Sri Karan Narendra Agriculture University,

Jobner-303 329, Jaipur (Raj.)

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Entrepreneurship Short Course Training
Trichoderma for Plant Diseases Management

Department of Pathology

Sri Karan Narendra Agriculture University, Jobner

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Entrepreneurship Short Course Training

“*Trichoderma* for Plant Diseases Management”

INTRODUCTION:

Globally, enormous losses of the crops are caused by the plant diseases. The loss can occur from the time of seed sowing in the field to harvesting and storage. Important historical evidences of plant disease epidemics are Irish Famine due to late blight of potato (Ireland, 1845), Bengal famine due to brown spot of rice (India, 1942) and Coffee rust (Sri Lanka, 1967). Such epidemics had left their effect on the economy of the affected countries.

When a plant is suffering, we call it diseased, i.e. it is at ‘dis-ease’. Disease is a condition that occurs in consequence of abnormal changes in the form, physiology, integrity or behaviour of the plant. A plant is diseased when it is continuously disturbed by some causal agent that results in abnormal physiological process that disrupts the plants normal structure, growth, function or other activities. This interference with one or more plant’s essential physiological or biochemical systems elicites characteristic pathological conditions or symptoms.

Causes of Plant Diseases

Plant diseases are caused by pathogens. Hence a pathogen is always associated with a disease. In other way, disease is a symptom caused by the invasion of a pathogen that is able to survive, perpetuate and spread. Further, the word “pathogen” can be broadly defined as any agent or factor that incites 'pathos or disease in an organism or host. In strict sense, the causes of plant diseases are grouped under following categories:

1. Animate or biotic causes: Pathogens of living nature are categorized into the following groups.

(i) Fungi (ii) Bacteria (iii) Phytoplasma (iv) Phanerogams (v) Algae (vii) Protozoa

(vi) Rickettsia-like organisms (viii) Nematodes

2. Mesobiotic causes : These disease incitants are neither living or non-living, e.g.

(i) Viruses (ii) Viroides

3. Inanimate or abiotic causes: In true sense these factors cause damages (any reduction in the quality or quantity of yield or loss of revenue) to the plants rather than causing disease. The causes are: (i) Deficiencies or excess of nutrients (e.g. 'Khaira' disease of rice due to Zn deficiency) , (ii) Light , (iii) Moisture (iv) Temperature (v) Air pollutants (e.g. black tip of mango) (vi) Lack of oxygen (e.g. hollow and black heart of potato) (vii) Toxicity of pesticides (viii) Improper cultural practices (ix) Abnormality in soil conditions (acidity, alkalinity)

Agricultural production in India increased dramatically during the last four decades, leading to an era of food self-sufficiency. The remarkable growth was achieved through the uptake of newer technologies in the form of high yielding crop varieties, chemical fertilizers and pesticides, as well as from the expansion of cropped area. Nevertheless, the growth in agricultural production needs to be sustained to meet the food demand of ever-increasing population. Since the prospects for bringing additional land under cultivation are limited, growth in agricultural production has to come from productivity increases. In other words, technology will be a key to future growth of agriculture. Insect pests, diseases and weeds inflict enormous losses to the potential agricultural production. Anecdotal evidences also indicate rise in the losses, despite increasing use of chemical pesticides. At the same time, there is a rising public concern about the potential adverse effects of chemical pesticides on the human health, environment and biodiversity. These negative externalities, though, cannot be eliminated altogether, their intensity can be minimized through development, dissemination and promotion of alternative technologies such as biopesticides and bioagents. The Government of India had adopted IPM as a cardinal principle of plant protection in 1985. Notwithstanding these initiatives, adoption of IPM has not been encouraging as biopesticides capture hardly 2 percent of the agrochemical market. Keeping these facts in mind, Department of Plant Pathology, SKN College of Agriculture formulated an **Entrepreneurship Short Course Training for Final year UG students** under IDP-NAHEP with the title "Trichoderma for Plant Diseases Management".

"The Impact of Plant Disease on Food Security"

A vast number of plant pathogens from viroids of a few hundred nucleotides to higher plants cause diseases in our crops. Their effects range from mild symptoms to catastrophes in which large areas planted to food crops are destroyed. Catastrophic plant disease exacerbates the current deficit of food supply in which at least 800 million people are inadequately fed. Plant pathogens are difficult to control because their populations are variable in time, space, and genotype. Most insidiously, they evolve, often overcoming the resistance that may have been the hard-won achievement of the plant breeder. In order to combat the losses, they cause, it is necessary to define the problem and seek remedies. At the biological level, the requirements are for the speedy and accurate identification of the causal organism, accurate estimates of the severity of disease and its effect on yield, and identification of its virulence mechanisms. Disease may then be minimized by the reduction of the pathogen's inoculum, inhibition of its virulence mechanisms, and promotion of genetic diversity in the crop.

- The novel technologies in all areas of agriculture have improved agricultural production, but some modern practices affect the environment.
- The recent challenge faced by advanced farming is to achieve higher yields in environment-friendly manner

Principles of Plant Disease Management.

Healthy plants look good, grow well, and are productive. Plants remain healthy as long as conditions favor normal plant growth and development. Sometimes plants are unhealthy, and this occurs when something irritates the plant. The irritation may be somewhat continuous, acting over an extended period, or it may occur nearly instantaneously. Continuous irritation causes disease; instantaneous irritation causes injury.

Disease frequently is expressed by production of symptoms. Some common types of symptoms on specific parts of plants include rotting, stunting or swelling of roots; cankering, rotting, discoloration, distortion, elongation, or stunting of stems; wilting, spotting, blighting, rusting, mottling, discoloration, distortion, or stunting of leaves; and spotting, blighting, stunting, discoloration, distortion, or mottling of fruit.

Common agents that cause plant disease and injury both include nonliving (abiotic) and living (biotic) agents. Nonliving factors that cause disease include harmful levels of temperature, moisture, light, nutrient, pH, air pollutants, and pesticides. Living pathogens that cause disease include fungi, bacteria, viruses, viroids, mycoplasmas, and nematodes; most pathogens are microscopic and derive their food by growing in or on the host plant.

General management considerations: the practical reason for studying crop diseases is to develop economical measures for control. Controls must be based on knowledge of the specific disease, pathogen life cycles, the time and the method of infection, the plant parts affected, the method of causal agent dissemination, and certain other agronomic and economic considerations.

Certain guiding principles must be kept in mind; these include:

1. The cost of the measure must be less than the expected return.

2. The measure must not be too complicated and dangerous to use.

3. The measure must not aggravate other pest problems in the operation, and when possible, should complement other production practices.

In managing plant diseases, total plant populations are more important than individual plants; since damage or loss of a few scattered plants is insignificant, controls are directed at saving most of the population. The success of a management tactic may be judged in several ways, including extent of reduction in number of diseased plants and by an increase in size and vigor of a crop; ultimately, control must be reflected in an increase in yield, quality, and income.

Management of a plant disease means reduction in the amount of damage caused. It is estimated that the U.S. loses four billion dollars annually due to plant diseases. Complete control is rare, but profitable control, when the increased yield more than covers the cost of disease management, is quite possible. Commercial growers now average a return of four dollars for each dollar invested. The six fundamental principles of disease management are exclusion, eradication, protection, resistance, therapy, and avoidance of insect vectors and weed hosts.

1. **Exclusion** means preventing the entrance and establishment of pathogens in uninfested crops in a particular area. It means using certified seed or plants, sorting bulbs before planting, discarding any that are doubtful, possibly treating seeds, tubers or corms before they are planted, and most especially, refusing obviously diseased specimens from dealers. For example, tare soil returned to trucks at sugarbeet dump stations should never be returned to production fields because of contamination by nematode and rhizomania diseases from other infested fields.

In order to prevent the import and spread of plant pathogens into the country or individual states, certain federal and state laws regulate the conditions under which certain crops may be grown and distributed between states and countries. Such regulatory control is applied by means of quarantines, inspections of plants in the field or warehouse, and occasionally by voluntary or compulsory eradication of certain host plants. Plant quarantines are carried out by experienced inspectors, stationed in all points of entry into the country, to stop persons or produce likely to introduce new pathogens. Similar quarantine regulations govern the interstate, and even the intrastate, sale of nursery stock, tubers, bulbs, seeds, and other propagative organs, especially of certain crops, such as potatoes and fruit trees. For example, a Michigan quarantine prohibits the entry of seed potatoes produced in regions infested with rhizomania disease of sugar beet unless accompanied by a certificate indicating the production field has tested free of the disease.

2. **Eradication** involves the elimination of a pathogen once it has become established on a plant or in a field. It can be accomplished by removal of diseased plants, or parts, as in roguing to control virus diseases or cutting off a cankered tree limb; by cultivating to keep down weed hosts and deep ploughing or spading to bury diseased plant debris; by rotation of susceptible with nonsusceptible crops to starve out the pathogen; and by disinfection, usually by chemicals, sometimes by heat treatment. Spraying or dusting foliage with sulfur after mildew mycelium is present is eradication, and so is treating the soil with chloropicrin to kill nematodes and fungi. Soil treatment with various nematicides (Telone II, Temik 15G, Counter 15 and 20G) is useful to control sugar beet nematodes.

Tan spot, caused by the fungus Pyrenophora triticirepentis, is a major leaf spot disease of winter wheat in the Great Plains of North America. It has become an increasing problem in wheat cropping systems using conservation tillage. This disease can be managed by applying a three-year conservation tillage rotation system called ecofallow. Ecofallow is defined as crop rotation system of controlling weeds and conserving soil moisture with minimum disturbance of crop residue. In this system, corn or sorghum is seeded directly into winter wheat stubble in a winter wheat-grain sorghum/corn-fallow rotation. The uniqueness of this system is that one crop is planted directly into the residue of a different crop rather than into the residue of the same crop. This crop rotation-fallow system effectively breaks disease cycles, such as tan spot, which involve pathogens that survive in crop residue

3. **Protection** is the use of some protective barrier between the susceptible part of the suspect or host and the pathogen. In most cases this is a protective spray or dust applied to the plant in advance of the arrival of the fungus spores; sometimes it means killing insects or other inoculating agents; sometimes it means the erection of a windbreak or other mechanical barrier.

Fungicidal sprays that act as protectants are used to control Cercospora leaf spot of sugar beet, especially in those fields where inoculum has carried over from the previous year. The principle of protective fungicides is to disrupt the natural sequence of infection. These fungicides act on the leaf surface to kill the newly germinated spores. Flowable sulfur is used as a protectant fungicide to control powdery mildew of sugar beet.

There is a long list of chemicals available in the literature that can be used in present-day protective spraying and dusting, along with eradicant chemicals. The commercially sold chemicals are provided with instructions or notes on compatibility and possibilities of injury. A commercial grower can do his plants irreparable harm instead of the good he intends if he doesn't follow the instructions supplied. Spraying is never to be undertaken lightly or thoughtlessly. Read all of the fine print on the label; be sure of the dosage and the safety of that particular chemical on the plant species to be protected.

4. **Disease-resistant and tolerant varieties** are the cheapest, easiest, and most efficient way to reduce disease losses. Varieties should be selected that possess resistance or tolerance to one or more disease organisms. For some diseases, such as the soilborne vascular wilts and the viruses, the use of resistant varieties is the only means of ensuring control. Certified seed of resistant varieties is available and sold commercially. The use of varieties of plants resistant to

particular diseases has proved to be very effective, i.e., stem rust of wheat, rust of dry bean, and Rhizoctonia root rot of sugar beet. Most plant breeding is done for the development of varieties that produce greater yields of better quality. When such varieties become available, they are then tested for resistance against some of the most important pathogens present in the area where the variety is developed and where it is expected to be cultivated. If the variety is resistant to these pathogens for that area, it may be released to the growers for immediate production. If, however, it is susceptible to one or more of these pathogens, the variety is usually discarded, or sometimes it is released for production if the pathogen can be controlled by other means, e.g., chemical, but more often it is subjected to further breeding in an attempt to incorporate into the variety genes that would make it resistant to pathogens without changing any of its desirable characteristics.

There are degrees of resistance to certain diseases, some varieties being completely immune, others partially susceptible. Resistant varieties may become susceptible to new races of a pathogen, i.e., dry bean varieties Beryl and Olathe were resistant to rust races present at the time of their release, but are now susceptible to new rust races.

5. **Therapy** is used on individual plants and can't be used on a large scale. It is achieved by inoculating or treating the plant with something that will inactivate the pathogen. Chemotherapy is the use of chemicals to inactivate the pathogen, whereas heat is sometimes used to inactivate or inhibit virus development in infected plant tissues so that newly developing tissue may be obtained which is free of pathogen. Thermotherapy involves the exposure of diseased plants or parts of them to hot water or high air temperatures for different periods of time. Loose smut of wheat is controlled by treating the seeds with hot water, but modern resistant varieties are a simpler method of control. Hot water treatment has been used to kill nematodes in bulbs, corms, tubers, and fleshy roots while they are in a dormant condition.

Dormant chrysanthemum stools can be rid of foliar nematodes by submerging in water at 112°F (44°C) for 30 minutes.

6. **Control of insect vectors and weed hosts**: certain insects, especially aphids, beetles and leafhoppers, are known to transmit viruses and mycoplasmas from infected plants to healthy plants. Perennial weeds, including pokeweed, milkweed, Johnson grass, and horse nettle, serve as overwintering reservoirs of some viruses. Curly top in sugarbeet is a leafhopper-transmissible viral disease, and weeds play a significant role in its spread. Some of the important weeds involved in the spread of curly top disease are certain species of Chenopodium, Russian thistle, Amaranthus, deadly nightshade, shepherd's pursed, and knotweed. In some cases, aphids feed on some of the early-appearing weeds and then move to new crop plantings, thus introducing viruses which are then spread in secondary cycles within the planting. Sugarcane mosaic virus (SCMV), an aphid-transmitted virus in maize in the U.S. corn belt, is thought to spread from local weed reservoirs. Spread of SCMV depends upon three conditions: the coincident presence of large numbers of aphids and moderate numbers of infected source plants; when moderate numbers of aphids coincide with large numbers of source plants; or when large numbers of both vectors and source plants coincide. Johnson grass is found to be an important source of primary inoculum for SCMV in several areas. Bean yellow mosaic virus (BYMV) is a common problem in bean growing areas. Forage legumes (red clover) are found to be the source of primary inoculum for aphids to carry BYMV into bean fields. For lettuce mosaic virus, only 10 to 15 seconds of feeding are needed for an aphid to acquire the virus; then another 10 to 20 seconds on another plant suffices for the aphid to transmit the virus. Diseases in which the aphid-virus relationship is important include lettuce mosaic, celery mosaic, spinach blight, cucurbit mosaic, pea mosaic, sugar beet mosaic, and tomato mosaic. Several grassy weed species host the wheat curl mite vector of wheat streak mosaic virus and build up inoculum for transmission into adjacent fields of wheat. Many broad

leaf weed species build up inoculum of nematodes, root rot fungi, and leaf spot fungi that attack sugar beets, dry beans, and corn. All growers should practice good control of insects and weeds.

Plant disease symptoms and Signs

Symptoms are the plant's expression of being diseased. Examples of symptoms include: blights, cankers, galls, rots, necrosis, and spots. Symptoms are expressed either locally or systemically, and they frequently reflect the structural, functional, or physiological systems disturbed. Diseases that produce few noticeable symptoms are termed "symptomless". Signs are the physical evidence of the pathogen (primary or secondary, vegetative and/or reproductive structures). Some examples include: conks, mildew, mycelium, ooze, pycnidia, and rhizomorphs.

Diagnosis of plant disease is based on looking for symptoms and signs:

Blight: sudden death of twigs, foliage, and/or flowers.

Blotch: large and irregular-shaped spots or blots on leaves, shoots, and stems.

Canker: dead places on bark and cortex of twigs or stems; often discolored and raised or sunken.

Chlorosis: yellowing of normally green tissue due to reduced chlorophyll content, such tissue is chlorotic.

Damping-off: destruction of seeds in the soil, or seedlings near the soil line, resulting in reduced stand, or the seedling falling over on the ground

Decline: progressive, gradual weakening and death of a plant or population of plants

Dieback: progressive, gradual weakening and death of individual branches of a plant, often leading to decline

Distortion: malformed plant tissues

Flagging: the loss of rigidity and drooping of leaves and tender shoots preceding the wilting of a plant.

Fleck: a minute spot

Galls: abnormal, localized swellings or tumors, on leaf, stem or root tissue

Gum: complex of sugary substances formed by cells in reaction to wounding or infection gummosis: production of gum by or in a plant tissue

Inoculum: amount of pathogen available for infection

Leaf spot: a self-limiting lesion on a leaf

Lesion: a localized area of discolored, diseased tissue

Malignant: tissue that divides and enlarges autonomously, forming a tumor or gall

Masked symptoms: virus-induced plant symptoms that are normally, but appear when the host is exposed to certain environmental conditions of light and temperature

Mildew: a plant disease in which the pathogen is seen as a growth on the surface of the host; e.g., downy mildew, powdery mildew, caused by very different fungi, but both having the name Mildew.

Mosaic: symptom of certain viral diseases of plants characterized by intermingling patches of normal green and light green or yellowish colors

Mottle: an irregular pattern of indistinct light and dark green areas

Mummy: a dried shriveled fruit

Mycelium: masses of fungal threads (hyphae) which compose the vegetative body of the fungus

Necrosis: death of tissue

Necrotic: dead or discolored brown to black

Ooze: a mass of bacterial cells usually embedded in a slimy matrix appearing on the diseased plant surface, often as a droplet; or, a flux, a viscid mass of juices composed of host and parasite substances occasionally found exuding from a diseased plant

Pycnidia: minute, usually globose and black, fungal asexual fruiting structures formed on plant surfaces

Rhizomorphs: string-like strands of fungal mycelia sometimes found under bark of trees

Ring spot: a circular area of chlorosis with a green center; a symptom of many virus diseases

Rot: the softening, discoloration, and disintegration of succulent plant tissue as the result of fungal or bacterial infection

Russet: brownish roughened areas on skin of fruit as a result of cork formation

Rust: a type of disease caused by a specific group of fungi, often producing orange-red "rust" colored spores.

Scab: a roughened crust-like diseased area on the surface of a plant organ; a disease in which such areas form

Sclerotia: tough structures produced by fungi for long-term survival.

Signs: visible evidence of the pathogen; signs are not the same as symptoms

Spots: circular or irregular lesions on above ground tissue

Tip blight: death of shoot tips

Tumor: a malignant overgrowth of tissue

Vein banding: retention of bands of green tissue along the veins while the tissue between veins has become chlorotic

Vein clearing: destruction of chlorophyll adjacent or in the vein tissue as a result of infection by a virus or other pathogen

Wilt: loss of rigidity and drooping of plant parts generally caused by insufficient water in the plant

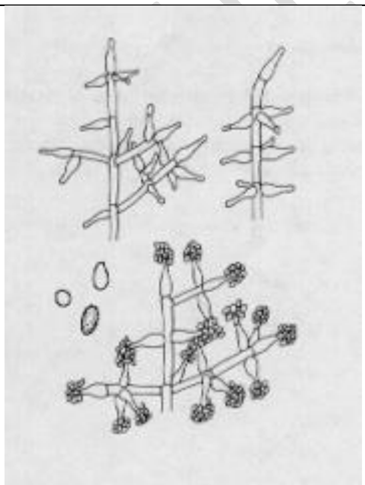
Witches' broom: broom-like growth or massed proliferation caused by the dense clustering of branches in woody plants

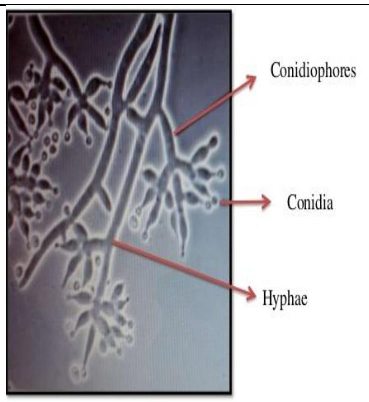
Yellows: a group of systemic mycooplasma-caused diseases often resulting in wilt, witches broom, or decline.

Trichoderma for Plant Disease Management and Food security.

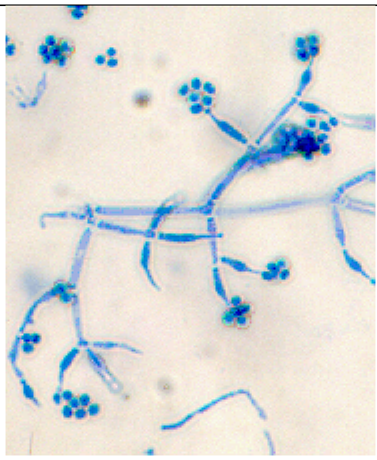
Identification of *Trichoderma*:

The members are saprophytes, found in soil and several species are found to be antagonistic by producing non-volatile antibiotics against a range of plant pathogens. These are easily recognized by rapidly growing white, yellow or green colonies.





Morphological structure of *Trichoderma*



Classification of Trichoderma Fungi

Kingdom: Fungi kingdom composed of multicellular and unicellular organisms that obtain their nutrients from organic matter

Phylum: Ascomycota - Fungi that are characterized by their ascus, a sac-like structure for reproduction

Class: Euascomycetes - Fungi that tend to form lichen with other organisms

Order: Hypocreales - fungi with brightly colored sphaeriaceous (structures that produce spores are brightly colored)

Family: Hypocreaceae - contains species with perithecial ascomata that are brightly colored (red, yellow etc)

Genus: *Trichoderma*

Conidiophores: hyaline, erect,solitary or aggregated into tufts, much branched with phialides in singles or in groups(non-verticillate).

Conidia- hyaline,grey, one celled,ovoid borne in small terminal clusters as balls on phialides.

Eg: *Trichoderma viridi, T.harzianum* – biocontrol fungi.

Isolation and purification of antagonistic fungi from Rhizosphere soil

Soil contains varied group of microorganisms' viz., bacteria, actinomycetes, algae and protozoa. The total number and types of microorganisms present in soil vary depending on chemical composition, physical conditions, organic matter contents, agricultural practices, etc. Some of the microbes cause diseases in plants like damping - off, root rots, wilt etc. However, a large number of microbes play an important role in decomposition of composts and plant residues and biogeochemical cycle.

Requirements: Various media like Potato Dextrose Agar, Czapek-dox and Martins' Rose Bengal Agar medium, Fusarium selective medium, Trichoderma selective medium, etc; for fungi, Sterilized Petri plates, Sterile distilled water blanks (90 ml in each 250 ml. conical flask), Soil samples.

Composition of *Trichoderma* selective medium (g/liter)

Ammonium nitrate	1.0g
Dipotassium Hydrogen phosphate	0.9g
Magnesium sulphate	0.2g
Potassium chloride	0.15g
Glucose	3.0g
Agar	15.0g
Rose Bengal	0.15g
Chloromphenical	0.25g
Metalaxyl	0.30g
Pentachloronitrobenzene	0.20g
Distilled water	1000 ml

Procedure:

Prepare the Petri plates containing agar media. Add 10g field soil (air dry) in 90 ml distilled sterilized water in conical flask and shake it for 3 to 4 minutes so that microbes get suspended in water. This will make 10^1 dilutions.

From 10^1 suspension, take 10 ml and transfer it to 90 ml sterilized distilled water which will make 10^2 dilutions. Serial dilutions are made up to 10^7 following the said procedure.

For determining fungal population, transfer 0.2 ml suspension of 10^3,10^4 and 10^5 dilutions to plates containing PDA and spread it uniformly using glass spreader.

INTERACTIONS INVOLVED IN BIOCONTROL MECHANISMS

From the plant's perspective, biological control can be considered a net positive result arising from a variety of specific and non-specific interactions. The types of interactions are referred as parasitism, antagonism, competition, and predation etc.

Parasitism: It is a relationship in which two phylogenetically unrelated microorganisms coexist over a prolonged period of time. In this type of association, one organism, usually the physically smaller of the two (called the parasite) benefits and the other (called the host) is harmed to some measurable xtent. The activities of various hyperparasites, i.e., those agents that parasitize plant pathogens, can result in biocontrol. And, interestingly, host infection and parasitism by relatively avirulent pathogens may lead to biocontrol of more virulent pathogens through the stimulation of host defense system.

Antagonism (ammensalism): one microorganism produces a substance that is inhibitory to other microbial population results in a negative outcome for later one. Production of oxygen may alter the population of obligate aerobes. Ammonia produced during decompositions of proteins and amino acids at concentrations inhibitory to nitrite oxidizing populations of *Nitrobacter*.

Competition: Competition is a relation within and between species which results in decreased growth, activity and/or productiveness of the interacting microorganisms. For example biocontrol can occur when non-pathogens compete with pathogens for nutrients in and around the host plant.

Predation: It refers to the killing of one microorganism by another for consumption and sustenance. While the term predator typically refer to animals that feed at higher trophic levels in the macroscopic world, it has also been applied to the actions of microbes, e.g. protists, and mesofauna, e.g. fungal feeding nematodes and microarthropods, that consume pathogen biomass for sustenance. E.g. *Dinidium nasutum* preys on *Paramecium*. Biological control can result in varying degrees from all of these types of interactions, depending on the environmental context within which they occur. Significant biological control, as defined above, most generally arises from manipulating mutualism between microbes and their plant hosts or from manipulating antagonism between microbes and pathogens.

ADVANTAGES

• Environment friendly and leave behind no toxic residues.

• Target specific pathogen and avoids unnecessary affect on beneficial microflora and microfauna.

• Most of them are easily culturable in the lab, with minimum space.

• Inexpensive to produce large quantities of inoculum.

• Its mimicry of nature by releasing them into an open environment.

• Biological control could reduce the use of many pesticides and herbicides hence, which could eliminate the overuse of chemicals by farmers and further reduces cost of cultivation.

DISADVANTAGES

• Necessity for careful and correct time of application.

• Host specificity of most pathogens, narrows down its use.

• Necessity to maintain a pathogen in a viable condition.

• Difficulty in producing some obligate and facultative pathogens on a large scale.

• Requirement of favourable environmental conditions for the pathogens to act, multiply and execute its mode of action.

• Potential biological control agents need to be subjected to extensive testing and quarantine before release into any new environment.

Mass production of *Trichoderma* bioagents and their field evaluation

The purpose of production is to produce the greatest quantity of efficacious propagules in the shortest period of time.

Requirements: Potato dextrose broth media, Petriplates, autoclave, laminar flow, inoculation needle, culture of antagonists, incubator, talc powder, Carboxy methyl cellulose.

Preparation of talc based formulation of *Trichoderma viride* and *T. harzianum*- In this case *Trichoderma viride* and *T. harzianum* are grown on potato dextrose broth media. One hundred milliliter of the broth media are transferred to 250 ml Erlenmayer flasks and sterilized at 15 p.s.i. for 15 minutes. The flasks containing the sterile potato dextrose broth media are inoculated with fresh culture of *Trichoderma viride* and *T. harzianum* separately and incubated at 25^0C for 10 days. Potato dextrose broth containing the fungal biomass is homogenized and mixed with talc powder in 1:2 ratio and dried in shade. The mixture is sieved to obtain a fine powder. Carboxy methyl cellulose is added at 6 g kg^{-1}talc preparation as sticker and mixed thoroughly.

Commercial –scale mass production

- Wettable powder (WP)
- Water –dispersible granules (WDG)
- Oil –based emulsifiable suspension (ES)
- Aqueous suspension (AS)
- Granular (G)
- Oil-flowable (OF)
- Whole culture (WC)
- Shellac latex formulation

Based/Carrier materials

- Talc (Magnesium silicate)
- Peat
- Multani soil
- Kaolin (Aliminium silicate)
- Bentonite (Montmorillonite)
- Plant based substrates
- Manures and agricultural bi-products

The commercial success of biocontrol agents requires

i) Consistent and broad spectrum action.

ii) Safety and stability.

iii) Longer shelf life.

iv) Low capital costs.

v) Easy availability of career materials.

vi) Economical and viable market demand.

Formulation

Formulation is blending of active ingredients such as fungal spores with the inert material such as diluents and surfactants in order to alter the physical characteristics of to a more desirable form. A final formulation must:

i) Be easy to handle.

ii) Be stable over a range of -5 to 35°C.

iii) Have a minimum shelf-life of two years at room temperature.

Standards for Trichoderma formulations

i.Colony Forming Units (CFUs) of Trichoderma spp. should be a minimum of 2x106 CFU per ml or gm on selective medium.

ii.Pathogenic contaminants such as Salmonella, Shigella or Vibrio should not be present. Other microbial contaminants need not exceed 1x104 count ml/gm**.**

iii. Maximum moisture content should not be more than 8% for dry formulation of fungi.

Registration of bioagents

Two important factors in the registration of bio agents are the toxicity and environmental fate.

Under Section 9(3) of Pesticide Act of India 1968, information required for the registration of any bio pesticide are:

i) Systemic name and common name of the bio control agent

ii) Natural occurrence

iii) Morphological description of the of the bio agent

iv) Details of manufacturing process

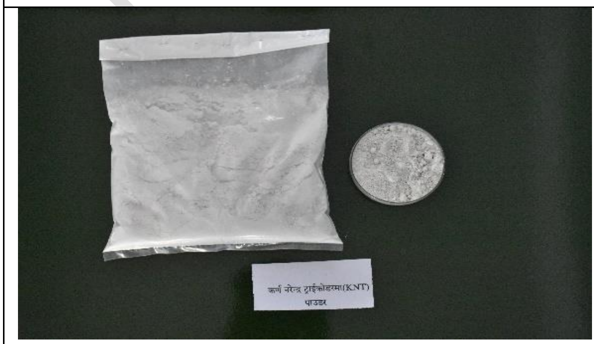
v) Mammalian toxicity

vi) Environmental toxicity

vii) Residual analysis

MODE OF APPLICATION OF TRICHODERMA FOR DISEASE MANAGEMENT

For successful diseases control, delivery and establishment of Trichoderma to the site of action is very important. The most common methods of application of Trichoderma are seed treatment, seed biopriming, seedling dip, soil application and wound dressing.

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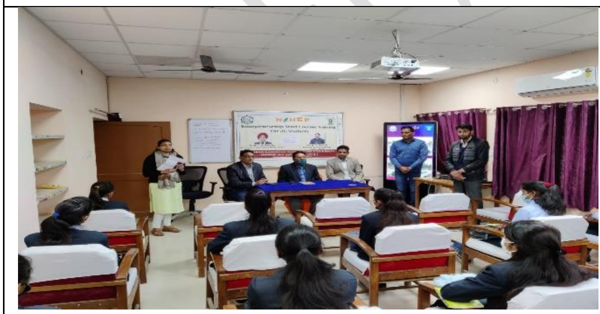

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