic acids. They are able to produce and tolerate highly acidic conditions below upto pH 1.5 with the action of acid they solubilize large amount of Zn, Cu, Fe, Co etc. what ever, micro-nutrient present in the soil in elementary or complex form. The liquid inoculum of above bacteria are very effective in all types of soils. During multiplication, bacteria produce large amount of acid, hence, the pH become low up to 2.5-4.5. Use of 100ml/acres in field along with 500kg of FYM/cow dung or 100 ml inoculum with 100 its water spray on field or through drip irrigation improves the quality / yield of crops.

(i) Manganese solubilizer *(Pencillium citrinum)*

Pencillium citrinum a fungal culture present in soil is known to be solubilize manganese from the low grade manganese ores and from the soil if it present. The fungus produces reductive compounds such as organic acids (oxalic acid, citric acid and which help in solubilizing of manganese. Temperature required for multiplication is $32^0 \pm 2^0$c. During multiplication, pH became low upto 5.5.

9. ROLE OF LIQUID BIOFERTILIZERS IN VARIOUS CROPS

- Increased crop yield
- Reduces chemical N, P & K fertilizers
- Increases seed quality of the crops
- Benefits the next crop too, due to its residual effect
- Enhances soil health and soil fertility
- Output returns economically profitable
- Besides N, P & K nutrition, growth hormones secreted increases crop productivity
- Disease and pest occurrence reduced

Hence, Biofertilizers play important role in agriculture to maintain sustained crop productivity and balanced soil nutrition. The supporting field data presented in the tables 18 - 24.

Table - 18 Effect of Liquid Biofertilizers on yield of some cultivated legume / non-legume crops (RBDC results)

Location	Crop	(Control) Yield (Q/ha)	(Treated) Yield (Q/ha)	% increase	Year
Deogarh	Wheat	11.2	15.4	37.5	2002
Kaladi, Phulbani	Maize	15.20	15.60	2.63	2001
Gundrigaon, Phulbani	Maize	15.06	15.36	2.00	2000
Katingia, Phulbani	Brinjal	33.12	42.40	28.00	2001
Sundarpali, Phulbani	Paddy	11.00	11.50	4.54	2000
Sasaikuti, Phulbani	Turmeric	22.00	24.00	9.74	2000
Katrajhari, Nayagarh	Paddy	13.60	19.60	44.11	2001
Dhanupara, Bihar	Paddy	18.08	20.50	13.38	2001
Sayabalpur, Bihar	Paddy	8.75	11.50	31.42	2000
Kanitiakatene	Paddy	34.5	40.00	16	2002
Kulagada	Paddy	45.0	55.00	22.2	2000
Karikol	Paddy	42.0	52.00	23.8	2002
Dalaiguda	Paddy	35.00	48.00	37.0	2001
Samantrapur	Paddy	38.00	44.00	15.78	2000
Tangara	G.nut	11	17.50	59.09	2001
Sinshour	Paddy	37.00	42.00	13.5	2000
Basudeipur	Paddy	25.87	41.25	59.45	2002
Chipilima	Paddy	51.40	72.80	41.6	2000

Hatipale	Paddy	65.60	90.00	38.46	2000
Kulakaijunga, Jagatsingpur,	Paddy	13.00	15.00	20.0	2000
Jamugaon, Jagatsinghpur	Paddy	13.75	17.25	25.45	2002
Kusapur, Chattrapur	g.nut	13.87	17.40	25.68	2001
R.K. Gram, North Andaman	Paddy	48.40	59.20	22.90	2000
Rampur, Andaman & Nico.	Paddy	40.8	44.4	8.82	2002
Temple Myo, South Andam	Paddy	28.40	33.20	16.90	2000
Gobardhansole, Mayurbhanj	Mung	6.52	7.65	17.33	2000
Chandua, Mayurbhanj	Mung	6/75	7.57	12.14	2001
Subadega, Sundargarh	Paddy	11/46	12.60	9.94	2002
Sundargar, West Bengal	Paddy	10.83	12.27	13.29	2002
Dharmapuri, Tamil nadu	Paddy	09.15	10.83	**8**	2004
Anaimalai, Tamil Nadu	Paddy	12.25	13.39	**9**	2004
Harihara, Karnataka	Paddy	17.0	25.0	**8.69**	2003
Makanahally, Karnataka	Paddy	16.0	20.0	**25.0**	2003
Seegepalya, Karnataka	Paddy	17.0	21.0	**23.5**	2003
Gulbarga, Karnataka	G.nut*	6.96	7.10	**2.01**	2003
Pandavapura, Karnataka	Ragi	20.0	23.5	**17.5**	2003

*Rainfed

Table - 19 National Level On-Farm Trial on Liquid *Rhizobium* Inoculation of Groundnut During Kharif 2001

Table - 19 National Level On-Farm Trial on Liquid *Rhizobium* Inoculation of Groundnut During Kharif 2001

Sl. No.	KVK/State	No. of OFT conducted / allotted	Percent Pod yield Increase over control						
			LRI-1		LRI-2		Mean of LRI	CRI	
			Range	Mean	Range	Mean		Range	Mean
1	Chomu (Tankarda) Rajasthan	5/5	0-13	5.56	4-15	10.39	8.00	2-14	7.5
2	Sattur (Mysore) Karnataka	13/20	0-30	13.77	0-24	12.24	13.00	0-24	12.0
3	Randheja Gujarat	5/5	0-7	3.74	0-7	4.9	4.32	4-13	8.5
4	Vridhachalam Tamil Nadu	5/5	1-4	2.45	3-7	5.0	3.73	1-3	1.7
5	Sirohi Rajasthan	6/6	2-17	9.92	1-16	8.5	9.21	0-13	7.4
6	Amaravati Maharastra	5/5	11-16	14.62	27-55	40.2	27.41	0-15	6.0
	Total	**39**		**8.35**		**13.54**	**10.95**		**7.18**

(Source : S.V. Hegde 2002)

Table - 20 National Level On-Farm Trial on Liquid *Rhizobium* Inoculation of Soybean During Kharif 2001

Sl. No.	KVK/State	No. of OFT conducted / allotted	Percent Pod yield Increase over control						
			LRI-1		LRI-2		Mean of LRI	CRI	
			Range	Mean	Range	Mean		Range	Mean
1	Amaravati Maharastra	5/5	16-43	29.5	31-83	55.4	42.45	5-30	15.70
2	Seoni Madya Pradesh	2/5	38-53	26.6	—	—	26.6	10-13	11.67
	Total	**7/10**		**28/1**		**55.4**	**34.53**		**13.69**

(Source : S.V. Hegde 2002)

Table - 21 National Level On-Farm Trial on Liquid Rhizobium Inoculation of Pigeonpea During Kharif 2001

Sl. No.	KVK/State	No. of OFT conducted / allotted	Percent Pod yield Increase over control						
			LRI-1		LRI-2		Mean of LRI	CRI	
			Range	Mean	Range	Mean		Range	Mean
1.	Sirohi (Rajasthan)	6/6	1-8	4.36	Nil	Nil	2	1-8	4.23
2.	Gulbarga (Karnataka)	5/10	0.5-16.7	13.2	14.2-19.0	16.6	14.9	5.8-12.1	8.7
3.	Bidar (Karnataka)	5/5	1.4-31.2	23.1	8.6-25.0	16.0	19.5	3.1-16.7	10.46
	Total	**16/21**	**—**	**13-6**	**—**	**10.9**	**12.3**	**—**	**07.8**

(Source : S.V. Hegde 2002)

Table - 22 National Level On-Farm Trial on Liquid Rhizobium Inoculation of Soybean During Kharif 2001

KVK/State	No. of OFT conducted / allotted	Percent Pod yield Increase over control						
		LRI-1		LRI-2		Mean of LRI	CRI	
		Range	Mean	Range	Mean		Range	Mean
Amaravati Maharastra	5/5	16-43	29.5	31-83	55.4	42.45	5-30	15.70
KVK Gulbarga (Karnataka)	9/10	5.1-10.7	7.7	10.1-19.8	13.8	10.7	3.4-8.6	5.4
Hanumanamatti (Karnataka)	5/5	25.0-40.6	34.5	5.5-25.0	14.8	24.50	16.6-28.1	20.7
Sattur (Karnataka)	10/10	00-16.7	7.5	00.16.7	0.8.7	08-10	00-25	13.0
Total	**24/25**	**—**	**16.6**	**—**	**12.4**	**14.5**	**—**	**13.0**

(Source : S.V. Hegde 2002)

Table - 23 Performance of Liquid Rhizobium Biofertilizer on Redgram (Arhar) in India during Kharif 2000

Location	Seed Yield Kg/Ha		
	Control	Lipha Tech	Increase Over Control (%)
Sehore (MP)	1378	1713	24.3
Akola (MS)	1013	1333	31.6
Bangalore	874	1036	18.53

(Source : S.V. Hegde 2002)

Table - 24 Field performance summary of the biofertilizer Inoculant Formulation Compared to Uninoculated and Local Inoculant Products*

Response Indicator (n)	Response of liquid inoculant above			
	Control		Local Inoculant	
	Relative Frequency	Percent Increase	Relative Frequency	Percent Increase
Seed Yield[1] (n=37)	97	90	68	6
Total Seed N (26)	96	112	50	3
Nodule No. (n=39)	97	>10000	77	20
Nodule wt. (n=42)	97	>1000	71	14

(Source : S.V. Hegde 2002
*Personal communication from Dr. Paul Singleton, NifTAL, Hawaii, USA).

10. BASIC LABORATORY FACILITIES REQUIRED FOR LIQUID BIOFERTILIZER / BIOCONTROL AGENTS PRODUCTION (3 lakh Ltr per annum) / and QUALITY CONTROL

Since Biofertilizer production involves microorganisms which are cultured and tested periodically under sterile conditions, the following equipments and laboratory facilities are essential.

1. Laminar Air Flow Cabinet (Horizontal type with .2 micron German made happa filter) - 2 nos.
2. Phase Contrast microscope-1no.
3. BOD Incubators-2 nos.
4. Fermentors with attachment of computer for reading the pH and temperature 5000 lit. capacity of each - 4 nos.
5. Horizontal autoclave - 1 no.
6. Orbital Shaker (small) - 1 no.

7. Bottle filling machine, automatic - 1 no.
8. Air filter system
9. Reverse Osmosis Process
10. Demineralised water plant

The total cost of the project including building and machinery will be about 5 crores (personal estimation may vary the cost depends upon quality of the equipment and the laboratory location is proposed to set). The production unit/quality control laboratory should have sufficient space with properly fitted equipments and rooms should be maintained clean and contamination free. Further, undisrupted electricity, water supply should be ensured. Biofertilizer production/quality control must be handled by trained person.

11. QUALITY MANAGEMENT

Biofertilizer quality management can be effectively operated considering the following aspects :

1. Commitment of the organization like RCOF and State Govts.
2. Procurement of quality mother culture from appropriate source
3. Establishment of appropriate laboratory
4. Recruitment of qualified personnel
5. Adoption of standard protocols
6. Regular maintenance of the laboratory
7. Procurement of relevant instruments, glass wares and chemical, manufacturing biofertilizers as per BIS standards

12. GENERAL MICROBIAL PROTOCOLS OBSERVED TO TEST THE QUALITY OF LIQUID BIOFERTILIZERS

I. General Tests

Prescribed marking on the Biofertilizer bottle like name of the product, crop for which intended, name and address of the manufacturer, batch number, date of manufacture, date of expiry, methods of use and handling instructions etc.

II. Qualitative Tests

a. Serial Dilution Technique by Plate Count: By this method, the total number of viable bacteria present in the Biofertilizer bottle could be known. Usually 10 ml of Liquid based Biofertilizer is removed from the bottle to be mixed in 90 ml of sterile water and shaken thoroughly. After homogenization from this, 1 ml is taken and added with 9ml of sterile water. Likewise, serially it is diluted under sterile conditions till 10^{-10}. Finally, usually 10^{-6} to 10^{-10} dilutions are chosen and out of each dilution 1.0 ml of aliquot is inoculated in sterile solidified enrichment media specific to the test microorganism and spread uniformly. The Petri plates are inverted and incubated in BOD incubator at $\pm 28^{0}$C. The plates are observed periodically for the appearance of bacterial colonies.

b. Colonies appeared in the plates are observed for colony morphology, shapes like either round, round with scalloped margin, round with raised margin, wrinkled, concentric, irregular and spreading, filamentous, round with radiating margin, filliform, complex etc. The margin of appeared colony like either smooth, wavy, lobate, irregular, ciliate, branching, wooly, is observed. The elevation of the colony either like flat, raised, hilly, convex, drop like is observed.

c. One of the isolated colonies is removed and spread in a loop on the slide and gram stained.

d. The colonies observed are stained and its morphological characters like cellular shape, size, flagella, attachment of flagella etc. are studied preferably under a phase-contrast microscope and strain is identified.

e. Specific confirmation tests for each microorganism are done to check its authenticity.

III. Quantitative Tests

a. Through plate counts, the total number of colonies appeared are counted in an electronic colony counter and depending on the dilution factor, the colonies formed are enumerated.

b. From the broth, the total microbial populations could be counted directly under a microscope using a Petroff-Hauser Counter otherwise through a Haemocytometer and populations are enumerated. This test does not confirm the purity.

c. From the broth, samples are taken and its optical density is measured in a spectro photometer against its grown media as check and indirectly, the population is extrapolated through it standard.

d. The nitrogen fixing potentiality/phosphate solubilizing ability of the strains could be tested using standard procedures.

However, in the absence of adequate laboratory facilities to carry out sophisticated microbiological tests a simple "GROW OUT" test could be conducted to test its ability for the respective crop marked in the packet through different parameters of crop response and indirectly the quality of the Biofertilizer packet is partially could be assessed.

13. DO'S AND DON'TS FOR ENTREPRENEURS, DEALERS AND FARMERS

Do	Don't
❖ Keep Biofertilizers bottles away from direct heat and sunlight. Store it in cool and dry place. ❖ Sell only Biofertilizers bottles which contain batch number, the name of the crop, the date of manufacture and expiry period. ❖ If the expiry period of the bottles is over, discard it. It will not be effective. ❖ Keep Biofertilizers bottles away from fertilizer or pesticide containers should not mixed directly.	❖ Don't store Biofertilizers bottles under heat and sunlight. ❖ Don't sell Biofertilizers bottles after their expiry period is over. ❖ Don't prick holes into the bottles or puncture them. ❖ Do not mix the Biofertilizers with fungicides, insecticides, herbicides and chemical fertilizers.

14. LIQUID BIOFERTILIZER APPLICATION METHODOLOGY

There are three ways of using Liquid Biofertilizers

1. Seed treatment
2. Root dipping
3. Soil application

(i) Seed Treatment

Seed Treatment is a most common method adopted for all types of inoculants. The seed treatment is effective and economic. For small quantity of seeds up to 5 kgs quantity. The coating can be done in a plastic bag. For this purpose, a plastic bag having size (21"x10") or big can be used. The bag have to be filled with 2 kg or more seeds. The bag has to be shut in such a way to trap the air as much as possible. The bag have to be twisted for 2 minutes or more until all the seed were uniformly wetted. The bag have to be opened, inflated the bag again and shaked gently. Stop shaking after each seed got a uniform layer of culture coating. The bag has to be opened and spread the seed under a shade for 20-30 minutes for dry. For large amount of seeds counting can be done in a bucket and inoculant can be mixed directly with the hand. Seed Treatment with *Rhizobium, Azotobacter, Azospirillum, KMB* along with PSM.

The seed treatment can be done with any of two or more bacteria. There is no side (antagonistic) effect. The important things has to be kept in mind that the seeds must be coated first with *Rhizobium or Azotobacter or Azospirillum* when each seeds get a layer of above bacteria then PSM and KMB. inoculant has to be treated on outer layer of the seeds. This method will provide maximum number of population of each bacteria required for better results. Mixing the any of two bacteria and the treatment of seed will not provide maximum number of bacteria of individuals.

ii) Root Dipping

Application of *Azospirillum* / KMB / PSM with the paddy transplanting / vegetable crops this method is needed. The required quantity of *Azospirillum* / KMB / PSM has to be mixed with 5-10 ltr of water at one corner of the field

and all the plant roots have to be dipped for minimum $^1/_2$ an hour before sowing.

iii) Soil Application

P.S.M. has to be used as a soil application use 200ml of PSM per acre. Mix PSM & KMB with 400 to 600 kgs of Cow dung FYM along with $^1/_2$ bag of rock phosphate if available. The mixture of PSM and KMB, Cow dung and rock phosphate have to be kept under any tree or ceiling for over night and maintain 50% moisture. Use the mixture as a soil application in rows or during leveling of soil.

15. DOSAGE OF LIQUID BIOFERTILIZER IN DIFFERENT CROPS

Recommended Liquid Biofertilizers and its application method, quanity to be applied for different crops as follows :

Crop	Recommended Biofertilizer	Application Method	Quantity to be applied
FIELD CROPS			
PULSES			
Chickpea, pea, G. nut	Rhizobium	Seed	200ml/acre
Soybean, beans		treatment	
Lentil, lucern,	Rhizobium	Seed	200ml/acre
Berseem, green gram		treatment	
Black gram, cowpea			
Pegion pea.			
CEREALS			
Wheat, oat, barley	Azotobacter/ Azospirillum	Seed treatment	200ml/acre
Rice	Azospirillum	Seedling treatment	200ml/acre
OIL SEEDS			
Mustard, seasum	Azotobacter	Seed	200ml/acre
Linseeds		treatment	
Sunflower, castor	Azotobacter	Seed treatment	200ml/acre

MILLETS			
Pearl millets, finger millets, Kodo millet	Azotobacter	Seed treatment	200ml/acre
maize & Sorghum	Azospirillum	Seed treatment	200ml/acre
FORAGE CROPS & GRASSES			
Barmunda grass, Sudan grass, Napier Grass, Paragrass, Star grass etc.	Azotobacter	Seed treatment	200ml/acre
OTHER MISC. PLANTATION CROPS			
Tobacco	Azotobacter	Seedling treatment	500ml/acre
Tea, Coffee	Azotobacter	Soil treatment	400ml/acre
Rubber, Coconuts	Azotobacter	Soil treatment	2-3 ml/Plant
AGRO-FOREST-RY/FRUIT PLANTS			
All fruit/agro-forestry (herb, shrubs, annuals & perennial) plants for fuel wood, fodder, fruits, gum, spice, leaves, flowers, nuts & seeds purpose	Azotobacter	Soil treatment	2-3 ml/plant at nursery
Leguminous plants / Trees	Rhizobium	Soil treatment	1-2 ml/plant

Note : Doses recommended when count of Inoculum is 1x10^{9}cells / ml. If the population is less than 10^8 cells / ml doses will be ten times more besides above said Nitrogen fixers, Phosphate solubilizers and Potash mobilizers at the rate of 200 ml/acre could be applied for all crops.

16. SOME PROPOSED NORMS AND RECOMMENDATIONS FOR BIOFERTILIZERS

1. The definition of Biofertilizer as " the product containing carrier-based (solid or liquid) living microorganisms which are agriculturally useful in terms of nitrogen fixation or phosphorus solubilization, Potassium mobilizing etc. to increase the productivity of the soil and/crops ".

2. The opinion that there is need to regulate the number of strains in respect of all biofertilizers, maintenance of huge number of strains is not practical. It has also been decided to recommend appropriate 1-3 strains for each region. The recommendation may be made by AICRP-BNF, ICAR based on their research work in consultation with NBDC. Manufacturers will be allowed only to use authenticated strains. **NCOF/RCOF will be registering strains & making them available to others.** It is also necessary to document authenticated strains in terms of their capacity on nodulation, nitrogen fixation, P-solubilization, potash mobilizing, biomass increase.

3. i. There is a need of separate registration committees of Central Government and State Governments.
   ii. All manufacturers should be registered with DAC/ RCOF.
   iii. No new Biofertilizer be allowed without the approval of DAC/RCOF.
   iv. RCOF shall be provided with full details on strain no., equipments, technical man power & field performance data at the time of registration/sample test.
   v. If a sample fails, the re-testing will be allowed for testing the same in any reference laboratory other than the one where the sample was failed earlier.
   vi. NCOF/RCOF will reference laboratory.

4. A Biofertilizer needs to be legally controlled with any available Act like FCO, BIS, Weight and Measurement, Consumer Protection Act etc. As many of the participants have no clear idea regarding FCO, they wanted that, it is the issue which will be decided by DAC/NCOF after proper examination of all available acts.

Substandard of sample (range)	Proposed penalty
10-25%	Permitted to sale, but warning will be issued to improve the quality
More than 25%	Selling will be stopped
More than 50%	Registration should be revoked

6. BIS specification for all remaining biofertilizers like BGA, Azolla, *Acetoabacter diazotrophicus* and VA-Mycorrhiza Potash mobilizing bacteria and all liquid formulation prepared and submitted, matters to be take up.
7. All production units would be visited once in a year by concern RCOF.
8. Liquid Biofertilizer should be encouraged. The minimum CFU for liquid Biofertilizer will be 10^9/ml. Other parameters will be the same as made for other biofertilizers.
9. Any new Biofertilizer product needs permission from DAC/NCOF/RCOF. Plant extracts/sea weed extracts/growth regulators of biological origin in general should not be considered as Biofertilizer.
10. There should not be any compulsion to procure mother culture for higher rates or region wise.

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