Biopesticides: their role in sustainable agriculture, production technology and entrepreneurship opportunities in Assam

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ABSTRACT

Ordeals and dilemmas accompany the life of farmers in a flood prone state like Assam. Despite technological advances, their farming wisdom has remained traditional. Chemicals are a mere means of managing pests and diseases in their fields. But with changing times, the preference of consumers towards organic produce is growing day by day as they are becoming more conscious of food quality, their health as well as sustainability of the environment. Organic agriculture is the answer to address the issues thrown up as the after effects of pesticide application are becoming more potent.

Thus, in these changing times, the demand for biopesticides as alternative pest control measures is gaining popularity. Biopesticides are any formulations which are derivatives of biological origin containing either plant products, microorganisms, insects or their combinations which helps in mitigating these pest problems. The sources of biopesticides are readily available and easily biodegradable, exhibiting different modes of action and are less expensive with low residual toxicity. Neem, Tobacco, Garlic, Onion, Citronella, Jatropha etc. are potent sources of biopesticides which are already under commercialization. Different species of *Trichoderma*, *Bacillus* sp. etc. have also been isolated with potent anti-microbial activity.

With the government giving more emphasis on turning the entire North East India into an organic region, the demand for biopesticides have increased manifold owing to increasing pest and disease problems. The locally available producers are not able to provide for even 1 per cent of the biopesticide needs of the state. Thus, this has opened a huge window of opportunity for the development and industrialization of the bio pesticide industry in Assam.

INTRODUCTION

Our nation has pursued the policies of intensive use of agro-chemicals in the last 30-40 years to meet the challenges of increasing the agricultural production. Level of consumption of agro-chemicals is sometimes held as yardstick of agricultural development. Use of agro-chemicals along with other technologies like improved hybrids/varieties and irrigation has indeed elevated our country to self-sufficiency in food production.

But, in the journey of ever challenging agricultural development, we have reached a stage- where the basis of production itself is in perilous situation. Because, the use of agro-chemicals has damaged our eco-system and delicate balance between various components of eco-system. The biological basis of fertility imparts self-supporting feature in soil. Reducing organic carbon status on one hand and treating the soil as mere physical medium to supply the nutrients on the other- have ignored the biological basis of soil fertility. Similarly, the pest control by pesticides alone is akin to chemical invasion on eco-system. A pest is part of biological equilibrium in an eco-system and killing the pest by pesticides not only damages the eco-system but also kills predators and natural enemies of pests.

Use of fertilizers and pesticides had their designated aims of increased productivity and reduced damage due to pests respectively. But, the productivity of many crops has not shown proportionate improvement in the last 10-15 years- despite the increased use of fertilizers. Similarly, extensive use of pesticides has not reduced the losses due to pests. With these two facts in background- a stage has now reached to review whether promoting the use of agro-chemicals is appropriate strategy or not.

Assam and for that matter the entire NER has been pursuing eco-friendly agriculture right from the era of green revolution. Though low input-low output agriculture practices in the region has now become a cause of concern particularly on the face of increased food demand, the region holds the key to triple its food production capacity with higher doses of environment-friendly inputs in the form of scientifically produced bio inputs. Having experienced the fatigue in the green revolution belt, NE states like Sikkim, Mizoram, etc. have already declared organic mode of food production as an agricultural policy. All these

developments have necessitated production of bio inputs for supporting the causes of organic agriculture and productivity increase.

WHAT ARE BIOPESTICIDES?

Biopesticides are products and by-products of naturally occurring substances such as insects, nematodes, microorganisms, plants as well as semiochemicals. Based on the nature and origin of the active ingredients, biopesticides fall into several categories such as botanicals, antagonists, growth promoters, predators and pheromones. Plants and microorganisms are the major sources of biopesticides due to the high components of bioactive compounds and antimicrobial agents. The active compounds in plants include phenols, quinones, alkaloids, steroids, terpenes, alcohols and saponins.

HARMFUL EFFECTS OF CHEMO-INTENSIVE AGRICULTURE

In the journey of ever challenging agricultural development, the policies of intensive use of agro-chemicals were pursued. With the advent of green revolution in India during 1960s, the level of consumption of agro-chemicals has been held as yardstick of agricultural development. Use of agro-chemicals along with other technologies like improved chemo-responsive hybrids/varieties and irrigation has elevated our country to self-sufficiency in food production. However, the price paid to achieve the green revolution was beyond imagination as the agro-chemicals also damaged the eco-system and delicate balance between various components of eco-system. Use of chemicals has also a saturation point somewhere, as revealed by recent studies that, extensive use of pesticides in the last 10-15 years has not reduced the losses due to pest damages. So, a stage has now reached to review whether promoting the use of these toxic chemicals is appropriate strategy or not.

The word pesticide means “pest killer”. Depending on the kind of pest against which they are effective, pesticides are known as insecticide, bactericides, fungicides, nematicides, herbicides, and so on. Hundreds of pesticides are produced annually throughout the world, and many of the newer pesticides are much more toxic, i.e., they are effective at a much lower concentration and faster in action than earlier pesticides.

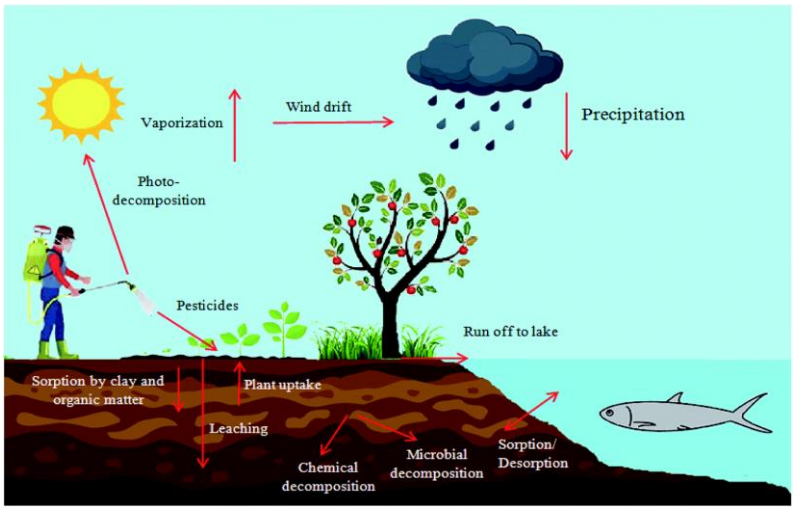
THE PESTICIDAL MENACE:

` There are harmful effects associated with the use of synthetic pesticides such as toxicity and poisoning. Synthetic pesticides also lead to environmental pollution due to the unbiodegradable nature of their constituent compounds. The first significant concern about

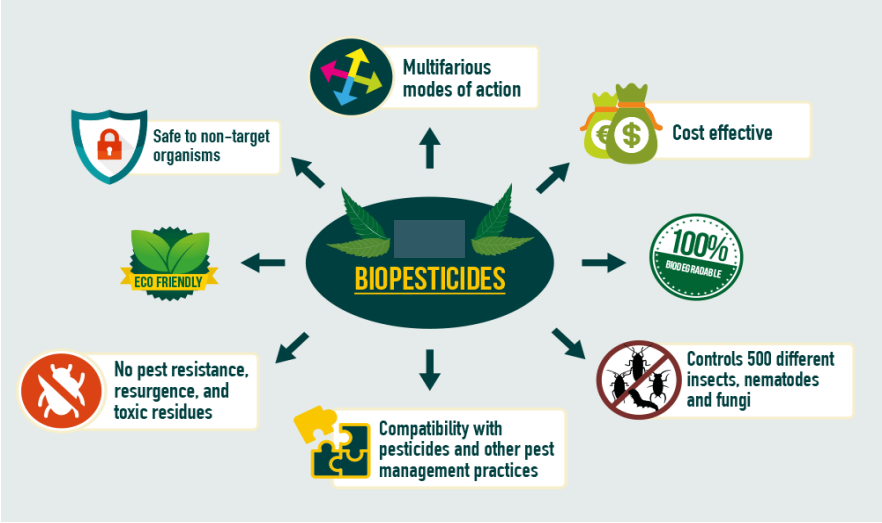
using pesticides was felt during the 1950s, but the obvious benefits from controlling insects and diseases in plants, animals, and humans were so overwhelming that such concerns never reached the public imaginations. At this juncture, the book "Silent Spring," written by Rachel Carson (1962), vividly described the dangers of polluting the environment with poisonous chemicals and documented several cases of bird and fish deaths due to pesticides accumulation through the food chain. Carson's book generated a great deal of controversy but also a much greater awareness of the possible adverse effects of pesticides such as DDT. By the mid-1960s, all pesticides containing mercury were banned by the U.S. government, and soon afterward, DDT and chlorinated hydrocarbons were also banned. DDT has been reported to have carcinogenic properties leading to its ban from agricultural use. Laws were passed that prohibited the use of pesticides causing cancer in laboratory animals or mutations in microorganisms. All existing pesticides were subjected to a new, stricter review, and those found to be carcinogenic or mutagenic were banned and removed from the market. Since the mid-1980s, approximately 85-90% of the pesticides or pesticide uses previously available for plant disease control have been banned by the U.S. government or discontinued by the manufacturers. In the meantime, the requirements for less toxic, more specific pesticides have increased. In the process of managing target pests, synthetic pesticides kill non-target beneficial organisms such as pollinators, predators and antagonists thereby disrupting biodiversity. Exposure to some pesticides has also been reported to retard growth, induce chemical and structural changes in body organs as well as disturb immune responses. They also reduce resistance of animals to disease-causing pathogen infections. Continuous exposure to pesticides such as chlorpyrifos causes gene mutations, genetic damages, reproductive health problems and chronic diseases such as asthma, hypertension and cancer.

ALTERNATIVE TO CHEMO-INTENSIVE AGRICULTURE:

While the population pressures are mounting on limited agricultural lands demanding the increase in the productivity, the increased awareness of hazards of chemicals is discouraging their usage. This in turn may reduce the present level of productivity in short run. It is in this context, use of environment friendly approaches of crop pest management has been found to be relevant as a part of sustainable agricultural system. In this regard, the promising approaches in-elude conventional breeding and genetic engineering of disease-resistant plants, application of disease-suppressing cultural practices, RNA and gene-silencing techniques, and biological management system, wherein biological agents antagonistic to the microorganisms that cause plant diseases are used.



Harmful effects of chemical pesticides in environment



Beneficial effects of biopesticides

BIOLOGICAL CONTROL:

Biological control is the reduction of inoculums density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists".

For millions of years, microorganisms are naturally present in the natural habitats and parasitizing non-cultivated and cultivated plants. Also, they have been interacting with their pathogenic counterparts and governing the infection process in plants to a certain level. But, the phenomenon of controlling one organism by the other, called biological control,

attracted attention of workers quite late only during 1920s and 1930s. The first turning point in this area occurred in 1963 when contemporary leaders of plant pathology assembled at University of California, Berkeley (USA) to lay down a formidable foundation to the science of biological control of plant pathogens. This international symposium was held on **"Ecology of Soil-borne Plant pathogens-Prelude to Biological control".** Since then more than 15 International Symposia have been held on this phenomenon and a number of volumes appeared in the following years. The first book on biological control "**Biological Control of Plant Pathogens**" (**Baker and Cook,** 1974), Baker and Cook's contribution in the field of biological control is very significant as they did brilliant job by analyzing critically all available work and developed theories and concepts of biological control.

BOTANICAL BIOPESTICIDES

Botanical pesticides can either be plant extracts or essential oils [28]. They are obtained from plants parts such as leaves, barks, flowers, roots, rhizomes, bulbs, seeds, cloves or fruits which are either fresh or dried

Table.1 shows examples of plants reported to have potential as sources of botanical pesticides and respective target pests.

Table 1: Plants with potent bio-pesticidal activity and respective target pests

SOURCE PLANT	TARGET PEST	HOST
Azadirachta indica	*Aphids craccivora; Aphis gossypii; Myzus persicae*	*Vigna unguicilata Gossypium hirsutum*
Allium sativum	*Rhizoctonia solani; Colletotrichum sp.*	*Triticum sp.; Capsicum sp.*
Curcuma longa	*Ralstonia solanacearum*	*Solanum lycopersicum*
Jatropha curcas	*Aphis fabae; Meloidogyne incognita*	*Vigna unguicilata; Solanum melongana*
Zingiber officinale	*Fusarium oxysporum; Trichoplusia binotalis*	*Solanum lycopersicum; Brassica oleracea*

MICROBIAL BIOPESTICDES

There are certain microorganisms which too are reported to have potent biocontrol activities some of which are given in table 2.

Table 2: Potent microbial bio-pesticides

MICROORGANISM	TARGET PEST	HOST
Trichoderma spp.	*Ralstonia solanacearum*	*Solanum lycopersicum* *Capsicum chinens Jacq.*
Beauveria spp.	*Dendrolimus tabulaeformis*	*Phaseolus vulgaris*
Bacillus thuringiensis	*Ralstonia solanacearum*	*Solanum lycopersicum* *Capsicum chinens Jacq.*
Pseudomonas fluorescens	*Ralstonia solanacearum*	*Solanum lycopersicum*
Verticillium spp.	*Trialeurodes vaporariorum* *Bemisia tabaci*	*Phaseolus vulgaris* *Euphorbia ulcherrima*
Metarhizium anisopliae	*Musa domestica*	*Citrus sinensis*

MERITS OF BIOCONTROL AGENTS/BIO PESTICIDES

1. Biocontrol agents/Biopesticides being integral part of natural biodiversity are safer to the environment
2. These agents could give protection to the crop throughout the crop period.
3. They are not phytotoxic.
4. They multiply easily in the soil has no residual problem.
5. They not only control the disease and pests but also enhance the root and plant growth by way of encouraging the beneficial soil micro flora.
6. It is comparatively cheaper than any other pest management methods.
7. They are easy to manufacture and can be combined with bio-fertilizers
8. They are harmless to human beings, animals and other natural fauna and flora.

MICROBIAL BIO AGENTS

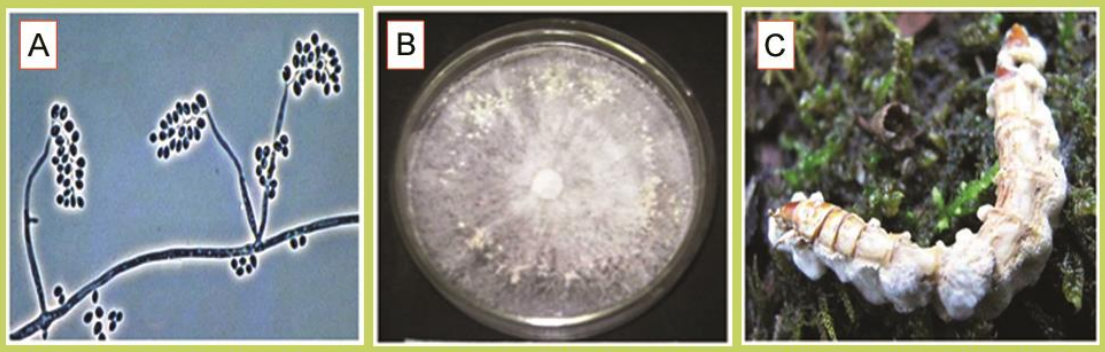


Fig. 1. *Beauveria bassiana* (A) Micrograph (B) Pure culture on PDA medium (C) mummified caterpillar killed by *B. bassiana*

Photo courtesy: Department of Plant Pathology, Assam Agricultural University University of Guelph, Canada, Doctor's Creek walking track, Reefton

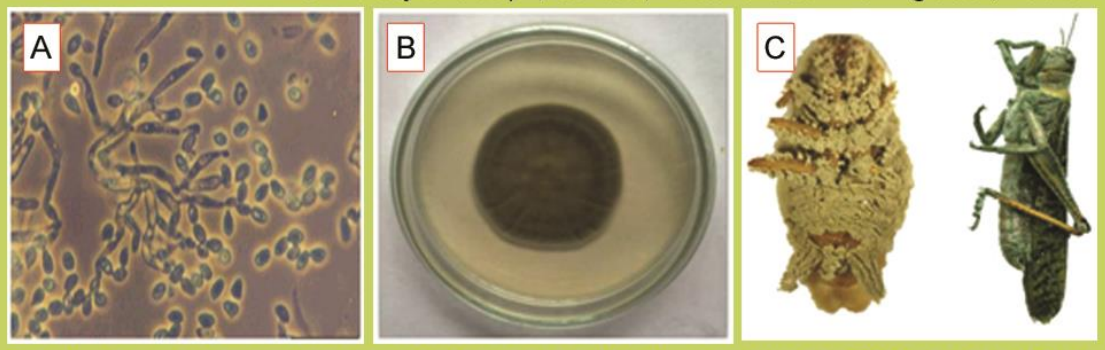


Fig. 2. *Metarhizium anisopliae* (A) Micrograph (B) Pure culture on PDA medium (C) *M. anisopliae* killed cockroach and grasshoppers

Photo courtesy: Department of Plant Pathology, Assam Agricultural University. Chengshu Wang and Yuxian Xia - PLoS Genetics Svetlana Y. Gouli_Organization_University of Vermont_Country United States

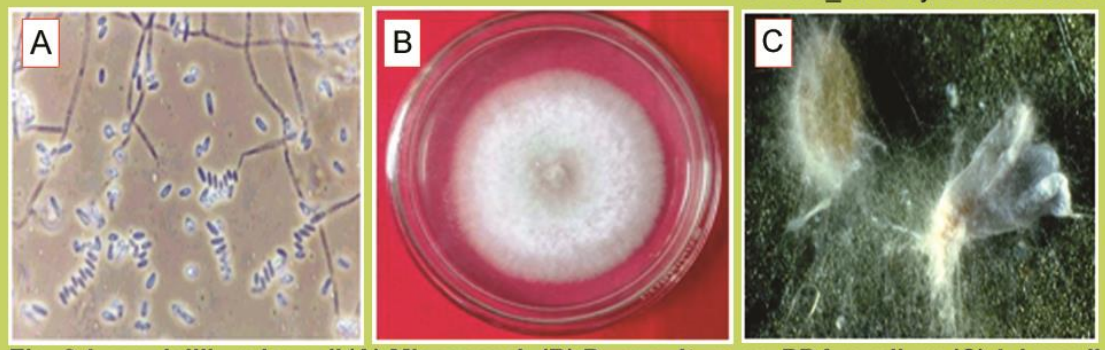


Fig. 3 *Lecanicillium lecanii* (A) Micrograph (B) Pure culture on PDA medium (C) *L lecanii* killed white fly

Photo courtesy: Department of Plant Pathology, Assam Agricultural University Svetlana Y. Gouli, University of Vermont, United States

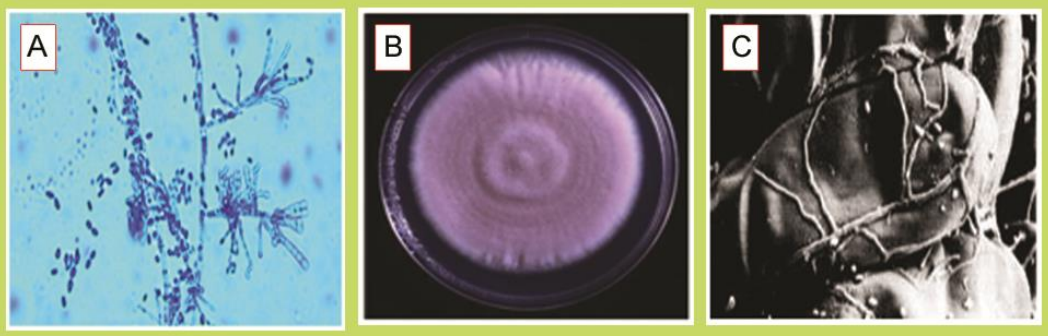


Fig. 4. *Paecilomyces lilacinus* (A) Micrograph (B) Pure culture on PDA medium (C) Mycelium of *P. lilacinus* growing on eggs of root knot nematode

Photo courtesy: Department of Plant Pathology, Assam Agricultural University, Rita Holland

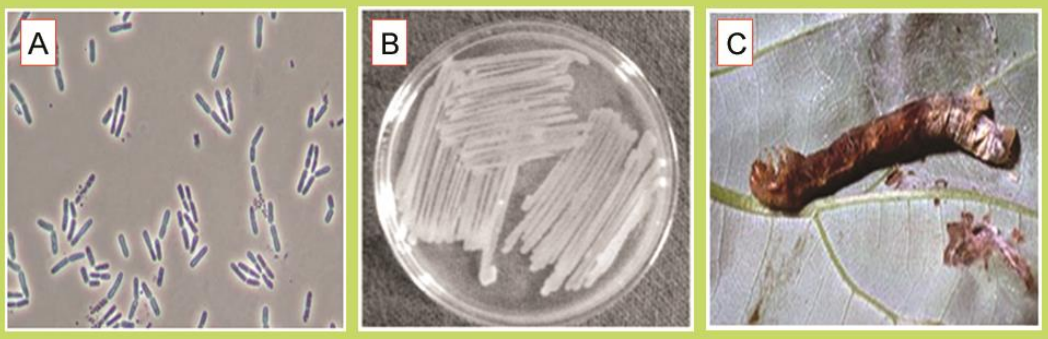


Fig. 5. *Bacillus thuringiensis* (A) Micrograph (B) Pure culture on NA medium (C) *B. thuringiensis* killed caterpillar

Photo courtesy: Department of Plant Pathology, Assam Agricultural University, Brent Selinger

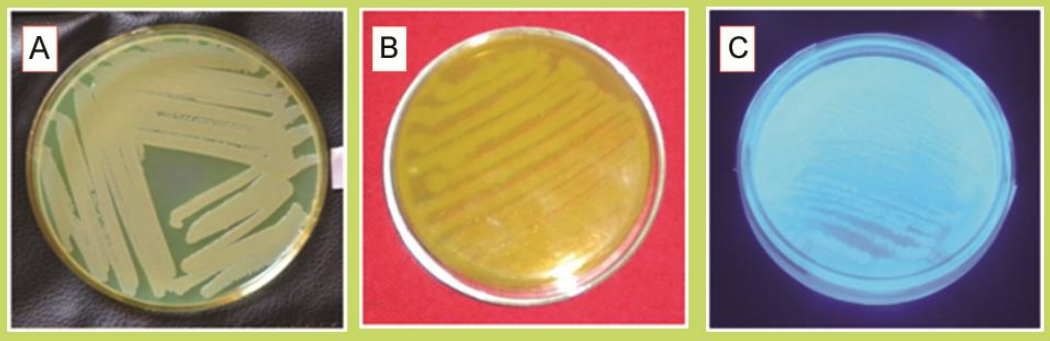


Fig. 6. *Pseudomonas fluorescens* (A) Pure culture on King's B medium (B) inverted plate showing yellow-green, fluorescent pigment (C) *P. fluorescens* under UV light (D) broth culture

Photo courtesy: Department of Plant Pathology, Assam Agricultural University

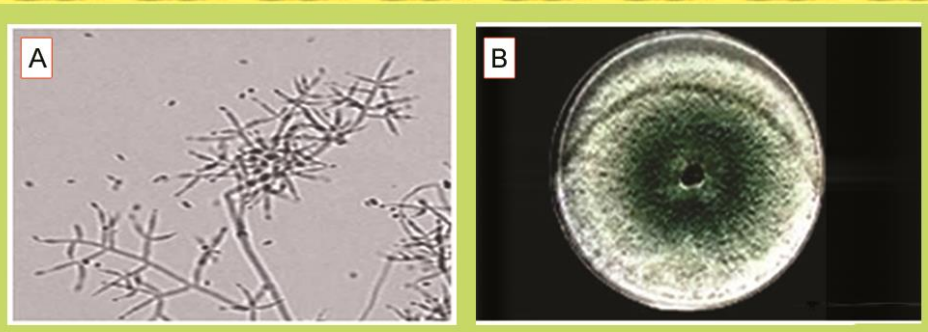


Fig. 7a. *Trichoderma harzianum* (A) Micrograph (B) Pure culture on PDA medium
Photo courtesy: Department of Plant Pathology, Assam Agricultural University, Dayamí Yohana Rodriguez Batista and Jack Cardenas, Research Institute of Plant Protection, Havana

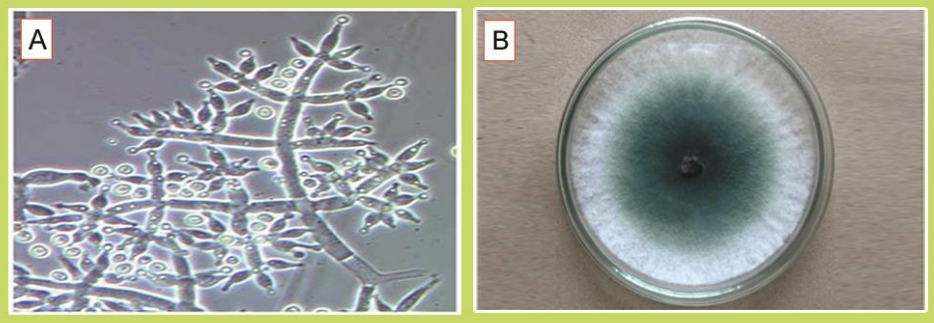


Fig. 7b. *Trichoderma viride* (A) Micrograph (B) Pure culture on PDA medium
Photo courtesy: Department of Plant Pathology, Assam Agricultural University, US Department of Agriculture, Agricultural Research Service, Systematic Botany and Mycology Laboratory

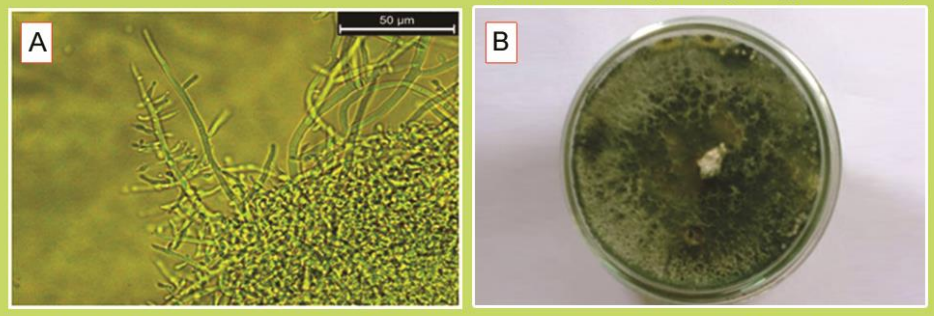


Fig. 7c. *Trichoderma parareesei* (A) Micrograph (B) Pure culture on PDA medium
Photo courtesy: Department of Plant Pathology, Assam Agricultural University

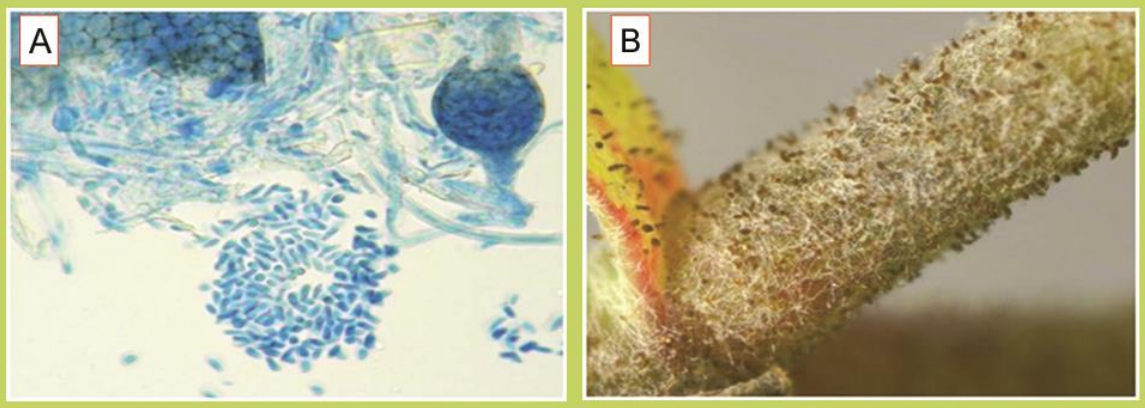


Fig. 8. *Ampelomyces quisqualis* (A) Micrograph (B) mycelium and pycnidia covering plant stem

Photo courtesy: discoverlife.org

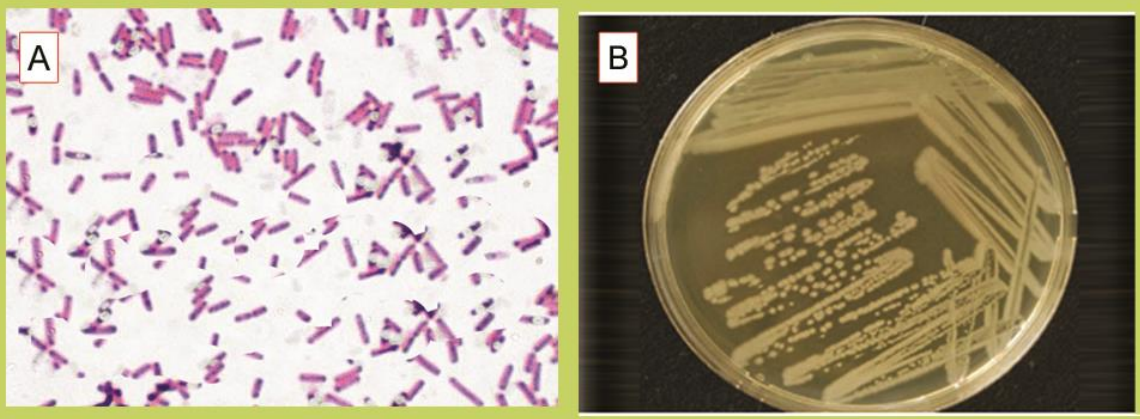


Fig. 9. - (A) Micrograph (B) Pure culture on Nutrient Agar Media

Photo Courtesy: textbookofbacteriology.net

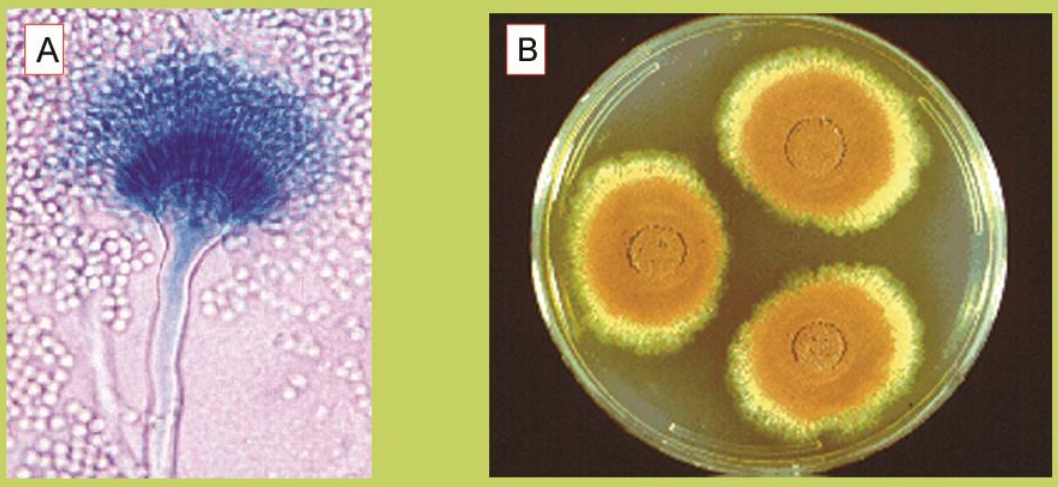


Fig. 10. *Aspergillus terreus* (A) Micrograph (B) Pure culture on PDA medium

Photo Courtesy: mycology.adeilede.edu

TYPES OF BIOLOGICAL CONTROL

There are three broad types of Biological control:

I. Conservation
II. Classical biological control and
III. Augmentation

I. Conservation biological control

The conservation of natural enemies is probably the most important and readily available biological control practice available to growers. Natural enemies occur in all production systems, and are adopted to the local environment as well as to the target pest, and their conservation is generally simple and cost-effective. These natural controls are important and need to be conserved and considered for pest management decisions.

II. Classical biological control

Classical biological control aims at introducing the exotic natural enemies of inadvertently introduced alien organisms in order to re-establish the balance between the pests and natural enemies. Introduction of host-specific organisms from the country of origin of the pests offers some highly effective and environmentally friendly solutions to the problem of invading alien pests.

II. Augmentation

This third type of biological control involves the supplemental release of natural enemies. Relatively few natural enemies may be released at a critical time of the season (inoculative release) or literally millions may be released (inundative release). Additionally, the cropping system may be modified to favor or augment the natural enemies.

MODE OF ACTION OF BIOLOGICAL CONTROL AGENTS (BCAs)

BCA reduce disease of the target crop usually by one or more of the following mode of action: antagonism, hypo virulence, and the induction of host resistance.

Antagonism:

Antagonism is a "type of symbiosis (living together of two unlike organisms) in which one organism is harmed by the other either by the latter being parasitic or predatory on former, or through competition for food in short supply, or through secretion of certain toxic substance," The antagonistic action can be broadly divided into three categories

(i) Direct "parasitism" or "predation" of other organisms over pathogenic ones (exploitation).
(ii) Active demand of nutrient over supply, a situation which results primarily from quicker and greater utilization of available nutrients by BCA with the result that pathogens face lysis or suppression due to starvation (competition).
(iii) Suppression of pathogenic organisms due to secretion of toxic or inhibitory compounds by BCA (antibiosis).

Exploitation (Parasitism / Predation):

Exploitation is an antagonistic condition wherein an organism directly harms another organism to get benefit out of the harm done to the organism. This phenomenon is operated through **parasitism and predation**.

A **parasite** develops some sort of etiological relationship with its host and the latter is exploited slowly, whereas a **predator** physically eliminates its prey (host) by direct feeding on it without establishing any etiological relationship. Predators are the organisms that prey and feed on other organisms. They often feed on various stages of the host (pest): eggs, larvae, pupae and adult. Each predator kills and feed on a number of prey individuals during their development (larvae to adult). Most adults are also predators.

Parasitoids are the organisms which during the larval stages feed on pests (external parasitoids) or in the pest (internal parasitoids). They complete their development on a single host, killing it. Some wasps paralyze insects and seal them in a nest with an egg. The emerging larva then feeds on the paralyzed victim.

Pathogens are agents that cause disease in organisms. Examples include fungi, bacteria, viruses and nematodes.

Mycoparasitism, hyperparasitism, direct parasitism or interfungus parasitism. :

The mechanism of biocontrol is also called **destructive mycoparasitism**. This is a parasitism of a pathogenic fungus by another fungus. Most of plant diseases are due to fungi, and large number of fungi parasitizing on fungi are known. When one fungus parasitizes another, the phenomenon is called **mycoparasitism, hyperparasitism, direct parasitism, or interfungus parasitism**. It involves direct contact between the fungi resulting in death of the plant pathogen, and nutrient absorption by the parasite.

Competition

Competition is an ability of BCA to compete successfully with pathogenic organisms. They compete with each other for carbon, nitrogen, oxygen, iron and other micronutrients. Successful competition occurs at the infection court, preventing the ingress of the pathogen, and sometime limits reproduction of the pathogen. Thus, competition occurs for both nutrients and infection sites (space).

Antibiosis

Antibiosis is defined as inhibition of the growth of one microorganism by another as a result of diffusion of an antibiotic. Antibiotic production is important for survival of microorganisms through elimination of microbial competitors. In biological control, a**ntibiosis** is that antagonistic condition, where suppression of pathogenic microorganisms occurs due to secretion of toxic or inhibitory compounds (antibiotics) by BCA. Such compounds range from hydrogen cyanide (HCN) to enzymes. Bacteria are the microbial BCAs that are known to produce most diverse range of antimicrobial compounds, like Bacilysin, fengumycin, Zwittermicin (*Bacillus*). The first commercial BCA, which has been used successfully to control crown gall disease by *Agrobacterium tumefaciens* was strain K84 of *Agrobacterium radiobacter*.

Hypovirulence

Hypovirulence is the phenomenon of reduced virulence of a pathogen strain than normal ones developed as a result of its infection by double-stranded RNA (dsRNA). When a hypovirulent strain is co-inoculated with highly virulent strain of a fungus, the latter became hypovirulent normally by hyphal contact (anastomosis). Some transmissible factor moves from the hypovirulent strain into the more aggressive one.

Induction of Host Resistance

Induction of resistance in host in an alternative, and quite different, mode of action of BCA for suppression of disease in plants. Rhizosphere bacteria (rhizobacteria) applied to seeds or roots induce systemic resistance (ISR) response expressed against pathogens infecting aerial tissues. This induction ISR is expressed due to the production of a signal molecule(s) by the colonizing BCA, which activates systemic acquired resistance (SAR) pathway resulting in release of pathogenesis-related (PR) proteins.

Suppressive Soils

Soils, through the microorganisms they harbour or through other means, suppress the development of certain diseases caused by soil borne pathogens. Numerous non-pathogenic microorganisms, mostly fungi and bacteria, antagonize various plant pathogenic fungi, bacteria, and nematodes, and some of them have been shown to protect the host plant from infection by the pathogen.

Direct Protection by Biocontrol agents:

Biological control practices for direct protection of plants from pathogens involve the deployment of antagonistic microorganisms at the infection court before or after infection take place. The mechanisms employed by biocontrol organisms in weakening or destroying the plant pathogens they attack are primarily

i) Their ability to parasitize the pathogens directly,
ii) Production of antibiotics (toxins) against the pathogens,
iii) Their ability to compete for space and nutrients and to survive in the presence of other microorganisms
iv) Production of enzymes that attack the cell components of the pathogens,
v) Induction of defense responses in the plants they surround,
vi) Metabolism of plant produced stimulants of pathogen spore germination, etc.

Although thousands of microorganisms have been shown to interfere with the growth of plant pathogens in the laboratory, greenhouse, or field and to provide some protection from the diseases caused by them, strains of relatively few microorganisms have been registered and are available commercially for use so far. The most commonly used microorganisms include three fungi:

a) *Gliocladium virens,* sold as GlioGard for the control of seedling diseases of ornamental and bedding plants.
b) *Trichoderma harzianum,* sold as F-Stop, for the control of several soil borne plant pathogenic fungi
c) *Trichoderma harzianum/T. polysporum,* sold as BINAB T, for the control of wood decays.

The other three commercially available microorganisms are bacteria:
d) *Agrobacterium radiobacter* K-84, sold as Gallex or Galltrol for use against crown gall
e) *Pseudomonas fluorescens,* sold as Dagger G for use against *Rhizoctonia* and *Pythium* damping-off of cotton
f) *Bacillus subtilis,* sold as Kodiak and used as a seed treatment.

CHARACTERISTICS OF AN EFFECTIVE BIO CONTROL AGENT

An effective biological control agent (BCA) that results in protection against disease caused by plant pathogenic organisms must have the following characteristics:

- BCA must be able to control the pathogen by inhibiting its development, making it vulnerable to other members of the prevailing microflora or killing it.

- Mechanisms by which the inhibitions is done include, competition for nutrients or potential sites of penetration of the host, production of antibiotics or lytic enzymes. Parasitism or inhibition of the mechanism by which the pathogen attacks its host, e.g, inhibiting enzymes necessary for penetration or destroying toxins.

- BCA must be able to establish itself at the appropriate location and at a sufficient density to give effective control. For air-borne pathogens, the BCA must be able to compete with the naturally occurring microflora and withstand fluctuations in the microclimate normally associated with the crop. These may include high temperatures and high light intensities as well as wash-off by rainfall. For soil-borne pathogens, the BCA must be able to compete with the soil microflora and to grow in the rhizosphere competent.

GUIDELINE FOR ESTABLISHMENT OF A BIOPESTICIDE PRODUCTION UNIT

The basic objective of this chapter is to highlight out the minimum infrastructural and other facilities, equipments and legal formalities that are essential to install a production unit of microbial biopesticide unit.

Land selection:

Land is required for construction of basic infrastructures like, buildings, water, electricity and waste disposal facilities. The area selected for establishment of production unit must be sufficiently isolated from human habitat to ensure that there is no possibility of contaminant drifts. The land has to be located away from industrial unit to avoid pollution problems. A small scale production unit can also be started in a rented building to avoid land cost.

Water:

A perennial source of water free from any kind of pollutants is very important near the proposed production unit. The water quality should be scientifically tested.

Electricity:

Power supply line is essential for bio-pesticide units. Irregular electricity may cause damage to microbial culture; fermenting cultures *etc*. Preferably, the unit should be connected to a 440 V power line and must be equipped with electricity backup facilities like generators or other kind of electricity solutions for uninterrupted power supply.

Building and civil works:

Microbial bioformulation production involves culturing of microbial bioagents. Hence, it does not need any heavy construction work. A basic civil infrastructure appropriately fulfils the objective. The building must have a raw material storage room, chemical storage room, culture and media preparation room, sterilization room, inoculation and incubation room, quality evaluation room, packaging and storage room, office, staff room, lavatory *etc.*

To install a medium capacity biopesticide production unit, built up area of approx. (110x55) feet is sufficient to carry out different production related activities smoothly.

A typical design of a biopesticide production unit is given in this chapter showing various components of a production establishment. The unit should be built such a way that prevents dust contamination. Biopesticide production room and product storage room must be equipped with air conditioning facilities. It is very much important to have proper arrangement for waste disposal in compliance with pollution control norms.

Plant equipments and machineries:

In a small scale microbial biopesticide unit, large heavy plant or machineries are not required. The basic equipments and infrastructural facilities required for manufacturing microbial bio pesticides formulations are already discussed in previous chapters.

Raw materials:

For production of biopesticides raw materials like talcum powder, vermicompost, wheat bran/rice bran, various chemicals, glassware, plastic ware, polypropylene bags *etc.* are required. Before setting up a production unit make sure that all these materials are locally available in sufficient quantity.

Manpower:

Production of bio-pesticides is a labour intensive industry. There is need of both skilled and semi-skilled manpower. The numbers and position of manpower requirement depends on the production capacity of a unit. On a small scale production unit (two ton/month) following mentioned manpower are needed at different stages of production:

Positions	Numbers
Administrative manager	1
Scientific adviser	1
Technical staff	1
Skilled labour	2
Semi-skilled labour	5

Production capacity:

A biopesticide production unit can be setup on a small or large scale depending upon the market demand and technological expertise available. Medium to Small scale biopesticide

production facilities at regional level is particularly well fitted to meet the local demands, which can be undertaken by farmers, unemployed local youths and SHGs as a profession after receiving proper institutional training.

Medium and large scale production units are best suitable for large firms, agro-chemical companies, cooperative societies which are already engaged in the manufacture and distribution of agro-chemicals that are equipped with the in-house technical expertise and marketing resources. The demand for biopesticides are presently increasing and there is a wide gap between the production and demand, which can be bridged by setting up of smaller and medium biopesticide production units. So, there is a scope of these small and medium enterprises in the days to come as the biopesticide demand is on the increase every year.

Quality control:

It is very important to have effective quality control measures in a biopesticide unit. The Directorate of Plant Protection Quarantine and Storage, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India strictly enforced the quality control standards of commercial bioformulation. All the production units have to meet these standards and technical specifications to be eligible for registration under the Insecticides Act, 1968.

Biopesticide production unit and product registration:

It is illegal to deal with biopesticide production and sell without registration under the Insecticides Act, 1968. In this connection, The Directorate of Plant Protection Quarantine and Storage, Department of Agriculture and Cooperation, Ministry of Agriculture, GOI have issued guidelines/data requirements for registration of bio-pesticides in the country. At present, Neem based formulations, *Bacillus thuringiensis* and other microbial pesticides like fungi, NPV *etc*., are included in the schedule of Insecticides Act, 1968.

SELECTION (SCREENING) OF BIOLOGICAL CONTROL AGENTS

The environment harbours a multitude of potential BCAs. These may form part of the resident microflora of aerial and root surfaces of plants as well as existing independently in the soil. In order to exploit these resources efficiently it is necessary to select promising sources of potential BACs and to use appropriate screening procedures to single out the microorganisms responsible. Soils with a history of suppressiveness towards a given pathogen are obvious sources o potential BCAs.

DEVELOPMENT OF BIOLOGICAL CONTROL AGENTS

Biocontrol agents normally possess several of the following characteristics:

1. Ability to associate sufficiently closely with the plant to exert an effect on the pathogen, i.e. to be phyllosphere or rhizosphere competent.
2. Ability to compete with the pathogen for nutrients or niches, e.g. infection courts.
3. Production of antibiotic compounds.
4. Production of lytic enzymes effective against the pathogen.
5. Ability to parasitize the pathogen.
6. Ability to interfere with the reproduction of the pathogen.
7. Ability to interfere with the virulence mechanisms of the pathogen.
8. The induction of host defense mechanisms.

Since, no single BCA possesses all above mentioned characteristics, it is often advantageous to combine BCAs that exert control by different mechanisms.

APPLICATION AND ESTABLISHMENT OF BCAs

Biological control agents provide effective control only when they are applied at the right place at the appropriate time in sufficient amount and successfully establish there. This programme is not so simple and faces major difficulty. Application as seed dressing is very attractive but it is necessary for the shelf-life of the BCA to be sufficiently long and perfectly to match that of the seed itself. Development of the BCA plays important role here. Environmental impact is other aspect that needs care before the widespread application of any biological control agent. The BCA cannot affect population dynamics of other microorganisms in the changed environment.

Commercially Available BCAs

Hundreds of biological control agents have been demonstrated to reduce disease severity in the laboratory, greenhouse, or field. Many of them have shown very promising effect in their experimental trials, but have yet to make a significant impact in the market place. To date, however, very few BCAs have been registered and are commercially available for use. Some them are fungi and some are bacteria.

Although the actual use of commercially available BCAs is rather limited, it is expected that these and other such products will find wide acceptance in the near future. The main constraints in the slow progress in use of these products are the apprehension of the farming community (users) about both its efficacy and safety. It is definite and has been emphasized time and again that the future of plant disease management cannot rest on the shoulders of hazardous chemicals and therefore biological control has to play significant role in days to come.

Table 3: COMMERCIALLY AVAILABLE BCAS, THEIR TRADE NAME(S), AND THE TARGET PESTS

Sl. No.	BCA	Trade name (s)	Target
	Fungi/Bacteria		
1	*Trichoderma harzianum*	F-Stop	Several Soil-borne diseases, wood decays, mushroom bubble, silver leaf of fruit trees
2	*Trichoderma harzianum*/ *T. polysporum*	Binab-dry	
3	*Gliocladium virens*	GlioGard	Seedling diseases of ornamental
4	*Peniophora, Phlebia gigantean*	PG suspension	*Heterobasidium* rot
5	*Agrobacterium radiobacter* K84	Gallex, Galltrol, Nogall	Crown gall disease
6	*Pseudomonas fluorescens*	Dagger G, Biofor-PF2	*Rhizoctonia* and *Pythium* damping off
7	*Bacillus subtilis*	Kodiak, Quantum 400	Seedling diseases
8	*Streptomyces griseoyiridis*	Mycostop	Seedling diseases

PRODUCTION TECHNOLOGY OF VARIOUS BIOPESTICIDES

There are various bacteria as well as fungi which are being used in the production of various biopesticides, (*viz, Trichoderma sp., Verticillium sp., Metarhizium anisopliae, Beauveria bassiana, Bacillus thuringiensis, Pseudomonas fluorescens.*) The production technology of few of these common biopesticidal formulations are discussed below.

Strain Selection

The efficacy of a biopesticide formulation solely depends on strain selection of a bio agent. To produce an effective bioformulation the bio agent used must have higher antagonistic activity against the wide range of pathogens, good rhizosphere fitness, longer shelf life and higher multiplication rate. During last 10 years Department of plant Pathology, Assam Agricultural University have identified a number of *Trichoderma* strains which emerged as successful bio control agent in different crop field. These strains are very much suitable for commercial formulation preparation.

Liquid fermentation based bioformulation production protocol:

Generally, in liquid fermentation based technology biocontrol agents are mass multiplied in liquid medium followed by blending with solid substrate to prepare a formulation. Production technologies of the few bioformulations are discussed below.

The major steps involved in production process includes-

I. Selection of microbial bioagent strains

II. Preparation of substrate/carrier material and sterilization

III. Preparation of microbial inoculants, osmoticant, adjuvants and nutrient sources

IV. Blending/inoculation and incubation

V. Quantitative & qualitative evaluation

VI. Packet sealing and storage

Production technology of *Pseudomonas fluorescens* and *Trichoderma viride* based biopesticidal formulation

Description of the bacterial bioagent *Pseudomonas fluorescens*

Pseudomonas fluorescens is a gram negative, rod shaped bacterium under the genus *Pseudomonas.* It has multiple flagella, and it can be present in the soil, plant and water surfaces. *Pseudomonas fluorescens* is an obligate aerobe. The optimum growth temperature is between 25-30^{o}C. A number of *Pseudomonas fluorescens* strains present in the plant's rhizosphere (root zone) and produces a variety of secondary metabolites including antibiotics against soil borne plant pathogens. *P.fluorescens* can produce soluble, green fluorescent pigments when the iron concentration is low in the soil. Some strains of *Pseudomonas fluorescens are* use as bio-control agents against a number of Fungal plant pathogens like *Fusarium, Pythium, Rhizoctonia, Verticillium, Sclerotinia* etc and bacterial plant pathogens like *Ralstonia, Xanthomonas* etc as well as some phytophagous nematodes.

Description of the fungal bioagent *Trichoderma viride*

Trichoderma viride is a filamentous fungus under the family *Hypocreaceae. Trichoderma spp.* is present in nearly all soils and other diverse habitats. In soil, they frequently are the most prevalent culturable fungi. The fungus produces dirty green to faded green colour colonies on PDA media. The fungus produces ovoid conidia with size 3.5 x 2-4 µm. The tips of the phialids (an open-ended, tubular or flask-like conidiophore that produces conidia.) are oval. *T. viride* is one of the most effective and widely used antagonists against both fungal and bacterial pathogen. They antagonize pathogens by causing lysis due to host cell penetration, competition for space and food, antibiosis by producing a number of antibiotics.

Production of *Pseudomonas fluorescens and Trichoderma viride* based biopesticide (e.g: Biofor-Pf-2 and Biozin-PTB) using vermicompost and talc as carrier materials.

Materials required for preparation of inoculants and osmoticant, adjuvants and nutrient sources:

1. Potato Dextrose Agar broth (Potato- 200 g, Dextrose- 20 g, Agar Agar- 20 g and distilled water- 1 lit.)

2. King's B broth (Petone-20g, K_2HPO_4-1.5 g, $MgSO_4$-1.5g, Glycerol-15ml, distilled water-1 lit. sterilized at 121^oC for 15 minutes).
3. 1% mannitol solution (12g mannitol in 1 litre distilled water and sterilized at 121^oC for 15 minutes).
4. 1% CMC solution (10g Carboxy methyl Cellulose in 1 litre distilled water and sterilized at 121^oC for 15 minutes).
5. Pure tube culture of *Pseudomonas fluorescens* maintained in King's B media.
6. Pure tube culture of *Trichoderma viride* maintained in PDA media.
7. Conical flask
8. Measuring cylinder

Materials required for substrate preparation:

1. Vermicompost or good quality talcum powder
2. Polypropylene bags (23 x 30) cm size
3. Non-absorbent cotton
4. Rubber band
5. Sealing machine
6. Distilled water

Packet inoculation of *Trichoderma viride and Pseudomonas fluorescens*

1. 1% mannitol solution (12g mannitol in 1 litre distilled water and sterilized at 121^oC for 15 minutes).
2. 1% CMC solution (10g Carboxy Methyl Cellulose in 1 litre distilled water and sterilized at 121^oC for 15 minutes).
3. 1% humic acid
4. Pure tube culture of *Pseudomonas fluorescens* maintained in King's B media.
5. Pure tube culture of *Trichoderma viride* maintained in PDA media plates.
6. Sterilized vermicompost/talc powder.

Procedure:

(I) Substrate preparation and sterilization

1. Fill the polypropylene bags with 1 kg of finely sieved vermicompost or talc powder and add 100 ml clean filter water.

2. Put cotton plug in the mouth of the packet and close the mouth with a heat resistant rubber band or twine thread.
3. Sterilized the filled packets at 121^{o}C under 15 psi pressure for 15 minutes.

(II) Preparation of inoculants

(a) *Pseudomonas fluorescens*

1. Switch on the UV lamp of the laminar flow desk for half an hour before work.
2. Surface sterilize the working laminar air flow desk with 70% ethyl alcohol. Before that surface sterilize hands with a cotton swab dipped in 70% ethyl alcohol.
3. Switch on the laminar air flow and light the spirit lamp.
4. Keep the required materials like King's B broth, culture tube containing 24 hours old *Pseudomonas fluorescens* on the working desk of the laminar air flow.
5. Now carefully open the cotton plug of the culture tube containing *Pseudomonas fluorescens* near the spirit lamp and pour little amount of King's B broth media in to the tube aseptically.
6. Put again the cotton plug in to the tube as well as in to the conical flask containing King's B broth media immediately.
7. Shake the tube for a minute.
8. Transfer this suspension of King's B broth and *Pseudomonas fluorescens* from the tube conical flask containing King's B broth media.
9. Incubate the flask at 27±1^{o}C for 72 hrs.
10. Yellowish green coloration of culture broth indicates proper growth of *Pseudomonas fluorescens.*

(b) *Trichoderma viride*

1. Switch on the UV lamp of the laminar flow desk for half an hour before work.
2. Surface sterilize the working laminar air flow desk with 70% ethyl alcohol. Before that surface sterilize hands with a cotton swab dipped in 70% ethyl alcohol.
3. Switch on the laminar air flow and light the spirit lamp.
4. Keep all the required materials on the desk.
5. Sterilize the inoculating needle using 70% ethyl alcohol then flame the tip of the needle on spirit lamp.
6. Transfer *T. viride* from the tubes to Petri plates containing PDA media.

7. Incubate the freshly transferred plates at 27±1°C for 72 hrs in inverted position.

(III) Packet inoculation of *Trichoderma viride and Pseudomonas fluorescens*

1. 1% mannitol solution (12g mannitol in 1 litre distilled water and sterilized at 121°C for 15 minutes).
2. 1% CMC solution (10g Carboxy Methyl Cellulose in 1 litre distilled water and sterilized at 121°C for 15 minutes).
3. 1% humic acid
4. Pure tube culture of *Pseudomonas fluorescens* maintained in King's B media.
5. Pure tube culture of *Trichoderma viride* maintained in PDA media plates.
6. Sterilized vermicompost/talc powder.

Procedure

1. Switch on the UV lamp of the laminar flow desk for half an hour before work.
2. Surface sterilize the working laminar air flow desk with 70% ethyl alcohol. Before that surface sterilize hands with a cotton swab dipped in 70% ethyl alcohol.
3. Switch on the laminar air flow and light the spirit lamp.
4. Keep the chemicals and other materials like *Pseudomonas fluorescens* maintained in King's B media, culture plate containing *T.viride*, Mannitol, CMC, alcohol, cork borer, inoculating needle, forceps, *etc.*
5. Sterilize cork borer, forceps and inoculating needle using alcohol as well as by flaming in spirit lamp.
6. Fill three measuring cylinders with liquid culture of *P. fluorescens*, 1% mannitol solution, 1% CMC solution respectively and place these over the laminar desk.
7. Using the sterilized cork borer cut out 2 mm discs of *T.viride* from the culture plate.
8. Carefully open the cotton plug of the polypropylene packet containing vermicompost powder and keep the packet near the spirit lamp.
9. Pick one disk of *T.viride* (2 mm) from the petri plate with the help of a sterilized forceps and place it aseptically inside the polypropylene packet.
10. Now pour 10 ml each of *P. fluorescens* containing King's B broth, mannitol (1%), CMC (1%) in to the polypropylene packet.
11. Put on the cotton plug in the packet, tighten with rubber band.

12. Keep the inoculated packets in the incubator at 27±1^{o}C for multiplication of *P. fluorescens* and *T.viride.*
13. After 24 hr of incubation shake the packets manually or using mechanical shaker for uniform spread of *P. fluorescens* and *T.viride* in the vermicompost substrate. This operation should be repeated on each alternate day for 1 week.
14. After 1 week of incubation and continous shaking the inoculated packets should be brought out from the incubator and evaluate for presence of required population of both *P. fluorescens* (cfu/g) and *T.viride* (cfu/g).

a) Mass multiplication of *Trichoderma viride*

Preparation of mother culture

Molasses yeast medium is prepared as detailed below.

Molasses	: 30 g
Yeast	: 5 g
Distiller water	: 1000 ml

The medium is prepared and dispensed into conical flasks and sterilized at 15 lb pressure for 15 minutes in an autoclave. After the medium is cooled it is in inoculated with 10 days old fungal disc of *T. viride* and then incubated for 10 days for fungal growth. This serves as mother culture.

Mass multiplication:

Molasses yeast medium is prepared in fermenter and sterilized as described earlier. Then after the medium is cooled, the mother culture is added to the fermenter @ 1.5 lit / 50 lit of the medium and incubated at room temperature for 10 days. Then the incubated broth containing the fungal culture is used for commercial formulation preparation using talc powder.

b) Mass production of *Pseudomonas fluorescens*

Preparation of mother culture

Mother culture is prepared by using the king's B medium

Peptone	: 20.0 g
$K_2 HPO_4$	: 1.5 g
$Mg SO_4$	: 1.5 g
Glycerol	: 10 ml
Distilled water	: 1000 ml

The above broth is dispersed into conical flasks and autoclaved at 15 lb pressure for 15 minutes and cooled and inoculated with a loop of *P. fluorescens* and incubated for 2 days.

Mass multiplication:

The kings B medium is prepared and poured into the fermenter and sterilized at 15 lb pressure for 15 minutes. After the broth has cooled below the mother culture of *P.fluorescens* is added to the king's B medium in the fermenter at the rate of 3 lit for 40 lit of the broth. Then it is incubated in the fermenter for 2 days with frequent mixing of the broth by operating the stirrer. Then the broth containing the bacterial growth is collected in plastic buckets and used for mixing with talc powder for commercial formulation.

c) Mass multiplication of *Bacillus subtilis*

Preparation of mother culture

The nutrient broth medium is prepared as detailed below

Glucose	: 5.0 g
Peptone	: 5.0 g
Beef extract	: 3.0 g
Sodium chloride	: 3.0 g
Distilled water	: 1000 ml

The above medium is dispensed in conical flasks and autoclaved at 15 lb pressure for 15 mts. A loop of *B. subtilis* is inoculated into the medium and incubated for 2 days. This serve as the mother culture.

Mass multiplication:

The nutrient broth is prepared in fermenter and sterilized at 15 lb pressure for 15 mts. Then the mother culture is added @ 1 lit / 100 lit of the medium and incubated at room temperature for 2 days. The medium containing the bacterial growth of *B. subtilis* is used for mixing with talc powder.

PREPARATION OF TALC BASED PRODUCTS, AIR DRYING OF FORMULATION AND ESTIMATION OF MOISTURE CONTENT

a) *Trichoderma viride:*

The fungal biomass collected from fermenter is mixed with talc powder at 1:2 ratio. The mixture is air dried in shade and mixed with carboxy methyl cellulose (CMC) @ 5 g / kg the product. It is packed in polythene bags and should be used within 4 months.

Quality control parameters:

1. Fresh product should contain hot less than 28 x 10^6 cfu / g
2. After 4 months of storage at room temperature, the population should be 20 x 10^6 cfu/ g.
3. Maximum storage period in talc is 4 months.
4. The talc size should be 500 microns
5. The product should be packed in polythene bags
6. Moisture content of the final product should not be more than 20%

b) *Bacillus subtilis*

The broth containing the bacteria is collected from fermenters and mixed with 250 kgs of sterilized neat soil for 100 lit of broth. Then 37 kgs of calcium carbonate is added thoroughly mixed, dried is shade and packed in polythene bags. This can be stored upto 6 months.

c) *Pseudomonas fluorescens*

The broth containing the bacterial growth is collected from fermenter and added @ 400 ml / kg of talc powder. Then CMC is added @ 5 g /kg mixed well air dried to 20% moisture level and packed in polythene bags.

Quality control parameters:

1. Fresh produce should contain 2.5 x 10^8 cfu/g
2. After 3 months of storage at room temperature the population should be 8-9 x 10^7 cfu/g
3. Storage period is 3-4 months
4. Minimum population load should be 1.0 x10^8 cfu /g
5. Moisture content should not exceed 20% in the final product
6. Population per ml of the broth should be 2 x 10^8 cfu /g

Production of *Pseudomonas fluorescens* & *Trichoderma* based biopesticide at AAU, Jorhat

Weighing of substrates

Addition of Moisture

Putting cotton plug

Plugged packets

Carrying to autoclave room

Sterilization of substrates

Sterilized substrates

Bioagents

Arranging required materials on laminar air flow desk

Inoculation with bioagents (i)

Inoculation with bioagents (ii)

Addition of Nutrients

Addition of adjuvants

Incubation

Stirring of packets

Sealing of packets

Final packets of biopesticides

POTENTIAL BIOAGENTS USED IN BIOPESTICIDE PRODUCTION IN ASSAM AGRICULTURAL UNIVERSITY

Biofor-Pf 2 (Vermicompost Based)
Biozin –PTB (Talc Based)

Bioagents : ***Pseudomonas fluorescens and Trichoderma viride***

- Manages several soil borne diseases like Wilt of Tomato, Brinjal, Potato, Chilli, Ginger, Turmeric and Bhoot Jolokia caused by *Ralstonia solanacearum.*
- Also effective against diseases caused by *Xanthomonas* spp., *Rhizoctonia* spp., *Fusarium* spp., *Colletotrichum* spp. etc.

Bio-Meta (Talc Based)

Bioagent : ***Metarhizium anisopliae***

- Manages a number of insect pests like Red Ant, Red Spider Mite, Termites, Plant Hoppers, Diamondback Moth etc. of a number of crops like potato, mango, coconut, tea, coffee, cotton, cabbage, onion, sugarcane etc.

Bio-Ilium (Talc Based)

Bioagent : ***Verticellium lecanii***

- Can be used against Insect pests like Mites, White flies, Thrips, Aphids, Nematode pests etc. of various fruit crops like grape, guava, mango, banana and cococnut, potato, tomato, chilli, brinjal etc.

Bio-Time (Talc Based)

Bioagents : ***Pseudomonas fluorescens,Trichoderma viride and Metarhizium anisopliae***

- Manages several soil borne diseases like Wilt of tomato, Brinjal, Potato, Chilli and Bhoot Jolokia caused by *Ralstonia solanacearum.*
- Also effective against diseases caused by *Xanthomonas* spp., *Rhizoctonia* spp., *Fusarium* spp., *Colletotrichum* spp. etc. and a number of insect pests like Red Ant, Red Spider mite, Termites etc.

Bio-Sona (Talc Based)

Bioagent : ***Beauveria bassiana***

- Manages whitefly, aphids, thrips, psyllids, weevils, and mealy bugs of Vegetable and plantation crops, rice hispa, fruit borer of tomato and brinjal, Tea mosquito bug, Tea Loopers, pests of banana etc.

Bio-Zium (Talc Based)

Bioagent : ***Trichoderma harzianum***

- Manages Rhizome rot of ginger and turmeric, wilt and damping off disease of Bhoot jalakia, tomato, chilli, brinjal, wilt of pepper and betel vine and other soil borne plant diseases.
- Also can be used as an effective wound dressing.

Bio-Veer (Talc Based)

Bioagent : ***Trichoderma viride***

- Management of Rhizome rot of ginger and turmeric, wilt and damping off disease of Bhoot jalakia, tomato, chilli, brinjal, wilt of pepper and betel vine.
- Can be used as an effective wound dressing.

QUALITY CONTROL OF MICROBIAL BIOPESTICIDES THROUGH ASSESSMENT OF VIABLE POPULATION COUNT AND SHELF LIFE

Preparation of culture media: PDA (for fungal bioagents), King's B agar (for *P. fluorescens*) and Nutrient agar (for other bacterial bioagents) are prepared for inoculation.

Sterilization of water blank: Sterile water blanks can be prepared by taking 9ml of distilled water in each test tubes. Autoclave is used for sterilization of the water blanks.

SERIAL DILUTION OF MICROBIAL PESTICIDES:

Procedure:

1. Put on the UV lamp of inoculation chamber for 15 minutes before starting the isolation procedure. Swab the table top and also hands with 70% ethyl alcohol and air dry. Light the burner or spirit lamp, arrange sterile petri dishes near the burner.

2. Assemble the 9 ml sterile water blank culture tubes on a test tube rack and label as 10^{-1} to 10^{-9} .

3. Make ready agar media plates of PDA (for fungal bioagents), King's B agar (for *P. fluorescens*) and Nutrient agar (for other bacterial bioagents).

4. Carefully open the cotton plug of one randomly selected inoculated and incubated bioformulation packet from a production batch under laminar air flow cabinet and measure out 10g of formulation.

5. Replace the agar plug in the biopesticide packets.

6. Add this 10g to 100ml sterile distilled water and shake on a rotary shaker for 10 minutes at 200 rpm.

7. Pipette out 1ml of aliquot from this suspension using a sterile pipette and transfer it to first tube containing 9 ml sterile water.

8. Transfer 1 ml of aliquot from first tube to second and continue the dilution process up to 10^{-9} dilution.

9. Now draw 0.1 ml aliquot from each of 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} dilutions and spread on previously plated PDA (for fungal bioagents), King's B agar (for *P. fluorescens*) and Nutrient agar (for other bacterial bioagents) plates. For each dilution at least three replications should be maintained.

10. After proper spreading with a sterilized glass spreader mark the culture plates and incubate in an inverted position at 28 ± 1^{o}C for 3-7 days.

11. Bioagent colonies should appear on the inoculated plates after 24-72h of incubation so, examine all incubated plates daily starting from 2^{nd} day of incubation for the presence of individual colonies of the target organism growing throughout the medium.

12. Observe and count the number of colonies appeared on the agar surface with colony counter and record.

13. Calculate the cfu/g of bio formulation by following equation:

$$\text{Viable cells/g (cfu/g)} = = \frac{\text{Mean plate count} \times \text{dilution factor}}{\text{Dry weight of soil}}$$

14. For registration of a bioformulation Colony Forming Unit (CFU) count on selective medium should be minimum of $1x\ 10^{8}$ per ml or gm (for Entomopathogenic bioagent), $2x\ 10^{6}$ per ml or gm (for *Trichoderma* sp.).

REGISTRATION OF BIOPESTICIDES

It is illegal to deal with biopesticide production and sell without registration under the Insecticides Act, 1968. In this connection, The Directorate of Plant Protection Quarantine and Storage, Department of Agriculture and Cooperation, Ministry of Agriculture, GOI have issued guidelines/data requirements for registration of bio-pesticides in the country. At present, Neem based formulations, *Bacillus thuringiensis* and other microbial pesticides like fungi, NPV *etc.,* are included in the schedule of Insecticides Act, 1968.

PROTOCOL FOR REGISTRATION OF BIOPESTICIDES IN INDIA

The Organization for Economic Cooperation and Development (OECD) project on biopesticides was undertaken in 1999 to help the member countries to standardize the methods and approaches to access biopesticides. This has helped Governments to work together and assess pesticide risks to human and the environment. The OECD agreed guidelines (http://www.oecd.org/chemicalsafety/pesticides-biocides/biologicalpesticideregistration.htm) contain two formats:

1. For industry to use when making data submission (dossiers) for microbial and pheromones/semiochemicals, and
2. For governments to use when writing their evaluation reports.

THE CENTRAL INSECTICIDE BOARD (CIB)

In India, biopesticides fall under the Insecticide Act (1968) under which any microbial organism manufactured or sold for pest and disease control should be registered with the Central Insecticides Board (CIB) of the Ministry of Agriculture. The Central Insecticide Board & Registration Committee has formulated simplified guidelines for registration of biopesticides as compared to chemical pesticides. During provisional registration granted under Section 9 (3B) of The Insecticides Act, 1968, the applicant is allowed to commercialize the biopesticides, unlike chemical pesticide. Data on product characterization, efficacy, safety, toxicology, and labeling must be submitted while applying for registration.

THE ASSAM SCENARIO AND SCOPE FOR ENTREPRENEURSHIP DEVELOPMENT

Though there are about 140 biopesticide production units existing in the country as on today, they are able to meet the demand of only less than 1% of cropped area. There exists a wide gap, which can only be bridged by setting up of more and more units for production of biopesticides. This requires large scale investment and private participation. Some of the local small scale industries have already started production and marketing of *Trichoderma viride* (against few fungal diseases) and *Trichogramma* (against sugarcane early shoot borer). There is a scope to enhance production and use of biological control agents in the days to come as the demand is on the increase every year.

In Assam, Assam Agricultural University, being the only state agricultural university have taken an arduous task of developing the biopesticide industry in the state. Table 4.shows the requirements of biopesticide for organic conversion of cropped area in Assam.

Table 4: Biopesticide requirement for organic conversion of cropped areas in Assam

CROP	CROP AREA (000 ha)	REQUIREMENTS/ha (kg)	TOTAL REQUIREMENT(000 mt)
Rice	2500.00	37.00	92.50
Pulses	88.0	33.00	2.9
Ginger/Turmeric	15.00	33.00	0.5
Lemon	13.00	30.00	0.4
Potato	98.00	95.00	9.3
Chilli	18.00	47.00	0.8
Brinjal	16.00	47.00	0.8
Tomato	17.00	47.00	0.8
Garlic	9.00	15.00	0.1
Banana	858.00	52.00	44.6
Coconut	137.00	39.00	5.3
Mustard	170.00	20.00	3.4
Cauliflower	21.00	47.00	1.0
Cabbage	31.00	47.00	1.5
Tea(STG)	150.00	65.00	9.8
TOTAL	**4141.00**	**654.00**	**173.7**
Requirement of biopesticides for 100 per cent organic conversion in Assam			173.7
Requirement of biopesticides for 10 per cent organic conversion in Assam			17.37
Current production capacity of biopesticide units, DBT-AAU Centre			0.024
PRODUCTION DEFICIT			**17.346**

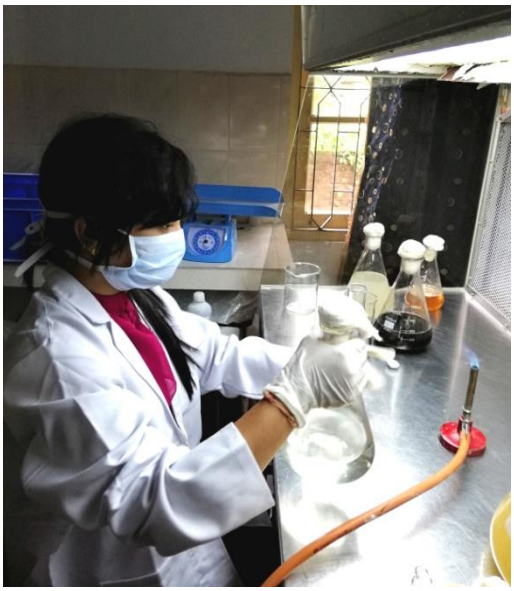
Thus, this shows a huge potentiality for developing entrepreneurs in the state of Assam in the biopesticide production sector. A few firms, *viz* Green Harvest Bio-Tech Pvt. Ltd., Ghy; Nirmal Seeds Pvt. Ltd. Ghy; Vishal Organic, Jorhat; Orgaman R&D Division, Jorhat; Eastern Biotech Solutions, Jorhat; Balaji Chemicals, Dibrugarh are already engaged in biopesticide production and commercialization throughout Assam.

The Assam Agricultural University, Jorhat readily developed technologies to intending user groups through technology transfer module by signing MoU under PPP mode

MoU signed between AAU & Orgaman, Jorhat

MoU signed between AAU & SLRD, Shillong

TRAINING TO DEVELOP ENTRPRENEURES ON BIOPESTICIDE PRODUCTION IN BIOPESTICIDE LABORATORY, PLANT PATHOLOGY, AAU, JORHAT



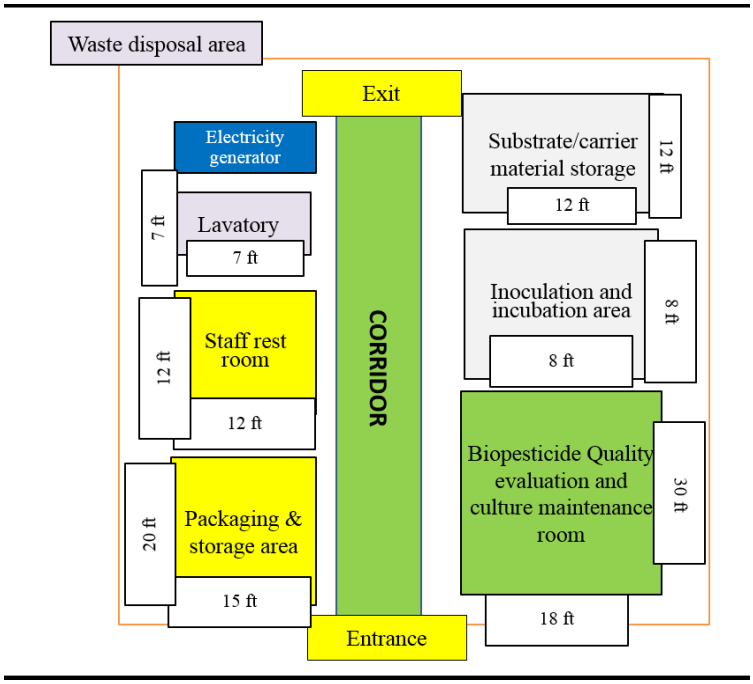
MODEL PROJECT FOR ESTABLISHMENT OF BIOPESTICIDE PRODUCTION UNIT

(Production capacity approx. 12 MTPA carrier based and liquid)

LOCATION OF BIOPESTICIDE UNITS

To achieve optimum results, bio-pesticide facilities are to be set up in areas which have appropriate climatic conditions. Because temperature control is less costly in locations where there are no extreme conditions. Besides the climatic conditions, the proximity of the location to the market is also important. However, care must be taken that the production facilities are set up at least a quarter of a mile away from farming areas, so as to prevent the contamination of production facilities by insecticides from the farming areas. Also, as air pollution can damage bio-pesticides, the production should be located away from industrial and urban areas.

LAYOUT



ECONOMICS

(1) List of Equipments/Plant and Machinery

Sl. No.	Equipments/Machinery/Civil works	Qnty. Required	Cost
1	Building	1	200000.00
2	Vertical autoclave	1	60000.00
3	BOD incubator	1	80000.00
4	Laminar air flow desk	1	70000.00
5	Hot air oven	1	45000.00
6	Refrigerator	1	12000.00
7	Manual sealing machine	1	2000.00
8	Small instruments	-	20000.00
Total (Rs.)			**489000.00**

(2) Consumables (per year requirement)

Sl. No.	Items	Monthly	Yearly
1	Stationeries	10000.00	120000.00
2	Chemicals/glassware	14000.00	168000.00
3	Carrier material (Talcum powder @ Rs. 14/kg)	28000.00	336000.00
Total (Rs.)		**52000.00**	**624000.00**

(3) Manpower (per unit month^{-1})

Sl. No.	Person	Positions	Emolument	Yearly
2	Skilled worker	1	6000.00	72000.00
3	Semi-skilled worker	1	4500.00	54000.00
Total (Rs.)			**10500.00**	**126000.00**

(4) Per unit monthly output of biopesticide involving one skilled and one semiskilled worker

Avr. Daily output	Monthly output	Yearly output
33.3 Kg	1 MT	12 MT

(5) Cost of Production (per annum)

Sl. No.	Particulars	Cost
2	Capital Cost (Depreciation cost of Non-movable items)	19450.00
3	Consumables	624000.00
4	Manpower	126000.00
Total (Rs.)		**769450.00**

Cost of Production (per month) = Rs 769450/12 = Rs. 64121.00

Cost of production (per kg biopesticide) = 64121/1000= Rs. 64.12 /Kg.

CONSTRAINTS

There are many constraints with respect to biological control and some important one are the following.

1. One of the major difficulties is the application of BCAs in getting them to the right place at the right time in sufficient density to be effective and then maintaining them there. For example, use of BCAs as seed dressings is very attractive but it is necessary for the self-life of the BCA to be sufficiently long to be practicable and preferably to match that of the seed itself. In initial field trials of *Bacillus cereus*, the bacterium was added to alfalfa seeds in 1% methylcellulose but later, it was found that clay-granule formulations applied in furrow provided the most consistent results.

2. Other difficulty is the apprehension of the growers about the efficacy of BCAs. For convenience, *Trichoderma harzianum* was formulated by a Japanese company in the form of dry powder spore and mycelia in rice bran and was named as 'Trichoderma Powder'. The later is still available in Japanese market and is used by growers but, as per estimate, only 0.2 ton of formulation was produced by the company in 1994. Similarly, only 0.6 ton of the formulation of *Agrobacterium radiobacter* was produced in 1992 for control of crown gall. However, these market potentials stand nowhere in comparison to fungicide market. This may have been due to the fact that the grower's confidence in such new products remains very less in spite of the fact that over 35% control of the disease was found under field conditions and no adverse effect of BCAs was observed on plants.

3. Safety is not least important. A biocontrol agent may be pathogenic to humans and other members of the biota. For instance, bacteria belonging to the complex *Burkholderia cepaci* are used as biocontrol agents and in bioremediation but some strains are plant pathogens and others are opportunistic pathogens of humans with cystic fibrosis

4. Industries have to play a major role for developing a marketable product of BCAs but, most of them are showing least interest probably because high expenditure in comparision to low profit. This can overcome only if the government institutions bear main responsibility to make his novel approach available by providing subsidies to producers as well as convince the growers to use BCA products.

FUTURE PROSPECTS

Increased population has led to agriculture dependant on chemicals. But its increased residual effects has led to serious health concerns throughout the world. At this need of the hour, the govt. has formulated strict food policies which has led to the ban of many such harmful chemical fertilizers and pesticides.

Owing to the rising food need and keeping in mind the health of the consumers, the use of bio pesticides have emerged as a promising alternative to chemical pesticides. It is

very much likely that in a place like Assam where there is a promising scope for organic conversion by the government, the bio pesticide industry is likely to flourish, leading to sustainable economic growth and agricultural production in the region.

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